Plant Physiol. (1994) 106: 1065–1072

Aerenchyma Carbon Dioxide Can Be Assimilated in *Typha latifolia* L. Leaves[1]

John V. H. Constable[2] and David J. Longstreth*

Department of Plant Biology, Louisiana State University, Baton Rouge, Louisiana 70803

Leaf structural characteristics and gas-exchange measurements were used to determine whether photosynthetic tissue of *Typha latifolia* L. (cattail) utilized CO_2 from the aerenchyma gas spaces, part of an internal pathway for gas transport in this wetland species. The partial pressure of CO_2 (pCO_2) in these aerenchyma gas spaces can be more than 10 times atmospheric pCO_2. The photosynthetic tissue occurred in structurally similar adaxial and abaxial palisades, which were distinctly separated from each other by the aerenchyma gas spaces. In each palisade there were three to four layers of tightly packed, nonchlorophyllous cells separating the photosynthetic tissue from the aerenchyma gas space. Different lines of evidence indicated that CO_2 conductance in the light was significantly greater across the epidermal surface than across the internal surface of both palisades. However, at an epidermal pCO_2 of 350 μbars and an internal pCO_2 of 820 μbars, the net rates of CO_2 uptake (P_N) across the epidermal and internal surfaces were about equal. P_N across the internal surface was greater than across the epidermal surface at higher internal pCO_2. Gas space pCO_2 can be greater than 820 μbars in the field, and therefore, P_N across the internal surface could be a significant proportion of epidermal surface P_N.

Typha latifolia L. (cattail), like many emergent wetland plant species, possesses aerenchyma tissue that forms an internal pathway for gas transport between the emergent leaves and the submerged rhizome (Sebacher et al., 1985; Constable et al., 1992). The transport of O_2 through the aerenchyma minimizes anaerobic respiration in below-ground tissue and may oxidize reduced and potentially toxic compounds in sediments surrounding the below-ground tissue (Armstrong, 1978; Dacey, 1980; Laan et al., 1989). In *T. latifolia* leaves, the aerenchyma gas space is composed of large channels divided by porous diaphragms that run parallel to the long axis of the leaf (Kaul, 1974). These channels account for more than 50% of a leaf's thickness and separate the leaf into distinct AD and AB palisades (Kaul, 1974; Pazourek, 1977; Constable et al., 1992). These structural features are in contrast to more typical leaves that lack aerenchyma gas spaces and have a continuous mesophyll between the AD and AB epidermal surfaces (Esau, 1977).

One potential consequence of the *T. latifolia* leaf structure is that CO_2 exchange can occur between two separate pools of CO_2: the normal atmospheric source where exchange is through stomatal openings in the epidermis and the aerenchyma gas spaces by way of a pathway that has not been characterized (Longstreth, 1989). In different species, the pCO_2 in the aerenchyma gas space can be several times the pCO_2 in the atmosphere (Dacey, 1981; Sebacher et al., 1985; Constable et al., 1992). If this CO_2 is consistently high and available to the photosynthetic tissue, then photorespiration may be significantly reduced in *T. latifolia*, a C_3 species (Ogren, 1984; Bowes, 1993). Although the response of plants to high pCO_2 is varied (Sage et al., 1989; Stitt, 1991; Bowes, 1993), a reduction in photorespiration can generally improve the efficiency of carbon fixation and increase growth of C_3 plants (Bowes, 1993). The natural diurnal pattern of pCO_2 in the aerenchyma gas spaces of *T. latifolia* leaves has been examined during the growing season (Constable et al., 1992). The pCO_2 of aerenchyma gas spaces reached values as high as 6000 μbars (Constable et al., 1992) and was always at least 100 μbars higher than that found in the intercellular spaces of leaves from plants lacking aerenchyma (Yoshie, 1986). Therefore, the pCO_2 in the aerenchyma gas space of *T. latifolia* leaves can be significantly higher than atmospheric pCO_2 under natural conditions.

This study was based on two general hypotheses. First, photosynthetic fixation of aerenchyma CO_2 is significant relative to fixation of atmospheric CO_2, at least when aerenchyma gas space pCO_2 is much higher than atmospheric pCO_2. Second, CO_2 conductance from the aerenchyma gas space to photosynthetic cells is less than the CO_2 conductance from the atmosphere to photosynthetic cells. The use of standard gas-exchange measurements of intact leaves for this study was problematic because this analysis depends on CO_2 uptake being from only one source, the air outside the leaf. Separate determinations of P_N across the epidermal surface and the internal surface facing the aerenchyma gas space were achieved by removing one palisade from a *T. latifolia* leaf and measuring gas exchange across each side of the remaining palisade layer. Microscopy was used to characterize the structure of the pathway for CO_2 movement from the aerenchyma gas space to the photosynthetic tissue of a palisade. These results demonstrate that *T. latifolia* can fix significant amounts of CO_2 from the aerenchyma gas space under conditions that can be found in nature.

[1] Supported by Louisiana Education Quality Support contract RD-A-06 (1991–1992).

[2] Present address: Desert Research Institute, Biological Sciences Center, 7010 Dandini Blvd., Reno, NV.

* Corresponding author; fax 1–504–388–8459.

Abbreviations: AB, abaxial; AD, adaxial; C_i, pCO_2 in leaf intercellular space; g_{wv}, water vapor conductance; pCO_2, partial pressure of CO_2; P_N, net rate of CO_2 uptake; SEM, scanning electron microscopy.

MATERIALS AND METHODS

Plant Material

Soil was removed from the roots of *Typha latifolia* plants collected at a site 1.3 km northeast of the Louisiana State University Ben Hur Research Farm in Baton Rouge, LA. Plants were potted in moist vermiculite in 20-cm-diameter plastic pots, fertilized with 14–14–14 Osmocote fertilizer (Sierra Chemical Co., Milpitas, CA), and grown in a greenhouse under ambient conditions of PPFD and temperature. Two weeks prior to all experiments, plants were transferred to a growth chamber with a 13-h photoperiod and day/night temperatures of 30/22°C. Growth PPFD on leaves was 180 to 300 μmol m^{-2} s^{-1}. Plants were watered with half-strength Hoagland solution (Epstein, 1972) every 3rd d, and water depth was maintained at approximately 2 to 4 cm below the surface of the vermiculite.

Leaf Structural Properties

Entire leaf cross-sections, approximately 3 × 4 × 15 mm, were fixed in formalin-acetic acid-alcohol (5% formalin, 5% acetic acid, 45% ethanol, 45% distilled water) and dehydrated in a graded ethanol series for light microscopy. The cross-sections were embedded in paraffin and sectioned at 12 μm on a rotary microtome, stained with toluidine blue, and photographed in bright-field with a camera mounted on a Leitz Ortholux II microscope. Depths of tissue layers and cell diameters (for circular cells) or cell lengths and widths (for noncircular cells) through the center of an aerenchyma gas space were measured at ×400. Diameters or lengths and widths were used to calculate cell areas in cross-section assuming chlorophyllous cells to be rectangles with semicircular ends and nonchlorophyllous cells to be circles.

For SEM, leaf palisades, approximately 4 × 5 × 3 mm, were fixed for 2 h in 2.5% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2), rinsed in distilled water, and dehydrated in a graded ethanol series. After critical point drying, sections were mounted on stubs, sputter coated with approximately 200 Å of gold-palladium, and viewed with a scanning electron microscope (model S-260; Cambridge Instruments, Cambridge, UK). Stomatal densities were measured on AD and AB epidermal surfaces from six mature leaves. Mean stomatal density was estimated for each surface from stomatal counts in each of 10 randomly placed 0.09-mm^2 quadrats on an −75 SEM image. The morphology of the epidermal and internal surfaces were also examined to evaluate the gas pathway across the internal palisade surface facing the aerenchyma gas space.

Chl concentration and Chl *a/b* were determined in 80% (v/v) acetone extracts by the method of Arnon (1949). Transmittance to different depths in leaves was examined with 10-cm-long sections from mature leaves taped to a 5-mm-thick piece of clear plexiglass. PPFD, provided by a carrousel projector (model Ektagraphic III E with a 300-W bulb; Eastman Kodak Corp., Rochester, NY) was 1428 μmol m^{-2} s^{-1} at the leaf surface, and PPFD at different distances through the leaf section was measured using a photodiode (Hamamatsu Corp., Bridgewater, NJ).

Gas-Exchange Measurements

Rates of CO_2 and H_2O vapor exchange were measured with a clamp-on cuvette in an open gas-exchange system. To separately measure gas-exchange rates of individual sides or surfaces of leaves, a leaf was placed between two plexiglass plates with central openings before clamping the leaf into the cuvette. This arrangement divided the cuvette into two chambers separated by the leaf. Flow rates and air pressures in the two chambers were balanced using a mass flow meter (model 8142; Matheson Gas Products, East Rutherford, NJ) and a water manometer in each gas stream. Two mixing systems (model 8141, Matheson Gas Products, and model B1–2-DP, Bingham Interspace Co., Hyde Park, UT), using proportional gas controllers to blend CO_2-free air and 1% CO_2, allowed independent control of pCO_2 (between 0 and 900 μbars) in each chamber. The gas streams were then bubbled through acidified water and brought to a dew point of 14.9 ± 0.3°C in a stainless steel condenser placed in a temperature-controlled water bath.

Projected leaf area was 3.25 cm^2 in the cuvette. Leaf temperature was 20 ± 2°C as measured with a fine-wire copper-constantan thermocouple. PPFD was provided by a carrousel projector with a 300-W bulb (model Ektagraphic III E, Eastman Kodak) and measured with a quantum sensor (model 190-SB; Li-Cor, Lincoln, NE). For some measurements, PPFD was varied by changing the distance between the projector and the cuvette or by placing neutral density filters (exposed photographic film) between the projector and the cuvette. The gas streams from the cuvette were either routed to a differential IRGA (model 225 MK II; Analytical Development Co., Hoddeson, UK) and dew point hygrometer (model 911; EG & G Corp., Waltham, MA) for measurement or vented to the room. Absolute pCO_2 was measured with an IRGA (model LCA-2; Analytical Development Co.). All gas lines carrying humidified air were made of stainless steel or Teflon tubing. The IRGAs, thermocouples, mass flow meters, and dew point hygrometer were connected by a data-handling system (model 91A; Cyborg Corp., Newton, MA) to a microcomputer (model II+; Apple Computer Corp., Cupertino, CA). Exchange rates of CO_2 and leaf conductance to water vapor (g_{wv}), a measure of stomatal conductance, were calculated according to the method of von Caemmerer and Farquhar (1981).

Two types of gas-exchange measurements were made. First, the responses to PPFD of AD and AB sides of intact leaves were measured. Second, the responses to pCO_2 of both surfaces of AD palisades were measured on leaves that were partially dissected. A portion of the AB palisade, slightly larger than the cuvette opening, was removed for these measurements. This dissection was necessary to permit control of pCO_2 on the aerenchyma gas space surface. The dissected portions of leaves had a single palisade bounded by epidermis (epidermal surface) and a surface that originally faced the aerenchyma gas space (internal surface). Dissected leaves had a water potential (measured with a pressure chamber) that was less than 0.1 MPa (n = 7) below that of control leaves after 3 h in the gas-exchange cuvette. Dissected leaves maintained a constant transpiration rate for the same period, indicating that water supply to the palisade had not

been substantially disrupted. Only the responses to pCO_2 by AD palisades are reported here, but the responses of AB palisades were qualitatively similar.

The g_{wv} of the internal surface could not be calculated accurately because dissection produced an uneven internal surface that was 4 to 5 times greater in area than the smoother epidermal surface. Therefore, the effect of O_2 concentration on epidermal P_N was measured to better evaluate the relative conductances of the epidermal and internal surfaces. The pCO_2 adjacent to the epidermal surface was 350 μbars and the internal surface was 900 μbars, and both partial pressures were obtained by mixing CO_2 in either air (21% O_2) or N_2 ($\leq$1.5% O_2).

RESULTS

Leaf Structural Properties

The AD and AB sides of leaves appeared similar, although they were separated by the large, parallel aerenchyma gas spaces that ran the length of the leaf (Fig. 1). Vascular bundles (which produced ridges on the epidermal surface) separated the photosynthetic tissue into distinct regions. The photosynthetic tissue consisted of two to three layers of palisade parenchyma cells and lacked spongy parenchyma cells (Fig. 1). The palisade cells were separated from the aerenchyma gas space by three to four layers of cells lacking chloroplasts (NL in Figs. 1 and 2). These nonchlorophyllous cells appeared to be tightly connected with no apparent openings (I in Fig. 2) to facilitate gas transfer between the palisade and the aerenchyma gas space. The depths of the nonchlorophyllous cells and the palisade cells at the center of a gas channel were similar for the AD and AB sides (Fig. 1A; Table I). The spherical, nonchlorophyllous cells were larger than the cylindrical palisade cells (NL in Figs. 1 and 2), and the area of nonchlorophyllous cells in cross-section was more than 4 times that of palisade cells (Table I). In the SEM, stomata occurred between the vascular ridges over the photosynthetic cells, and guard cells appeared "kidney" shaped rather than the typical "dumbbell" shape found in grass leaves (data not shown). There were no significant differences ($P \leq 0.01$) between the AD and AB palisade for seven leaf characteristics (Table I).

Gas-Exchange Measurements at Different PPFD

Measurements of intact leaves with the split-chamber cuvette permitted separate determinations of P_N for the illuminated and shaded palisades. When leaves were illuminated on the AD side, P_N of the AD palisade reached a maximum of 6.2 μmol m^{-2} s^{-1} at a PPFD of 500 μmol m^{-2} s^{-1}, whereas P_N of the AB palisade continuously increased to 1.4 μmol m^{-2} s^{-1} at the highest measurement PPFD (Fig. 3A). The epidermal g_{wv} for both palisades increased with measurement PPFD, although epidermal g_{wv} of the shaded AB palisade was much less than that of the illuminated AD palisade (Fig. 3C). A similar response was seen when leaves were illuminated on the AB side (Fig. 3, B and D). P_N of both palisades was PPFD saturated at approximately 500 μmol m^{-2} s^{-1}, although the maximum P_N for the illuminated AB palisade was 61% the rate of the illuminated AD palisade (Fig. 3, A

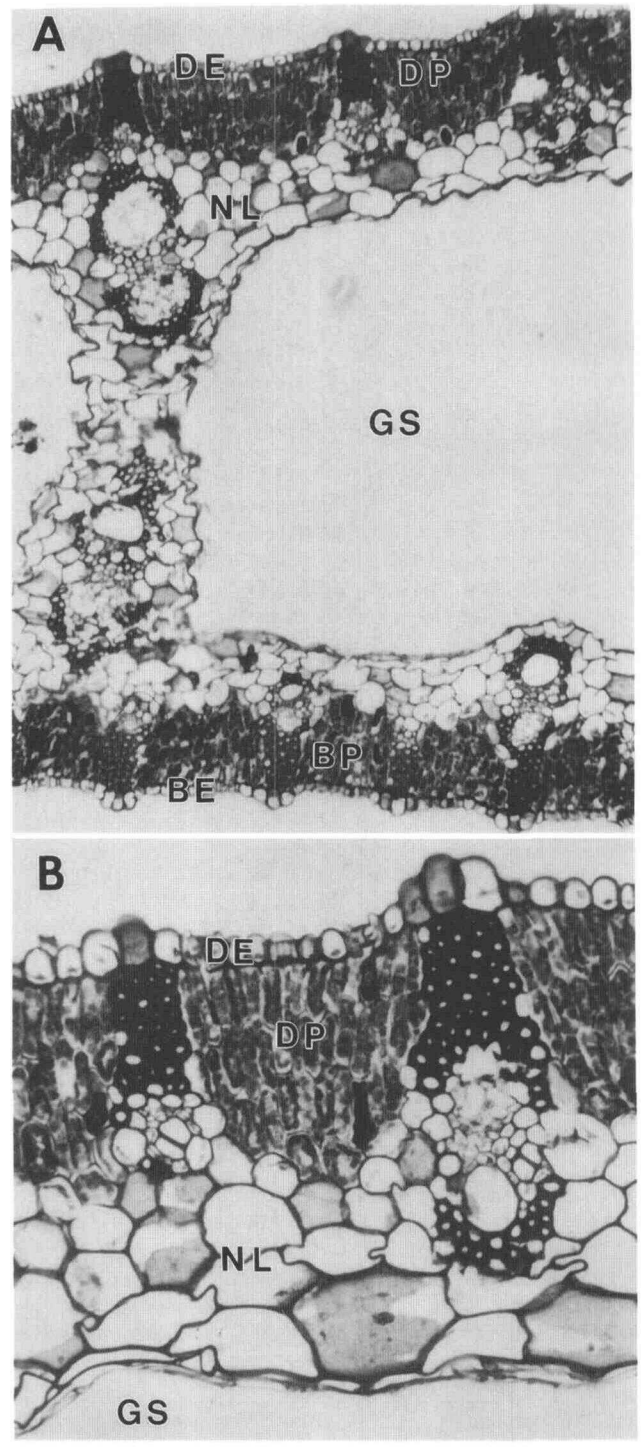

Figure 1. Cross-section (×85) of a *T. latifolia* leaf showing both palisades (A) and a closeup (×340) of the AD palisade (B). DE and BE indicate the AD epidermis and AB epidermis, respectively; NL is the nonchlorophyllous layers of cells and GS is the aerenchyma gas space. DP, AD palisade parenchyma; BP, AB palisade parenchyma.

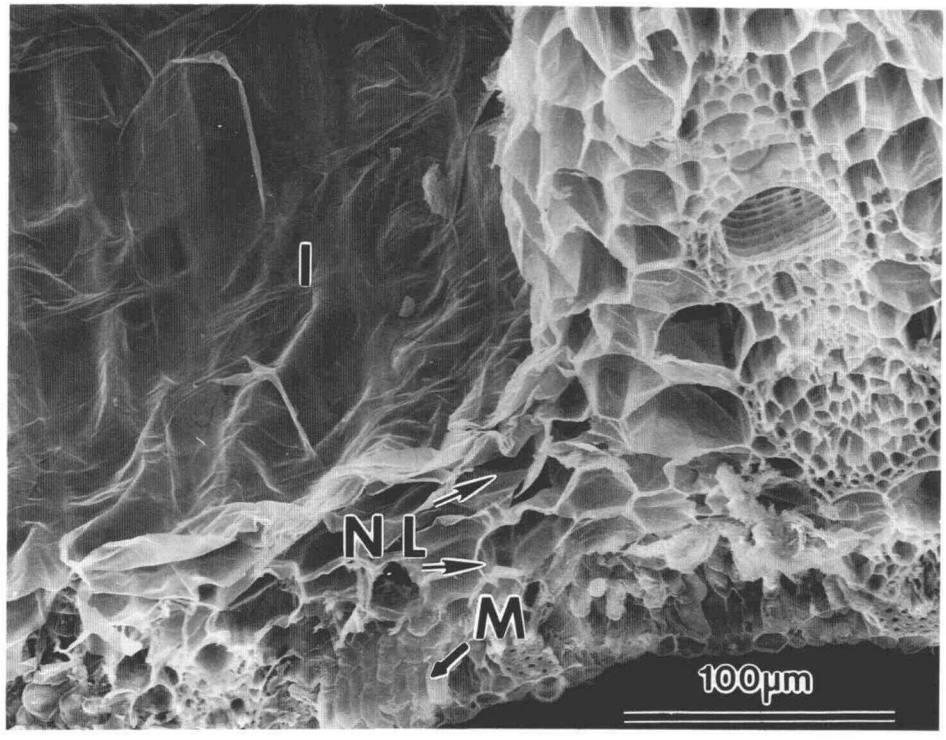

Figure 2. Scanning electron micrograph of a palisade section from a *T. latifolia* leaf looking down on the internal surface (I) of the nonchlorophyllous layers of cells (NL; arrows indicate individual cells). Note photosynthetic palisade mesophyll (M) below NL. Scale bar = 100 μm.

and B). Reversing the leaf orientation in the split-chamber cuvette produced the same differences between the AD and AB palisades. For a leaf from which the AB palisade was removed, the response of the remaining AD palisade was similar to that of the AD palisade in an intact leaf: P_N of the AD palisade from a dissected leaf was saturated at about 500 μmol m^{-2} s^{-1} and reached a maximum of 5.5 μmol m^{-2} s^{-1} across the AD epidermis (data not shown).

Light was transmitted through either palisade layer. About

Table I. *Comparison of leaf and cell characteristics for the AD and AB palisades from T. latifolia leaves*

Internal layer refers to nonchlorophyllous cells separating the palisade from the aerenchyma gas space. Values are means ± SE, n = 10. Significant differences between AD and AB means were tested with a t test, and the resulting probability for the null hypothesis (AD = AB) is shown (P).

Characteristic	AD	AB	P
Stomatal density (no. mm^{-2})	620 ± 116	550 ± 121	0.035
Depth of palisade (μm)	75 ± 3	83 ± 2	0.106
Depth of internal layer (μm)	88 ± 6	102 ± 4	0.199
Area of palisade cell (μm^2)	191 ± 5	195 ± 7	0.389
Area of internal cells (μm^2)	861 ± 80	905 ± 83	0.569
Total Chl (μg cm^{-2})	42 ± 1.9	49 ± 1.4	0.013
Chl *a*/*b* ratio	3.0 ± 0.11	3.0 ± 0.05	0.515

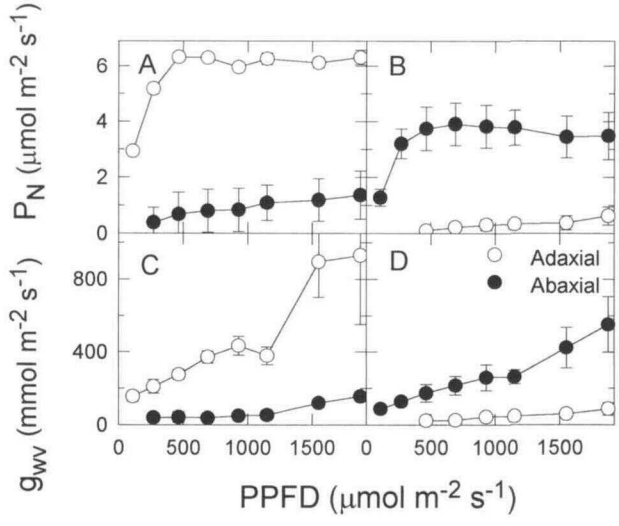

Figure 3. P_N (A and B) and g_{wv} (C and D) of isolated AD palisade (open symbols) and AB palisade (closed symbols) from *T. latifolia* leaves at different PPFD and an epidermal surface pCO_2 of 350 μbars. PPFD was directed toward the AD epidermis (A and C) or the AB epidermis (B and D). Values are means ± SE (n = 5–7).

Table II. *Transmittance through various depths of T. latifolia leaves*

A PPFD source was focused on the epidermis of either the AD or AB palisade, and the flux density was measured immediately below the palisade (first internal surface), below the palisade and across the aerenchyma gas space at the internal surface of the second palisade (second internal surface), and at the epidermal surface of the second palisade (second epidermis). Values are means ± SE (n = 8).

Measurement Location	Measured PPFD	
	AD	AB
	$\mu mol\ m^{-2}\ s^{-1}$	
First epidermis	1428 ± 1	1428 ± 1
First internal surface	161 ± 2	176 ± 5
Second internal surface	133 ± 3	124 ± 8
Second epidermis	21 ± 1	21 ± 1

11% of the PPFD incident on the epidermis of the AD palisade was measured below the AD palisade, and about 9% was measured at the internal surface of the AB palisade, across the gas space (Table II). Similar results were obtained when PPFD was directed toward the epidermis of the AB palisade. Therefore, about 9% of the PPFD on the epidermis of a palisade layer was transmitted to the internal surface of the other palisade layer.

Gas-Exchange Measurements at Different pCO_2 Values

The pCO_2 against the internal surface (facing the aerenchyma gas space) appeared to influence P_N across the epidermis when response to epidermal pCO_2 was measured (Fig. 4). The effect of varying the internal surface pCO_2 on epidermal P_N and g_{wv} was then measured with the epidermal

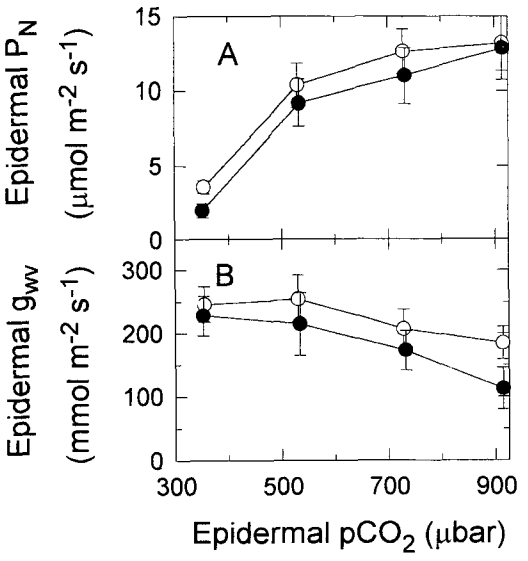

Figure 4. Epidermal P_N (A) and g_{wv} (B) at different epidermal surface pCO_2 for dissected *T. latifolia* leaves. Internal surface pCO_2 was 355 μbars (open symbols) or 930 μbars (closed symbols). Values are means ± SE (n = 4–10).

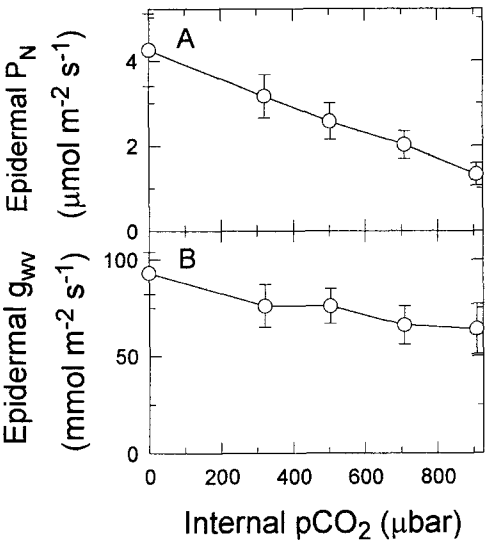

Figure 5. Epidermal P_N (A) and g_{wv} (B) of dissected *T. latifolia* leaves at an epidermal surface pCO_2 of 350 μbars and different internal surface pCO_2 values. Results are means ± SE (n = 4–6).

pCO_2 held constant at 350 μbars (Fig. 5). There was a negative and linear relationship between epidermal P_N and internal surface pCO_2 from about 0 to 900 μbars (Fig. 5A). Epidermal P_N at the highest internal pCO_2 was about 31% of the P_N where internal pCO_2 was 0. Epidermal g_{wv} also tended to decline as internal surface pCO_2 increased (Fig. 5B).

The pCO_2 on the epidermal surface was held constant at 350 μbars, and P_N of the internal surface was measured as internal surface pCO_2 was varied. The internal surface P_N was just positive when internal surface pCO_2 was about 320 μbars, but P_N increased in a linear manner through the highest internal surface pCO_2 used (Fig. 6). Under conditions used here, the maximum P_N across the internal surface (Fig. 6) was much less than the maximum P_N across epidermal surface (Fig. 4).

Epidermal P_N was also measured at a pCO_2 of 350 μbars on the epidermis and 900 μbars on the internal surface in either air (21% O_2) or N_2 (≤1.5% O_2). The maximum epidermal P_N occurred when CO_2 was mixed with N_2 on both

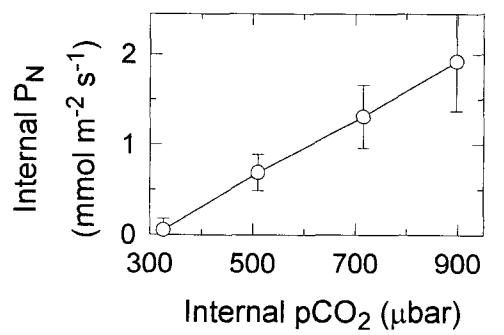

Figure 6. Internal surface P_N of dissected *T. latifolia* leaves at an epidermal surface pCO_2 of 350 μbars and different internal surface pCO_2 values. Results are means ± SE (n = 6).

Table III. *Effect of air (21% O_2) and N_2 (≤1.5% O_2) on epidermal P_N of dissected T. latifolia leaves*

Epidermal and aerenchyma gas space pCO_2 were held at 350 and 900 μbars, respectively, and mixed in air or N_2. Means with different letters indicate significant differences with a least significant difference test ($P \leq 0.05$). Values are means ± SE ($n = 6$).

Treatment		Epidermal P_N
Epidermis	Aerenchyma	
		μmol m^{-2} s^{-1}
350 μbars CO_2 in N_2	900 μbars CO_2 in N_2	3.0 ± 0.3 a
350 μbars CO_2 in N_2	900 μbars CO_2 in air	2.7 ± 0.3 ab
350 μbars CO_2 in air	900 μbars CO_2 in N_2	1.9 ± 0.2 bc
350 μbars CO_2 in air	900 μbars CO_2 in air	1.6 ± 0.3 c

surfaces (Table III). When the epidermal CO_2 was mixed in air instead of N_2, epidermal P_N was reduced 37% with the internal surface CO_2 mixed in N_2 and 47% with the internal surface CO_2 mixed in air. There was not a statistically significant reduction in epidermal P_N when internal surface CO_2 was mixed in air as opposed to N_2 (Table III).

DISCUSSION

Response to PPFD

The leaf of *T. latifolia* is unusual in that there are two distinct palisades separated by aerenchyma gas space (Fig. 1). Depending on the orientation of the leaf relative to a PPFD source, one palisade can be directly illuminated and the other shaded. The P_N of the directly illuminated and shaded palisades should produce an integrated response that reflects the relative degree of PPFD saturation for each surface. Although both AD and AB palisades PPFD saturate at approximately 500 μmol m^{-2} s^{-1} (Fig. 3), total P_N of the leaf would saturate at higher PPFD because the shaded palisade receives considerably less PPFD than the palisade receiving PPFD directly. In fact the intact leaf requires about 930 μmol m^{-2} s^{-1} before the combined P_N of both palisades is saturated (data not shown). PPFD is transmitted through the illuminated palisade, and at the highest PPFD, P_N by the shaded palisade could account for 15% of total leaf P_N (Fig. 3, A and B).

In these laboratory measurements, PPFD reaching the shaded palisade was primarily PPFD transmitted through the illuminated palisade because PPFD incident on the epidermis of the shaded palisade was less than 10 μmol m^{-2} s^{-1}. In natural stands of *T. latifolia*, however, PPFD normal to the soil surface frequently exceeds 1500 μmol m^{-2} s^{-1} for several hours per day (Constable et al., 1992). Although the relatively vertical orientation of *T. latifolia* leaves reduces PPFD interception at midday, the PPFD normal to the leaf surface can exceed 1000 μmol m^{-2} s^{-1} (J. Constable, unpublished data), which surpasses the PPFD necessary to saturate P_N found here (Fig. 3). In the field at 50 cm below the top of the canopy, indirect PPFD measured on the shaded palisade can be 200 μmol m^{-2} s^{-1} or about 12% of the maximum direct PPFD measured on the illuminated palisade, a value well below PPFD saturation (J. Constable, unpublished data). If 9% of PPFD incident on the illuminated palisade is transmitted to the shaded palisade (Table II), then at the highest PPFD transmitted PPFD could increase total PPFD intercepted by the shaded palisade by as much as 155 μmol m^{-2} s^{-1}. The total PPFD intercepted by the shaded palisade could then be 355 μmol m^{-2} s^{-1}, 200 μmol m^{-2} s^{-1} on the epidermal surface and 155 μmol m^{-2} s^{-1} on the internal surface. Therefore, the contribution to total leaf P_N by the shaded palisade would be significantly higher in the field than in these laboratory measurements.

The point of PPFD saturation was similar for the AD and AB surfaces, but PPFD-saturated P_N was higher for the AD surface (Fig. 3). The plants used here were grown in a growth chamber, and the leaves used in these measurements were oriented more vertically than horizontally; however, the AD surface received somewhat higher PPFD than the AB surface. The difference in PPFD-saturated P_N may have been due to development of the AD and AB palisades in slightly different PPFD environments, since increasing the growth PPFD produces higher P_N in many species (Björkman, 1981). The spatial separation of the two palisades of the *T. latifolia* leaf by the aerenchyma gas space can result in development under different PPFD environments and produce different photosynthetic characteristics for the two palisades.

Response to pCO_2

P_N occurred across the epidermal surface and across the internal surface facing the aerenchyma gas space (Figs. 4 and 6). The calculation of C_i was not possible because there were two potential CO_2 sources for a palisade, the atmosphere and the aerenchyma gas space. Therefore, the relationship of P_N to epidermal or internal surface pCO_2 was compared here. The relationship between epidermal surface pCO_2 and C_i could be close in these measurements because epidermal P_N was relatively low (Sage et al., 1989) and the effect of high pCO_2 on g_{wv} was not great (Figs. 4 and 5). The response of P_N and g_{wv} to changing pCO_2 across the epidermal surface was similar to that in other C_3 plants (Sage et al., 1989; Stitt, 1991). P_N can be CO_2 saturated at a C_i of 400 to 500 μbars in some species grown at atmospheric CO_2, and in most species, the C_i required for CO_2 saturation of P_N increases when plants are grown at a pCO_2 that is higher than normal atmospheric pCO_2 (Sage et al., 1989; Stitt, 1991). *T. latifolia* P_N required a pCO_2 of more than 700 μbars for saturation (Fig. 4A), resembling the response of species grown at high pCO_2. *Eriogonum inflatum* produces photosynthetic, hollow stems in the center of which pCO_2 can range from 3,000 to more than 14,000 μbars (Osmond et al., 1987). Stems of *E. inflatum* show increasing P_N with increasing pCO_2 through C_i of about 800 μbars. The epidermal g_{wv} for the *T. latifolia* leaf declined with increasing pCO_2 (Fig. 4B), a pattern found in many species.

CO_2 was taken up across the internal palisade surface facing the aerenchyma gas space, and this P_N increased linearly as internal surface pCO_2 was increased from about 320 to 900 μbars (Fig. 6). The P_N across the internal surface was significantly lower than P_N across the epidermal surface at comparable values of pCO_2 (Figs. 4 and 6), indicating a more significant barrier to CO_2 diffusion across the internal

surface. The g_{wv} for the internal surface was difficult to determine because the leaf dissection produced a ragged and ridged surface area that was 4 to 5 times greater than the projected leaf area. Based on the gas-exchange measurements and examination of the internal surfaces of dissected leaves with the light microscope, we estimated the conductance across the internal surface to be about one-third of that across the epidermal surface. The compensation point for pCO_2 of the epidermal surface (Fig. 4) appeared to be substantially lower than that for the internal surface (Fig. 6), which is consistent with higher conductance across the epidermal surface.

Differences in conductance across the epidermal and internal surfaces were also evaluated by manipulating $[O_2]$ over the two surfaces and observing the effect on epidermal P_N (Table III). The pathway across the internal surface involves CO_2 diffusion through the layers of nonchlorophyllous cells that fit together and have a generally unbroken surface facing the aerenchyma gas space (Fig. 2). This structure indicates that at least part of this pathway for CO_2 diffusion should involve CO_2 diffusion through the cell wall solution and/or symplast. Diffusion through an aqueous phase would be expected to substantially lower conductance relative to gaseous diffusion through stomata (Nobel, 1991). P_N can be represented as the product of leaf conductance to CO_2 and the pCO_2 gradient from outside to inside the leaf. The apparent conductance across the internal surface of the photosynthetic stem of *E. inflatum* also appears to be quite low, since P_N is quite low, but internal surface pCO_2 is several times that used in these experiments (Osmond et al., 1987) or that measured in field-grown *T. latifolia* leaves (Constable et al., 1992).

Epidermal surface P_N was inversely related to internal surface pCO_2 when the epidermal pCO_2 was held constant (Fig. 5A). This is potentially a response to the diffusion of CO_2 from the aerenchyma gas space into the intercellular space of the palisade. At low internal surface pCO_2, most of the CO_2 fixed by the palisade may come from diffusion through stomata in the epidermis, but as the internal surface pCO_2 increases, the rate of diffusion across the internal surface increases and the palisade requires less CO_2 from the epidermal surface. The reduction in epidermal g_{wv} with increasing internal surface pCO_2 (Fig. 5) is consistent with an increase in intercellular pCO_2 and partial stomatal closure. Whereas epidermal P_N decreases as internal pCO_2 increases, internal P_N increases and the sum of epidermal P_N and internal P_N remains relatively constant (Table IV). The internal surface P_N was not measured at an internal surface pCO_2 of 0, but the value calculated from the regression is a negative value that when added to the epidermal surface P_N gives a value similar to that found at other pCO_2 values (Table IV). We hypothesize that this apparent loss of CO_2 to the aerenchyma gas space is real and related to respiration by the nonchlorophyllous cells.

P_N was about equal across the epidermal and internal surfaces when epidermal surface pCO_2 was 350 μbars and internal surface P_N was 820 μbars (Figs. 5 and 6). When pCO_2 on the internal surface is greater than 820 μbars, more CO_2 may be fixed from the aerenchyma gas space than from the atmosphere. Based on field measurements of atmospheric and aerenchyma gas space pCO_2 and PPFD (Constable et al., 1992), the aerenchyma gas space may be the most important source of CO_2 for about 3 h in the morning in plants growing in the field. Although its importance would not be as great at other times of the day, P_N across the internal surface could still be a significant portion of total P_N throughout much of the daylight period because pCO_2 is generally equal to or greater than atmospheric pCO_2 (Constable et al., 1992). Although the focus of this study has been P_N, it should be noted that high pCO_2 affects other metabolic processes (Bowes, 1993). Elevated atmospheric pCO_2 has been shown to reduce dark respiration (Gifford et al., 1985; Amthor et al., 1992; Bunce, 1992) and alter uptake and reduction of NO_3^- (Pace et al., 1990). Such effects of high pCO_2 may also significantly affect the metabolism of *T. latifolia* plants, particularly through carbon partitioning.

Table IV. *Total P_N (epidermal and internal) in dissected T. latifolia leaves at different aerenchyma gas space pCO_2 values*

Epidermal P_N values are from Figure 5A and internal P_N values are from Figure 6. Epidermal pCO_2 was 350 μbars in both experiments.

P_N	Aerenchyma Gas Space pCO_2 (μbars)				
	0	321	503	708	908
	$\mu mol\ m^{-2}\ s^{-1}$				
Epidermal	4.3	3.1	2.6	2.0	1.4
Internal	−0.9[a]	0.1	0.7	1.3	1.9
Total	3.4	3.2	3.3	3.3	3.3

[a] Extrapolated value.

In conclusion, these results confirm our two hypotheses. First, photosynthetic fixation of aerenchyma CO_2 can be significant relative to fixation of atmospheric CO_2 when aerenchyma gas space pCO_2 is on the order of twice atmospheric pCO_2. Second, CO_2 conductance from the aerenchyma gas space to photosynthetic cells is much lower than the conductance from the atmosphere to the photosynthetic cells. Although aerenchyma is common in wetland species, *T. latifolia* appears somewhat unusual compared to other emergent wetland species such as *Phragmites australis* (Brix, 1990), in that aerenchyma gas spaces are continuous from leaves to the bases of plants (Constable et al., 1992; Bowes, 1993). Further studies are needed to understand the ecological consequences of CO_2 movement in aerenchyma for *T. latifolia* and other wetland species.

ACKNOWLEDGMENTS

We are grateful to Dr. Sharon Matthews from the Louisiana State University Basic Sciences Microscopy Center for performing the SEM and Dr. James V. Moroney for advice and comments concerning the manuscript.

Received January 21, 1994; accepted June 13, 1994.

LITERATURE CITED

Amthor JS, Koch GW, Bloom AJ (1992) CO_2 inhibits respiration in leaves of *Rumex crispus* L. Plant Physiol **98:** 757–760

Armstrong W (1978) Root aeration in the wetland condition. *In* DD Hook, RMM Crawford, eds, Plant Life in Anaerobic Environments. Ann Arbor Science Publishers, Ann Arbor, MI, pp 269–297

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol **24:** 1–15

Björkman O (1981) Responses to different quantum flux densities. *In* OL Lange, PS Nobel, CB Osmond, H Ziegler, eds, Encyclopedia of Plant Physiology, New Series, Vol 12A: Physiological Plant Ecology I. Springer-Verlag, New York, pp 57–107

Bowes G (1993) Facing the inevitable: plants and increasing atmospheric CO_2. Annu Rev Plant Physiol Plant Mol Biol **44:** 309–332

Brix H (1990) Uptake and photosynthetic utilization of sediment-derived carbon by *Phragmites australis* (Cav.) Trin. ex Steudel. Aquat Bot **38:** 377–389

Bunce JA (1992) Stomatal conductance, photosynthesis and respiration of temperate deciduous tree seedlings grown outdoors at an elevated concentration of carbon dioxide. Plant Cell Environ **15:** 541–549

Constable JVH, Grace JB, Longstreth DJ (1992) High carbon dioxide concentrations in aerenchyma of *Typha latifolia*. Am J Bot **79:** 415–418

Dacey JWH (1980) Internal winds in water lilies: an adaptation for life in anaerobic sediments. Science **210:** 1017–1019

Dacey JWH (1981) Pressurized ventilation in the yellow waterlily. Ecology **62:** 1137–1147

Epstein E (1972) Mineral Nutrition of Plants: Principles and Perspectives. John Wiley and Sons, New York

Esau K (1977) The Anatomy of Seed Plants, Ed 2. John Wiley and Sons, New York

Gifford RM, Lambers H, Morison JIL (1985) Respiration of crop species under CO_2 enrichment. Physiol Plant **63:** 351–356

Kaul RB (1974) Ontogeny of foliar diaphragms in *Typha latifolia*. Am J Bot **61:** 318–323

Laan P, Berrevoets MJ, Lythe S, Armstrong W, Blom CWPM (1989) Root morphology and aerenchyma formation as indicators of the flood-tolerance of *Rumex* species. J Ecol **77:** 693–703

Longstreth DJ (1989) Photosynthesis and photorespiration in freshwater emergent and floating plants. Aquat Bot **34:** 287–299

Nobel PS (1991) Physicochemical and Environmental Plant Physiology. Academic Press, New York

Ogren WL (1984) Photorespiration: pathways, regulation and modification. Annu Rev Plant Physiol **35:** 415–442

Osmond CB, Smith SD, Gui-Ying B, Sharkey TD (1987) Stem photosynthesis in a desert ephemeral, *Eriogonum inflatum*. Characterization of leaf and stem CO_2 fixation and H_2O vapor exchange under controlled conditions. Oecologia **72:** 542–549

Pace GM, Volk RJ, Jackson WA (1990) Nitrate reduction in response to CO_2-limited photosynthesis. Relationship to carbohydrate supply and nitrate reductase activity in maize seedlings. Plant Physiol **92:** 286–292

Pazourek J (1977) The volumes of anatomical components in leaves of *Typha angustifolia* L. and *Typha latifolia* L. Biol Plant **19:** 129–135

Sage RF, Sharkey TD, Seemann JR (1989) Acclimation of photosynthesis to elevated CO_2 in five C_3 species. Plant Physiol **89:** 590–596

Sebacher DI, Harriss RC, Bartlett KB (1985) Methane emissions to the atmosphere through aquatic plants. J Environ Quality **14:** 40–46

Stitt M (1991) Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. Plant Cell Environ **14:** 741–762

von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta **153:** 376–387

Yoshie F (1986) Intercellular CO_2 concentration and water-use efficiency of temperate plants with different life-forms and from different microhabitats. Oecologia **68:** 370–374