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Measurement of CO_2 and H_2O Vapor Exchange in Spinach Leaf Discs[1]

EFFECTS OF ORTHOPHOSPHATE

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ABSTRACT

A leaf chamber has been designed which allows the measurement of both CO_2 and water vapor exchange in *Spinacia oleracea* leaf discs. The center of the disc lies within a cylindrical gas chamber and its margins are enclosed within a cavity through which water or various metabolites can be pumped. In saturating light and normal atmospheres, the leaf discs have a relatively low resistance to H_2O vapor transfer (r_w = 1.87 seconds per centimeter) and can support high rates of photosynthesis for several hours. The abaxial surface of a disc had a higher resistance to water vapor transfer (r_w = 3.22 seconds per centimeter) than the adaxial (r_w = 2.45 seconds per centimeter) despite having a higher stomatal frequency (abaxial, 105/square millimeter; adaxial, 58/square millimeter). In 2% O_2, the discs required an internal concentration of CO_2 of 115 microliters per liter to support one-half of the maximal velocity of apparent photosynthesis (average value, 66 milligrams CO_2 per square decimeter per hour). In 20% O_2, the comparable values are 156 microliters per liter and 56 milligrams CO_2 per square decimeter per hour. In air, apparent photosynthesis saturated at intensities (750 microeinsteins per square meter per second) well below that of daylight but, when the internal CO_2 was raised to 700 to 900 microliters per liter, photosynthesis was not saturated even at daylight intensities (2025 microeinsteins per square meter per second). The distribution of Prussian blue crystals, formed after ferrocyanide feeding, showed that water entered the disc via the vasculature. When 25-minute pulses of orthophosphate were provided in the feeding solution, there were concentration-dependent increases in both r_w and r_m leading to inhibition of photosynthesis. The orthophosphate-dependent inhibitions were reversible.

Samples of leaf tissue in the form of discs, sections, or slices have been frequently employed in the study of photosynthesis and photorespiration (3, 6, 13, 16, 18, 19, 22, 25). One advantage is that uniform replicates can be obtained from an individual leaf of the same or different species and can often be assayed under identical conditions. In addition, various compounds can be fed through the cut surfaces, bypassing the selective permeability of the root system and minimizing the transport distance required for the intracellular distribution of a given reagent. Nevertheless, several problems have prevented the widespread use of leaf tissue pieces, especially in the measurement of photosynthetic gas exchange by IR gas analysis (19). Perhaps the most serious is that of providing the tissue samples with adequate water under conditions of rapid flow rate of gases and high-incident light intensities. This problem is compounded by the need for transpirational measurements which are necessary for the calculation of resistances to water vapor and CO_2 diffusion (7, 8, 20). This precludes the inclusion of a source of water within that part of the analysis chamber in which gaseous exchange is occurring.

Another difficulty relates to the possibility that sample preparation or the assay conditions themselves introduce variable and/or anomalous photosynthetic responses. This report describes how these difficulties have been largely overcome. It has three main aims: (a) the description of a leaf chamber which accomodates spinach leaf discs and allows for measurement of both CO_2 and water vapor exchange; (b) the characteristics of the photosynthetic and stomatal responses of leaf discs within the chamber; (c) the potential for the utilization of the chamber for feeding compounds to leaf discs.

MATERIALS AND METHODS

Plant Material. Spinach (*Spinacia oleracea*, United States Hybrid 424; Ferry Morse Seed Co., Mountain View, CA) was grown under glass in a mixture of Levington Compost and vermiculite in a ratio of 2:1.

Leaf Chamber Construction. The leaf chamber (Fig. 1) was constructed from clear Perspex (Plexiglas) and consists of two distinct components: an inner gas-exchange chamber and an outer feeding chamber. The inner chamber has two crescents of inlets and outlets which allow a uniform flow of gases over the surface of the disc. The inlet diameter is 3-fold smaller than the outlet, reducing the possibility of pressure build-up. There is no provision for stirring within the chamber, but the flow rate of gas (400–500 cm^3/min), in the small volume (3.5 cm^3) per half chamber, gives a relatively low boundary layer resistance (0.18 s/cm) when measured with a filter paper leaf model (20).

The outer chamber allows the cut edge of the leaf to be in contact with water or a solution. A water-tight seal was effected between the inner and the outer chamber by greasing the rubber inserts (Fig. 1, d and e) and sandwiching the leaf disc between them. The two halves of the chamber were then placed together and tightened with wing nuts.

IRGA[2] System. The complete system is shown in Figure 2. Gas was provided via tanks of 1,000 μl/l CO_2, 20% or 2% O_2, balance N_2. The CO_2 concentration required was set by means of the Analytical Development Co., Ltd. (ADC) gas diluter. The gas was humidified by passing it through two wash bottles at room tem-

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[2] Abbreviations: IRGA, infrared gas analyzer; r_w, water vapor transfer resistance; r_m, mesophyll resistance.

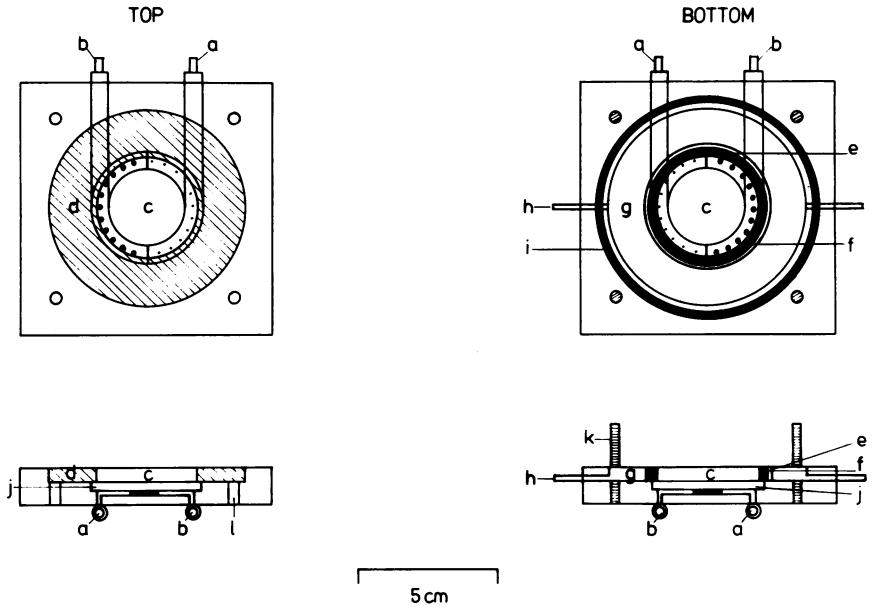

FIG. 1. Scale drawing of the leaf chamber. (a), Gas inlet; (b), gas outlet; (c), gas exchange chamber; (d), silicone rubber disc seal; (e), silicone rubber seal; (f), clear Perspex wall; (g), feeding chamber; (h), water inlet/outlet; (i), 'O' ring seal; (j), clear Perspex disc, drilled with the gas inlet and outlet jets; (k), threaded posts to clamp the top by means of wing nuts; (l), holes for threaded posts.

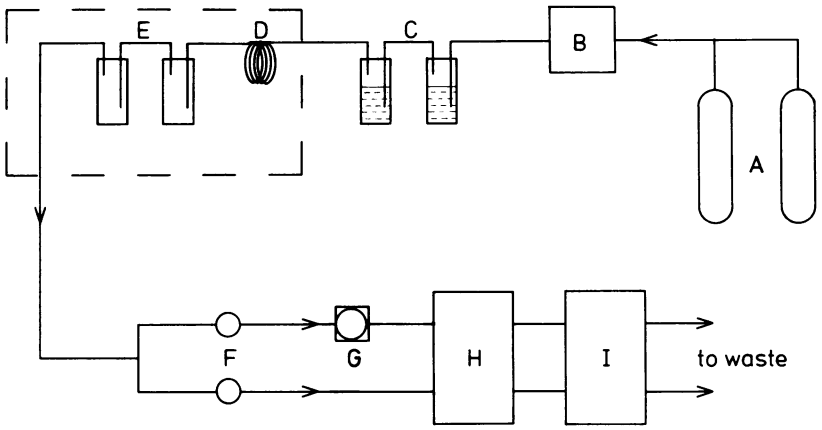

FIG. 2. Diagrammatic representation of the gas supply system. (A), gas cylinders of 1,000 μl/l CO_2, either 2% or 20% O_2, balance N_2; (B), CO_2 gas diluter (ADC); (C), water-filled dreschel bottles to saturate the gas; (D), cooling coil and E-traps both maintained at 9.5°C; (F), rotameters to set flow; (G), leaf chamber; (H), water vapor IRGA; (I), CO_2 IRGA.

perature, and the dew point of the moist gas was then set by passing the gas through a stainless steel coil and two glass traps immersed in a refrigerated water bath at 9.5°C giving 50.8% saturation at 20°C. The gas stream was split before passing through the two rotameters utilized to measure the flow rate.

The analysis stream passed through the leaf chamber, first to an ADC Mk. II water vapor IRGA then on to a Mk. III CO_2-IRGA, giving direct readout onto a chart recorder. The CO_2-IRGA was calibrated from standard CO_2 tanks bought from Rank Hilger. These were calibrated from gas certified by the National Physics Laboratory. The H_2O-IRGA was calibrated by means of an ADC water vapor generator. Leaf temperature measurements were made by means of a fine thermocouple of copper/constantan placed against the underside of the leaf disc in the gas exchange chamber. This was connected to a Comark electric thermometer and then to a recorder.

Illumination. The leaf chamber was illuminated by means of a Leitz Prado Universal projector, the light beam passing through a glass tank of water of path-length 20 cm and a 1-L distillation flask filled with water which was used as a lens. This system could give light intensities up to 2,025 $\mu E/m^2 \cdot s$ (400–700 nm) when measured through the Perspex window of the assimilation chamber.

Experimental Procedure. A disc of 5 cm diameter was cut under water and sealed in the chamber as above; distilled H_2O was then pumped into the system from the bottom by means of a peristaltic pump at a rate of 3.5 ml/min. When the feeding chamber was full and water was passing to waste, gas was passed over both surfaces of the leaf and out to the precalibrated IRGAs. When the IRGA traces had stabilized, the chamber was then illuminated at 1,500 $\mu E/m^2 \cdot s$ (unless light intensity was a variable parameter). Solutions could then be fed to the leaf disc by simply pumping them through the feeding chamber as and when required.

The method of following the movement of a given solute into the leaf disc and its subsequent distribution pattern was adapted from Burbano *et al.* (4). This involved feeding a 5% w/v solution of potassium ferrocyanide to the leaf disc for a given period. The disc was then washed and dropped into a boiling solution of 2% w/v ferric chloride in 80% ethanol, thus killing and clearing the leaf while converting the ferrocyanide to Prussian blue. After 5

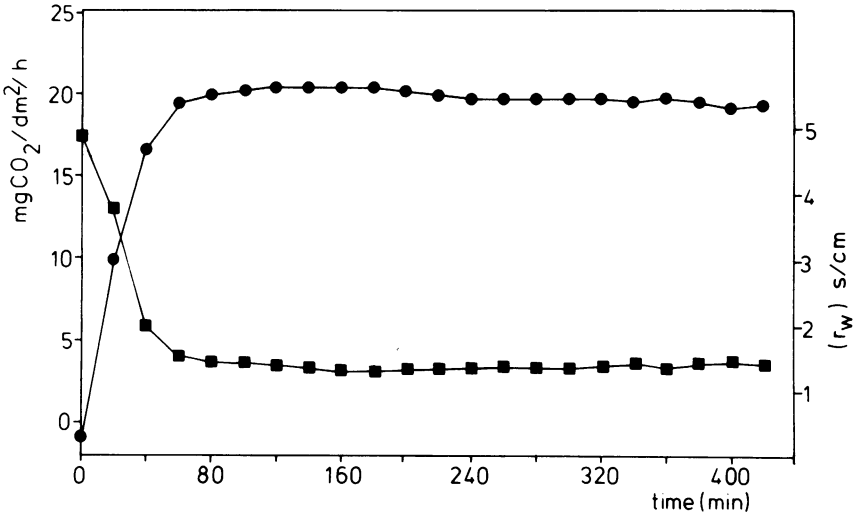

FIG. 3. Rate of CO_2 assimilation and r_w against time. Light intensity was 1,500 $\mu E/m^2 \cdot s$, in an atmosphere of 300 $\mu l/l$ CO_2, 20% O_2, balance N_2. CO_2 assimilation (●), r_w (■).

min, the disc was washed in boiling 80% ethanol.

Chl was determined by the method of Arnon (1) and sucrose according to Jones *et al.* (11).

Calculations. The resistances to CO_2 and H_2O vapor diffusion were calculated using a electrical analogy (7, 8, 20), and the results were processed by means of a Commodor CBM 3032 computer.

Suppliers. IRGA equipment was from ADC, Hoddesdon, Herts. Gas mixtures were from BOC Special Gases, London. Chemicals were from Sigma (London) Ltd., Poole, Dorset.

RESULTS

A Perspex (Plexiglas) leaf chamber has been designated which accomodates leaf discs and allows the simultaneous measurement of CO_2 and H_2O vapor exchange within an open gas exchange system (Figs. 1 and 2). When spinach leaf discs are enclosed within such a chamber under conditions of saturating light and normal atmospheric levels of gases, they are capable of sustaining high rates of photosynthesis and transpiration for extended periods of time. The data shown in Figure 3 are typical, although the actual rate of CO_2 exchange was somewhat lower than the average (Table I) (30 mg $CO_2/dm^2 \cdot h$). Under the environmental conditions imposed, the leaf discs displayed no evidence of water stress, as both the leaf temperature (25°C) and the r_w (1.4 s/cm) were maintained at nearly constant values throughout the 6-h experimental period. It should be noted that the r_w value given above is the average for the adaxial (upper) and abaxial (lower) surfaces and is calculated on the basis of only one surface area. Independent measurements of the r_w of the abaxial and adaxial surfaces of a series of discs showed that they were not equivalent with regard to r_w. It was consistently found that, despite having approximately half as many stomata, the adaxial surface generally had a lower r_w (Table II).

Table I. *Photosynthetic Gas Exchange Characteristics of Spinach Leaf Discs*

	2% O_2	20% O_2
APS_{max}[a]	66.4	55.9
APS_{325}	43.3 ± 1.56	30.3 ± 1.13
K_i[b]	115 $\mu l/l$	158 $\mu l/l$
r_w[c]	2.01 ± 0.28	1.87 ± 0.15
r_m[d] (150 $\mu l/l$)	3.75 ± 1.21	2.20 ± 1.34

[a] Apparent photosynthesis = mg $CO_2/dm^2 \cdot h$.

[b] K_i = CO_2 (internal) required for ½ APS_{max}.

[c] r_w (s/cm) calculated under saturating light and atmospheric levels of gases.

[d] r_m (s/cm) = $\frac{[CO_2]_i - \Gamma}{APS}$.

Table II. *Stomatal Frequency and Water Vapor Transfer Resistance in Spinach Leaf Discs*

Leaf Surface	Stomatal Frequency	r_w
	/mm²	s/cm
Adaxial	58.1	2.45
Abaxial	104.5	3.22

The route of entry of solution into the disc was determined by pumping a 5% ferrocyanide to the cut surfaces of normal leaf discs and those in which the vasculature was surgically interrupted. The analysis of the deposition of Prussian blue crystals, coupled with simultaneous r_w determinations, indicated that, in order to meet the transpirational demands of the leaf, the uninterrupted vasculature of the leaf had to be utilized (Fig. 4). To optimize this system it was, therefore, necessary to ensure that the midvein bisected the leaf disc that was to be placed in the chamber. This capacity to maintain a low and constant r_w is also likely to be the basis for the ability to attain uniform replicates. For example, the rate of apparent photosynthesis for a series of 12 discs ranged from 27 to 32 mg $CO_2/dm^2 \cdot h$ with an average of 30.3 mg/CO_2/$dm^2 \cdot h$ ± 1.13 SD.

Having established the uniformity and stability of the gas exchange characteristics of the discs, their responses to changes in light CO_2 and O_2 were then determined. Most of the information regarding the responses of discs to changes in CO_2 and O_2 is summarized in Table I. These data were derived primarily from plots similar to those shown in Figure 5, which displays the relationship between apparent photosynthesis and internal CO_2 at 2% and 20% O_2. At low concentrations of internal CO_2, the increases in rates of apparent photosynthesis were nonlinear functions of the internal CO_2 concentrations at both 2% and 20% O_2 (Fig. 5). The nature of these nonlinear responses, which are not atypical for C3 species (5, 7), made determination of initial slopes difficult. Therefore, methods of determining r_m or carboxylation efficiency which require these data, were not utilized. The r_m values were calculated at a limiting but defined internal CO_2 concentration (Table I). The nonlinear CO_2 response curves are not surprising if it is accepted that, under these conditions of limiting CO_2, changes in the assimilation rate are largely determined by the characteristics of ribulose-1,5-biphosphate carbox-

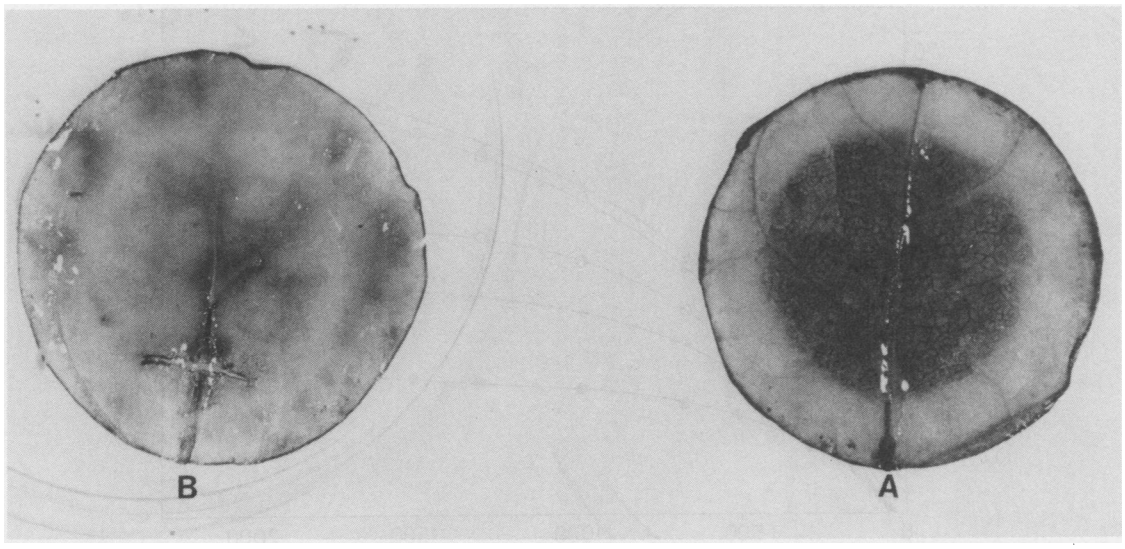

FIG. 4. Photograph of Prussian blue-stained leaf discs fed in the leaf chamber. Note the uniformity of distribution of solutes when the midvein is intact (A), and the effect of surgically interrupting the midvein (B).

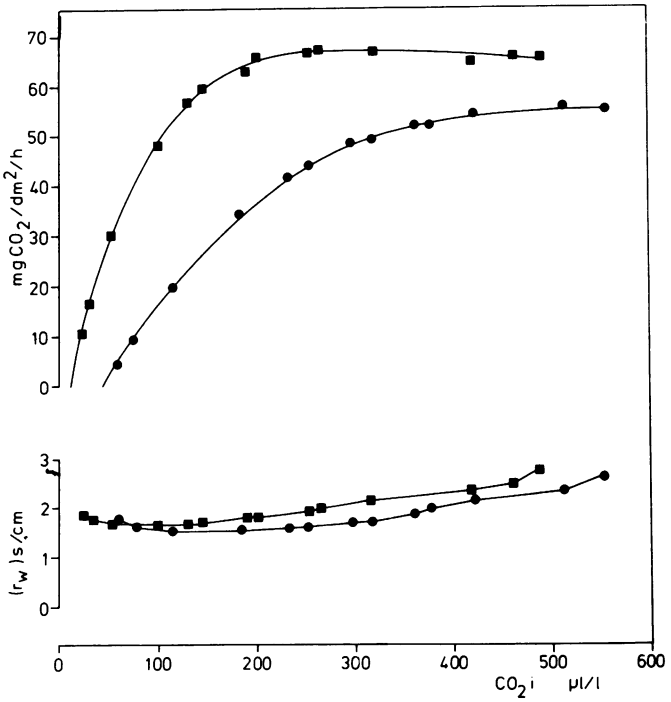

FIG. 5. Effect of internal CO_2 concentrations upon the rate of CO_2 assimilation and r_w in 2% and 20% O_2. Light intensity was 1,500 $\mu E/m^2 \cdot s$. 2% O_2 (■), 20% O_2 (●).

ylase oxygenase kinetics (7).

With increasing concentrations of CO_2, there is a transition to an apparent saturation with respect to CO_2. Under these conditions, the average maximal velocities in 2% and 20% O_2 were 66 and 56 mg $CO_2/dm^2 \cdot h$, respectively. The value of 66 mg $CO_2/dm^2 \cdot h$ can be considered to be near the upper limit for CO_2-saturated rates in spinach and is approximately equivalent to 300 μmol/mg Chl·h (0.5 mg Chl/10 cm^2). That this rate corresponds to that of electron transport in isolated chloroplasts under similar conditions of light and temperature (14) suggests that the transition to the saturation region is imposed by an electron transport limitation (14). Recent evidence also implies that, under certain conditions, the internal Pi concentration may prevent photosynthesis reaching the ceiling which would otherwise be imposed by electron transport (9).

As a measure of the apparent affinity of the leaf for CO_2, the internal concentrations of CO_2 needed to support one-half of the maximal velocity of photosynthesis were calculated to be 115 μl/l in 2% O_2 and 158 μl/l in 20% O_2. The implications of these maximal velocity and affinity data are that rates of 150 μmol/mg Chl·h must be supported by concentrations of CO_2 that are somewhat less than 4 μM. At one time, this would have been difficult to equate with the known characteristics of RuBP carboxylase (23) but, in recent years, the discovery of the activation requirements for the enzyme (15) has led to *in vitro* kinetics constants for the carboxylase (apparent K_m CO_2, about 10 μM; V_{max}, about 750 to 1000 μmol/mg Chl·h [7, 14]) which are consistent with these *in vitro* rates.

The r_w was sensitive to $[CO_2]_i$ in that it increased from an initial value of 1.8 s/cm (at a $[CO_2]_i$ of 25 μl/l) to 2.5 s/cm at a $[CO_2]_i$ of 550 μl/l. The r_w was generally found to be insensitive to O_2 at all CO_2 concentrations, although there were some indications of slightly higher r_w values under low O_2. (Recently, Korner *et al.* [12] compiled the available conductance [H_2O] values from the literature and arrived at value of 0.6 cm/s as the average conductance [r_w = 1.66 s/cm] for a diversity of plant species.)

The photosynthetic response of the leaf discs to increasing light intensities at several CO_2 concentrations is shown in Figure 6. It was found that, under low levels of CO_2, apparent photosynthesis exhibited light saturation at intensities well below that of daylight (2,000 $\mu E/m^2 \cdot s$). With increasing partial pressure of CO_2, there were concomitant increases both in apparent photosynthesis and the light intensity required for saturation, such that, at the highest concentrations of CO_2 utilized in these experiments, photosynthesis was nearly insensitive to increases in CO_2 and light saturation was not achieved, even at daylight intensities. These responses of the leaf disc to light and CO_2 are very similar to those reported by Gaastra (8) for intact spinach leaves.

A series of experiments were also undertaken in which various concentrations of Pi were provided in the feeding solution (Fig. 7). The Pi was provided in a 20-min pulse in an effort to minimize the principal drawback of the feeding technique, which is the steady accumulation of solute in the area exposed to the gas stream. This pulse, and the subsequent H_2O chase, must lead to a situation in which the extracellular concentration of Pi is constantly changing throughout the course of the experiment. Nevertheless, it was found that photosynthesis was inhibited by mM concentrations of Pi and that these inhibitions were associated

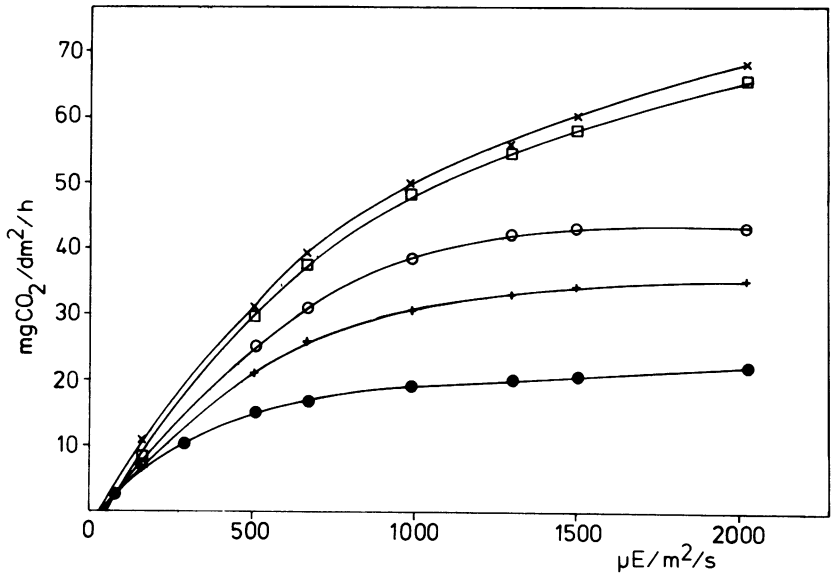

FIG. 6. Effect of light intensity on CO_2 assimilation in leaf discs of spinach at various CO_2 levels in 20% O_2, balance N_2. (×), 932 μl/l CO_2; (□), 707 μl/l CO_2; (○), 427 μl/l CO_2; (+), 329 μl/l CO_2; (●), 111 μl/l CO_2.

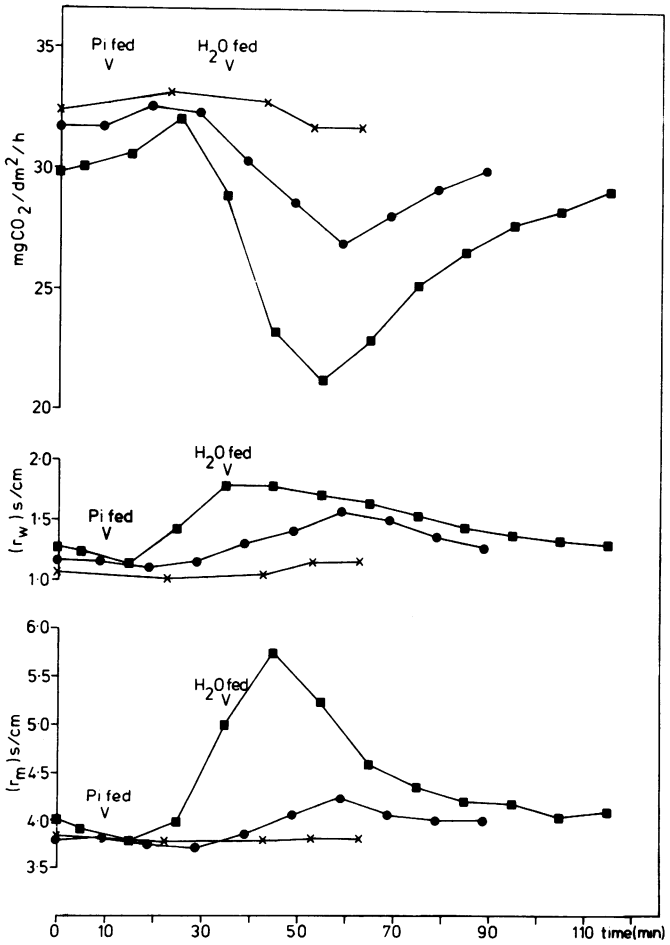

FIG. 7. Effect of Pi (pH 7) upon the rate of CO_2 assimilation in spinach leaf discs, stomatal resistance (r_w), and mesophyll transfer resistance (r_m). Light intensity was 1,500 μE/m^2·s in an atmosphere of 300 μl/l CO_2, 20% O_2, balance N_2. Five mM Pi (×), 10 mM Pi (●), 20 mM Pi (□).

with increases in both the stomatal and mesophyll resistances to CO_2 diffusion. The data suggest there was a differential effect of Pi. Thus, at the lowest concentration used (5 mM), the effect was primarily stomatal. With increasing concentrations of Pi, the r_m increased and the largest percentage increase in r_m was observed at the highest concentration of Pi employed.

DISCUSSION

The acquisition and analysis of photosynthetic gas exchange data from leaf tissue samples is primarily dependent on techniques which satisfy the transpirational demands of the leaf while simultaneously allowing measurements of temperature and rate of water vapor exchange. There have been several attempts to develop methods of providing water to leaf discs, etc. but these have not been coupled to simultaneous measurements of transpiration (3, 6, 13, 16, 19, 22). For example, dehydration has been spared by placing leaf discs or sections in contact with a water-saturated bibulous material in the hope that this would ensure adequate contact with the cut margin of the leaf (3, 6, 19, 22). Alternatively, sections of elongate leaves such as wheat have been allowed to stand in an aqueous reservoir within the gas exchange chamber (13) or leaf discs have been floated on a solution (10, 25). The results of these studies point to a need to maintain better contact between the cut surfaces and the water or feeding solution and also to the impossibility of measuring transpiration in these circumstances.

In the work reported here, an approach similar to that utilized by Natr and Glaser (16) was adapted for spinach leaf discs. The results clearly indicate that the technique of supplying water through the vascular tissues at the cut edge of a leaf disc, in a compartment spatially distinct from the gas exchange chamber, is more than enough to meet the transpirational demands of the tissue. Under saturating light and relatively rapid flow rate of gas, there was no evidence of water stress. It is likely that this ability to maintain an apparently favorable water potential is the basis of the uniformity of replicates and the high rates of photosynthesis. It is also likely that the low r_w is the key feature of a system which allows the leaf to maintain low leaf temperatures under high radiation in the absence of cooling water-jacket.

With regard to stability of the system, it is of interest that high rates of photosynthesis can be maintained for long periods without apparent inhibition by altered source to sink relationships. Recent work with soybeans has suggested that the inhibition of photosynthesis by interruption in the phloem is caused by stomatal closure following increases in ABA concentrations (21). The feeding experiments with Pi reported here have demonstrated that com-

pounds can be washed out of the leaf by utilizing the solution flowing through the cut edge. This suggests that at least part of the capacity of this system to maintain high rates of photosynthesis for extended periods of time is related to the leaching out of an inhibitor such as ABA or some other metabolite which might feed back on the disc. Sucrose was not detectable in the effluent.

Although there is some question as to whether or not the stomata on the abaxial and adaxial surfaces of leaves are inherently different in their response to environmental perturbations, it has generally been found that the abaxial surface has a lower r_w (2). Therefore, the observation that the adaxial surface of a spinach leaf disc has a lower resistance to water vapor transfer is somewhat anomalous. Possible reasons for what must be wider stomata on the upper surface might be the higher incident light intensities on this surface or the close association of the adaxial epidermal tissue with the hydrated leaf mesophyll. Recent work with peanut attributed lower adaxial resistance to the light intensity differences between the two surfaces (17). It has also been suggested that the adaxial stomata may be more responsive to the water status of the tissue (2).

However, in spite of what might be considered a somewhat unusual stomatal response, the responses of the leaf discs to CO_2, O_2, and light are consistent and generally similar to those of other C3 crop species grown under relatively high light and provided with adequate nutrition (5, 7, 8). These and other previously discussed results suggest that the method of preparing and assaying the discs does not induce any evidently anomalous photosynthetic responses.

Although there are a number of good reasons for employing tissue pieces, one of the more important is the ability to bypass the root system and to introduce inhibitors and metabolites into the tissue under study. The adequacy of the leaf disc chamber for this purpose was established by a series of experiments in which either ferrocyanide or Pi were provided in the solution bathing the cut edge of the leaf. The results from the ferrocyanide feeding experiments established that a solute can move through the xylem system and become rapidly and uniformly distributed throughout the portion of the leaf disc exposed to the gas stream.

When low concentrations (5–20 mM) of Pi were provided in the transpiration stream, photosynthesis was inhibited at all of the concentrations tested. These inhibitions were due to differential increases in both the stomatal and mesophyll resistances to CO_2 diffusion. The differential effect of Pi on the resistances is interesting because it suggests the possibility that there is a specific effect of elevated Pi levels on the stomata. Some additional evidence in this regard relates to the observations that mannose and deoxyglucose will induce wilting in those species in which these sugars are known to metabolically sequester Pi (10); although there may be little or no relationship between these observations, it is of interest to note that low Pi leads to an apparent loss of stomatal control and wide stomatal apertures and high Pi induces stomatal closure.

Previous work with isolated chloroplasts has established the central role of Pi in the regulation of carbon flux across the chloroplast envelope and the relatively narrow Pi optimum required for maximal photosynthetic activity (7, 24). Based on this evidence, it is reasonable to conclude for the experiments reported here that the increase in r_m is due, at least in part, to an increase in cytoplasmic Pi levels. This increase in Pi stimulates the exchange of chloroplastic triose-P for external Pi, ultimately depleting carbon pools of the photosynthetic carbon reduction cycle. The consequent inhibition of carboxylation leads to an increase in r_m.

LITERATURE CITED

1. ARNON DI 1949 Copper enzymes in chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
2. ASTON MJ 1978 Differences in the behaviour of adaxial and abaxial stomata of amphistomatous sunflower leaves: inherent or environmental? Aust J Plant Physiol 5: 211–218
3. ATKINS CA, DT CANVIN 1971 Photosynthesis and CO_2 evolution by leaf discs: gas exchange extraction and ion exchange fractionation of ^{14}C-labeled photosynthetic products. Can J Bot 49: 1225–1234
4. BURBANO JL, TD PISSOLATA, PR MAREY, JD BERLIN 1976 An application of the Prussian blue technique to a light microscope study of water movements in transpiring leaves of cotton (*Gossypium hirsutum* L.) J Exp Bot 27: 134–144
5. CANVIN DT, JA BERRY, MR BADGER, H FOCK, CB OSMOND 1980 Oxygen exchange in leaves in the light. Plant Physiol 66: 302–307
6. DILIEU T, DA WALKER 1981 Polarographic measurement of photosynthetic O_2 evolution by leaf discs. New Phytol, In press
7. EDWARDS GE, DA WALKER 1981 C3, C4 mechanisms, cellular and environmental regulations of photosynthesis. Blackwell Scientific Publications, Ltd., Oxford, In press
8. GAASTRA P 1959 Photosynthesis by crop plants as influenced by light, carbon dioxide, temperature and stomatal resistance. Meded Landbouwhogesch Wageningen 59: 1–68
9. HARRIS GC, JK CHEESBROUGH, DA WALKER 1983 The effects of mannose on photosynthetic gas exchange in spinach leaf discs. Plant Physiol 71: 108–111
10. HEROLD A 1978 Induction of wilting by mannose in spinach beet leaves. New Phytol 81: 299–305
11. JONES MGK, WH OUTLAW JR, OH LOWRY 1977 Enzymic assay of 10^{-7} to 10^{-14} moles of sucrose in plant tissues. Plant Physiol 60: 379–383
12. KORNER CH, JA SCHELL, H BAUER 1979 Maximum leaf diffusive conductance in vascular plants. Photosynthetica 13: 45–82
13. KUMARASINGHE KS, AJ KEYS, CP WHITTINGHAM 1977 The flux of carbon through the glycolate pathway during photosynthesis by wheat leaves. J Exp Bot 28: 1247–1257
14. LILLEY RM, DA WALKER 1975 Carbon dioxide assimilation by leaves, isolated chloroplasts, and ribulose biphosphate carboxylase from spinach. Plant Physiol 55: 1087–1092
15. LORIMER GH, MR BADGER, TJ ANDREWS 1976 The activation of ribulose-1,5-biphosphate carboxylase by carbon dioxide and magnesium ions. Equilibria, kinetics, a suggested mechanism and physiological implications. Biochemistry 15: 529–536
16. NATR L, J GLASER 1967 Carbon dioxide absorption and dry weight increase in barley leaf segments. Photosynthetica 1: 19–27
17. PALLAS JE, JR. 1980 An apparent anomaly in peanut leaf conductance. Plant Physiol 65: 848–851
18. RATHNAM CKM 1978 Metabolic regulation of carbon flux during C4 photosynthesis I. Evidence for parallel CO_2 fixation by mexophyll and bundle sheath cells *in situ*. Z Pflanzenphysiol 87: 65–84
19. SESTAK Z, J CATSKY, PG JARVIS 1971 Plant Photosynthetic Production. Manual of Methods. Dr. W. Junk NV, The Hague, pp 316–342
20. SESTAK Z, J CATSKY, PG JARVIS 1971 Plant Photosynthetic Production. Manual of Methods. Dr. W. Junk NV, The Hague, pp 556–631
21. SETTER TL, WA BRUN, ML BRENNER 1980 Affect of obstructed translocation on leaf abscisic acid and associated stomatal closure and photosyntheses decline. Plant Physiol 65: 1111–1115
22. TAMAS IA, RGA BIDWELL 1970 $^{14}CO_2$ fixation in leaf discs of *Phaseolus vulgaris*. Can J Bot 48: 1259–1263
23. WALKER DA 1973 Photosynthetic induction phenomena and the light activation of ribulose diphosphate carboxylase. New Physiol 72: 209–235
24. WALKER DA, SP ROBINSON 1978 Chloroplast and cell. A contemporary view of photosynthetic carbon assimilation. Ber Dtsch Bot Ges 91: 513–526
25. ZELITCH I 1979 Photorespiration: studies with whole tissues. *In* M Gibbs, E Latzko, eds, Photosynthesis II. Photosynthetic Carbon Metabolism and Related Processes. Encyclopedia of Plant Physiology, New Series, Vol 6. Springer-Verlag, New York, pp 353–367