PLANT WATER STATUS, LEAF TEMPERATURE, AND THE CALCULATED MESOPHYLL RESISTANCE TO CARBON DIOXIDE OF COTTON LEAVES

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Summary

The influence of plant water status and leaf temperature on the mesophyll resistance to CO_2 transfer for Deltapine cotton leaves was determined under conditions when the CO_2 supply was limiting photosynthesis. The mesophyll resistance was calculated from CO_2 response curves in normal air and oxygen-free air, under conditions when air was forced from the abaxial to adaxial side of the leaf to obtain a direct estimate of the CO_2 concentration at the mesophyll cell wall.

The mesophyll resistance was about 25% higher in normal air ($r_m \simeq 4$ sec cm^{-1}) than oxygen-free air ($r_m \simeq 3$ sec cm^{-1}), but neither variation in the relative leaf water content from 56 to 92% nor leaf temperature from 22·5 to 38°C affected the calculated mesophyll resistance in the oxygen-free air treatment.

Photorespiration was substantially inhibited by the oxygen-free air and was approximately linearly related to leaf temperature in both oxygen-free and normal air treatments. The temperature dependence of the CO_2 compensation point was explained by the influence of temperature on photorespiration.

I. Introduction

The rate of CO_2 exchange by leaves is determined by the rate of photosynthesis and respiration. During light-saturated photosynthesis, Rabinowitch (1951) has suggested that CO_2 uptake is dependent on the resistance to diffusion of CO_2 into the leaf and on the carboxylation reaction of photosynthesis. The components of the resistance to CO_2 diffusion in the gaseous phase are located in the boundary layer (r_a) which sheaths the leaf, and in the leaf itself (r_l). The leaf resistances are located in the cuticle (r_c), and in the stomatal pore and intercellular air spaces (r_s). The extent to which these resistances influence CO_2 exchange through controlling the CO_2 concentration at the mesophyll cell wall has been shown in a recent study on cotton leaves (Troughton 1969).

It has been more difficult to characterize the intracellular resistances to CO_2, which includes the solubility of CO_2, diffusion in the liquid phase, and the primary carboxylation reaction of photosynthesis. In this paper, this collection of resistances is termed the mesophyll resistance (r_m). The magnitude of this resistance has been in doubt because of the influence of CO_2 from respiration on the inward CO_2 flux (F) and on the assumed CO_2 concentration at the chloroplasts (Lake 1967; Zelitch 1967).

It is now apparent that respiration in the light (photorespiration) can be inhibited by oxygen-free air (McAlister and Myers 1940; Forrester, Krotkov, and Nelson

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1966; Hesketh 1967). Consequently, in oxygen-free air calculated estimates of the mesophyll and other resistances can be made from the relationship

$$F = (C_a - C_c)/(r_a + r_l + r_m), \tag{1}$$

where F is the flux of CO_2 into the leaf (g cm^{-2} sec^{-1}), r_a, r_l, and r_m (sec cm^{-1}) are as already defined, C_a is the CO_2 concentration in the bulk air (g cm^{-3}), and C_c is the CO_2 concentration at the site of the photosynthetic reactions (g cm^{-3}). A more direct measure of r_m can be made if the CO_2 concentration at the cell wall (C_w) is known. Then

$$F = (C_w - C_c)/r_m. \tag{2}$$

In this paper r_m is derived from the differential equation, obtained from CO_2 response curves:

$$r_m = \mathrm{d}C_w/\mathrm{d}F. \tag{3}$$

This has the advantage of being able to compare results in air (in which photorespiration was occurring) with results in oxygen-free air, and it effectively averages several F to C_w relationships obtained from one leaf or treatment. It does, however, assume that C_c is negligible compared with C_w and that r_m is independent of the CO_2 concentration. An estimate of r_m can be made by the use of equation (3) in the absence of photorespiration, and over the linear portion of the F to C_w relationship.

In this paper the influence of two plant parameters, leaf temperature and plant water status, on r_m are evaluated. Leaf temperature was varied because it was thought that this could be one method of distinguishing between the physical and biochemical components of the mesophyll resistance. Interest in the influence of plant water status on the mesophyll resistance was aroused because it had been suggested (Troughton 1969) that r_m was independent of the relative leaf water content (θ) down to values of the order of 75% (−15 bars). With more severe stress (θ = 75–56%) an increase in the calculated mesophyll resistance was observed but, because of the technique used, it was possible that the apparent increase in r_m was partly associated with the changes in leaf resistance or with respiration.

II. Methods

(a) *Plant Material*

Uniform plant material was obtained by growing cotton plants (var. Deltapine smooth leaf) from seed for about 6 weeks in an environment controlled at a day temperature of 30°C, night temperature of 25°C, and day length of 12½ hr of which 10½ hr were at a light level of 100 W m^{-2} (0·4–0·7 μ) produced by fluorescent tubes and the extra 2 hr by low light from incandescent lamps. Plants were fed a modified Hoaglands nutrient solution which was aerated and maintained near cabinet temperature. Leaves of similar appearance, area (180 cm^2), age, and insertion level on the stem of the plants were chosen for use in experiments.

(b) *Leaf Chamber Conditions*

Conditions in the single leaf chamber (Jarvis and Slatyer 1966) were rigorously controlled; the stem and rest of the plant were in a controlled temperature room at 26°C and only partially

illuminated. The roots were in aerated Hoaglands solution at a closely controlled temperature which could be varied to produce water stress in the plants when required.

Air drawn from outside the building was supplied to the plant overnight or when air was required for long periods. During experiments, air with closely controlled O_2 or CO_2 concentration was forced through the leaf by pressure from the abaxial to the adaxial side, at flow rates between 1·0 and 1·5 litre min^{-1}. The CO_2 concentration at the cell wall was controlled over a range likely to occur naturally (100–350 $\mu g\, l^{-1}$) by mixing CO_2-free air with air of known CO_2 concentration. Oxygen-free air (< 0·5% O_2) was prepared by mixing CO_2 with nitrogen in polyvinyl chloride balloons. CO_2 concentration was monitored with a conductivity cell analyser (Begg and Lake 1968), and the oxygen concentration of the oxygen-"free" air was checked with a paramagnetic oxygen analyser to confirm that the concentration was less than 0·5% O_2.

Light from an H.P.L.R. mercury vapour lamp was passed through a 1·5-cm water filter and an ultraviolet filter. Light was monitored by silicon solar cells above and below the leaf. All experiments were carried out when photosynthesis was light-saturated and for most experiments the light absorbed by the leaf was about 110 $W\, m^{-2}$. (All references to light levels apply to the range 0·4–0·7 μ.) Air flow rates were measured with capillaries and micromanometers and the output from all sensors was displayed on an integrating digital voltmeter.

(c) *Plant Water Status: Methods and Measurements*

Water stress in the plants was obtained by cooling the plant roots (Troughton 1969). Leaf thickness was monitored continuously by β-ray gauging and when necessary the water content of the leaf was derived from the measurements. After a CO_2 response curve had been obtained, the relative leaf water content of the leaf (θ) was determined from measurements of the fresh, turgid, and dry weight of the sample (Slatyer 1967).

(d) *Sequence of Measurements*

The chosen leaf, with a thermocouple inserted in a small vein, was allowed to equilibrate with the leaf chamber conditions overnight under a normal photoperiod. After 2 hr of light the following morning, air was forced through the leaf until there was a flow of 1–1·5 litre min^{-1} and a pressure less than 10 (and normally 5) cm of water gauge. Occasionally a leaf was put in the chamber and used the same day. The CO_2 concentration in air or nitrogen was changed once the environmental conditions and CO_2 exchange were constant.

III. Results

(a) *Shape of the CO_2 Response Curves*

In the course of the experiments numerous CO_2 response curves were obtained, all of which indicated that the curves were linear above the CO_2 compensation point (C_w at $F = 0$) in normal and oxygen-free air, at least over the range of CO_2 concentrations used.

Below the compensation point the shape of the CO_2 response curves in air was irregular. In some cases a linear relationship between F and C_w was observed to be of the same slope as that above the compensation point. More often it was non-linear, providing a lower value of F at $C_w = 0$ than that expected from extrapolation to $C_w = 0$ of the curve relating F to C_w above the compensation point (Fig. 1).

To determine the values of r_m, C_w at $F = 0$, and F at $C_w = 0$ a regression was calculated between F as the dependent variable and C_w for each leaf and treatment separately. For a given treatment, between 6 and 10 values of the relationship between F and C_w were obtained and the correlation coefficients for these regressions were always higher than +0·95.

(*b*) *Effect of Oxygen Concentration on the CO_2 Response Curve*

The effect of oxygen concentration up to 99% on the CO_2 response curve was tested on one leaf, on the same day, at a leaf temperature of 25°C and at 110 W m^{-2} (0·4–0·7 μ). The effect of increasing the oxygen concentration was to increase the CO_2 compensation point (C_w at $F = 0$), the photorespiration (F at $C_w = 0$), and the apparent calculated mesophyll resistance (Fig. 1). This was similar to the results of

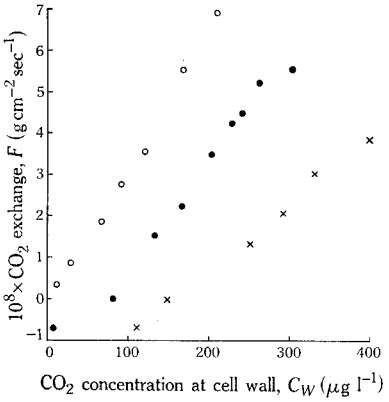


Fig. 1.—Influence of the CO_2 concentration at the cell wall of cotton leaves on the rate of CO_2 exchange at three levels of oxygen.
○ Zero O_2.
● 22% O_2.
× 44% O_2.

Forrester, Krotkov, and Nelson (1966) for soybean (Table 1). To determine the possibility of after-effects of high or low oxygen concentrations, the CO_2 response curve in air was rechecked on the day following the treatments, but no after-effects were evident (Table 1). Similarly, a check was made to show that a day of oxygen-free air had no deleterious long-term effects, r_m at the beginning of the treatment being 3·2 and the following day 3·01 sec cm^{-1}.

TABLE 1

INFLUENCE OF OXYGEN CONCENTRATION ON THE CO_2 RESPONSE CURVES

O_2 Concentration (%)	Time of Measurement	r_m (sec cm^{-1})	$10^8 \times F$ at $C_w = 0$ (g cm^{-2} sec^{-1})	C_w at $F = 0$ (μg l^{-1})	
				A*	B†
0	Day I	3·0	0·22	6·7	0
21	Day I	3·9	1·92	73·0	55
21	Day II	4·3	1·70	67·0	55
44	Day I	6·2	3·1	187·0	140
99	Day I	10·4	3·25	296·0	300

* Results from this experiment.

† Results from Forrester, Krotkov, and Nelson (1966).

(*c*) *Light Level and the CO_2 Response Curve*

It has been suggested previously that light level influences the mesophyll resistance (Bierhuizen and Slatyer 1964). Tests were made on two leaves at 25°C in oxygen-free air and over a range of CO_2 concentrations where the relationship between F and C_w was linear. As can be seen in Table 2 there was no significant effect of

light on r_m even when the light level was changed from 36 to 110 W m^{-2}. Furthermore the CO_2 response curves in air at 110 and 55 W m^{-2} were identical, as can be seen from the values of r_m, F at $C_w = 0$, and C_w at $F = 0$ (Table 2).

In these experiments the r_m values, in both air and oxygen-free air, were about 25% higher than those for leaves in the temperature experiments. This may have been caused by variability in the plant material, or by the method of leaf pretreatment. Because the stomata tended to close if the light level was lowered, plants used for these experiments were pretreated at low light (50 W m^{-2}). In all other experiments light levels were kept at high values (110 W m^{-2}) throughout.

TABLE 2

EFFECT OF LIGHT LEVEL ON THE CO_2 RESPONSE CURVES AT 25°C

Leaf No.	Light Level (W m^{-2}) (0·4–0·7 μ)	Treatment	r_m (sec cm^{-1})	C_w at $F = 0$ (μg l^{-1})	$10^8 \times F$ at $C_w = 0$ (g cm^{-2}sec^{-1})
1	110	Air	4·96	81·5	1·63
1	110	Zero O_2	3·78	10·7	0·33
1	55	Air	5·08	83·0	1·63
1	55	Zero O_2	3·91	9·0	0·25
2	110	Zero O_2	3·78	10·7	0·33
2	36	Zero O_2	3·92	1·6	0·11
2	36	Zero O_2	4·4	11·1	0·69

Light levels of 110 W m^{-2} were used in experiments on the temperature effect on r_m and levels of 80 W m^{-2} were used in experiments on water-stressed leaves to allow strict control of leaf temperature.

(*d*) *Leaf Temperature and the Rate of Respiration*

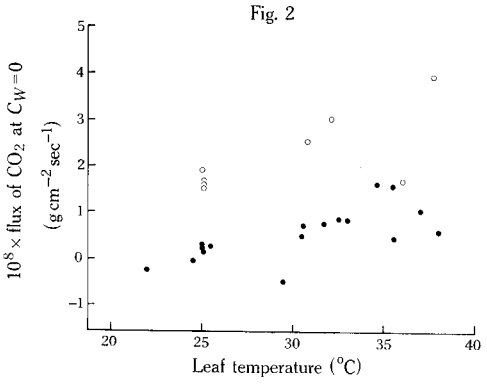
The estimated net flux at which C_w is zero (F at $C_w = 0$) was assumed to be the respiration rate of the leaves. The effect of leaf temperature on respiration in the light is shown in Figure 2. The oxygen-free air treatment has clearly reduced the CO_2 efflux compared with normal air, but respiration still occurs in oxygen-free air and is linearly related to leaf temperature in both air treatments. Scatter in the results prevents further analysis to elucidate the possibility that the source of CO_2 is different between the two treatments.

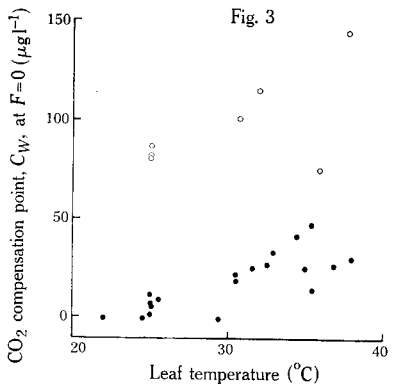
The rate of photorespiration (in normal air) at 30°C of about $2{\cdot}5 \times 10^{-8}$ g CO_2 sec^{-1} would suggest that photorespiration of leaves under normal conditions (high light and low stomatal resistance) would be about 25% of the net CO_2 exchange. This value is likely to be an underestimate if there is another component of respiration associated with the instantaneous rate of photosynthesis.

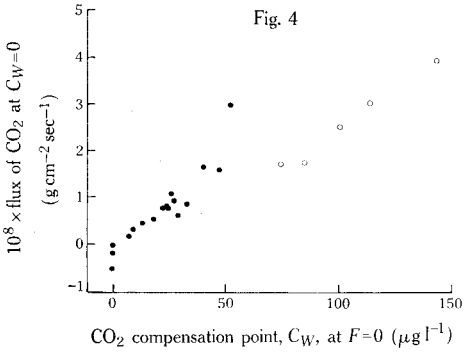
(*e*) *Leaf Temperature and the CO_2 Compensation Point*

The CO_2 concentration at which the CO_2 flux is zero (C_w at $F = 0$) is commonly called the CO_2 compensation point. Increasing the temperature increased the compensation point in both oxygen-free and normal air although the CO_2 concentration at $F = 0$ was about 70 μg^{-1} lower in oxygen-free than in normal air (Fig. 3).

The similarity between the response to temperature of the respiration rate and the CO_2 compensation point suggested that shifts in the compensation point could be explained by variation in photorespiration. As shown in Figure 4 there is a close relationship between C_w at $F = 0$ and F at $C_w = 0$ in both normal and oxygen-free air when the shift in these two parameters is caused by changing leaf temperature. The displacement of the normal air from the oxygen-free air results would be due to the differences in slope of the CO_2 response curves under the two treatments.







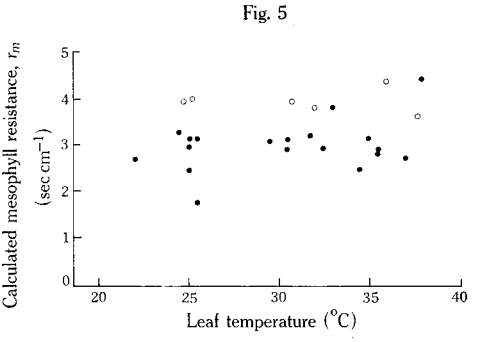


Fig. 2.—Temperature of cotton leaves and the net efflux of CO_2 at $C_w = 0$ in normal (○) and oxygen-free air (●).

Fig. 3.—Dependence of the CO_2 compensation point, C_w at $F = 0$, in air (○) and zero O_2 (●) on leaf temperature.

Fig. 4.—Relationship between the CO_2 compensation point, C_w at $F = 0$, and the net efflux of CO_2 at $C_w = 0$ in air (○) and oxygen-free air (●) for cotton leaves.

Fig. 5.—Calculated mesophyll resistance to CO_2 exchange in cotton leaves at a range of leaf temperatures. ○ In air. ● In zero O_2.

(*f*) *Leaf Temperature and the Calculated Mesophyll Resistance*

There was no effect of leaf temperature from 21·5 to 38·5°C on the calculated mesophyll resistance from either the oxygen-free or normal air treatments, as can be seen in Figure 5. However, the average mesophyll resistance in air of 4·2 sec cm^{-1}

was higher at all temperatures than the average value measured in oxygen-free air of $2 \cdot 9$ sec cm^{-1}.

A regression was calculated for the relationship between leaf temperature and the mesophyll resistance in oxygen-free air, providing

$$r_m = 0 \cdot 03T + 2 \cdot 05, \tag{4}$$

where T is the temperature in degrees centigrade. The slope did not differ from zero gradient at $P = 0 \cdot 01$.

(*g*) *Plant Water Status and the Mesophyll Resistance*

The effect of leaf temperature, oxygen concentration, and pretreatment, already reported, indicated the desirability of examining the influence of plant water status on the mesophyll resistance under the specific conditions of oxygen-free air at 25°C, and as soon after a period of high CO_2 exchange as possible. Usually a CO_2 response curve on a non-water-stressed leaf was determined, then the roots were cooled, the leaf water content reduced over a period of about an hour, then kept at the new water content for about an hour while a CO_2 response curve was determined. Consequently the water stress referred to in this paper is a short-term stress. The effect of θ on the mesophyll resistance could be observed either by measuring a CO_2 response curve as soon as θ had become steady at a new low level, or by observing F when C_w was constant but θ was changing.

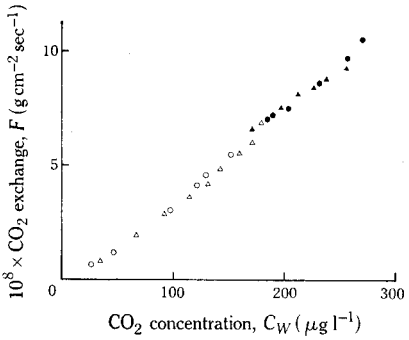


Fig. 6.—Photosynthesis at a range of CO_2 concentrations at the cell wall (C_w) for two cotton leaves in zero O_2 at 25°C and three levels of relative leaf water content.
● Leaf 1, θ = 92%.
○ Leaf 1, θ = 56%.
▲ Leaf 2, θ = 92%.
△ Leaf 2, θ = 69%.

Using the former procedure, CO_2 response curves were measured before water stress, and after the leaves were stressed to θ values down to 55%. In six experiments the average calculated mesophyll resistance for leaves when non-stressed was $3 \cdot 0$ sec cm^{-1} and, during water stress, $2 \cdot 6$ sec cm^{-1}. It is evident therefore that water stress, under the conditions of this experiment, did not increase the mesophyll resistance. Typical results, showing the slope of the $F : C_w$ relationship, are presented in Figure 6.

The lack of an effect of water stress on CO_2 exchange in oxygen-free air (when variation in the leaf resistance is eliminated) does not necessarily indicate that photosynthesis in normal air would be independent of θ. In oxygen-free air the stomata stayed open long enough to allow a CO_2 response curve to be obtained at low water contents, but in air the stomata reacted immediately to any change in leaf water

content. An alternative method of making preliminary measurements on the influence of water stress on CO_2 exchange is to use the CO_2 compensation point as a sensitive indicator of changes in photorespiration or the mesophyll resistance. In air at 25°C and without stress the CO_2 compensation value was 80 $\mu g\,l^{-1}$. Measurements on three leaves with θ less than 60% indicated that the value of C_w at $F = 0$ was the same as in unstressed leaves at 25°C. This would indicate that short-term water stress was unlikely to influence either photorespiration or the mesophyll resistance.

IV. Discussion

The results clearly show that the calculated mesophyll resistance was unaffected by short-term variations in the three main environmentally determined variables that affect plant growth, namely light, plant water status, and plant temperature, at least over the ranges observed in these experiments. Oxygen concentration of the air, over a wide range, did, however, affect the mesophyll resistance.

(a) *Characteristics of the CO_2 Response Curve*

The non-linearity of the CO_2 response curve in normal air, which was observed in these experiments, has also been observed by other recent workers (Holmgren and Jarvis 1967; Brix 1968; Heath and Orchard 1968). However, the non-linearity was confined to CO_2 concentrations below the compensation point which enabled the linear part of the curve to be used to determine the mesophyll resistance. The CO_2 response curve in oxygen-free air appeared to be linear down to low levels of CO_2 concentration, which suggests that the non-linearity in normal air is not directly a function of CO_2 concentration, nor a direct effect on photosynthesis. Consequently the effect is likely to be associated with respiration, recycling of CO_2 within the leaf, or variation in r_m with CO_2 concentration.

In these experiments the lowest measured values of the mesophyll resistance were about 2·4 sec cm^{-1}. However, even lower values might be expected for several reasons. For example, photorespiration occurred even in the oxygen-free air treatment and if there is an effect of photorespiration on the slope of the CO_2 response curve then r_m will be overestimated by about 5%. Also in the method of forcing air through the leaf there is a drop in CO_2 concentration, of up to 30% of C_a, across the leaf. This change in CO_2 concentration across the leaf can be accurately specified, but it is more difficult to accurately determine the average CO_2 concentration within the leaf (C_w). C_w in these experiments was derived assuming an exponential fall in CO_2 concentration through the leaf. A further possible cause of overestimating r_m may arise from the development of a variable boundary layer resistance at the cell wall. However, a series of tests with different flow rates through the leaf failed to show any significant change in CO_2 exchange at the same C_w.

It was observed, however, that if the stomata were allowed to open naturally and thereby increase the flow rate, there was a tendency for CO_2 exchange to increase with increase in flow. This suggested that the path of air through the leaf may be important, particularly in relation to dead-end cavities.

The slope of the CO_2 response curve was independent of the short-term changes in the three variables, leaf temperature over the range 22 to 38°C, plant water status from 92 to 56% θ, and light level from 110 to 36 W m^{-2} (0·4–0·7 μ). By confining

the range of leaf temperatures and by using high light and low CO_2 levels the likelihood of a possible effect of temperature on other processes involved in photosynthesis was reduced (Gaastra 1959), and under these conditions it was not possible to detect an effect of temperature on r_m. Results of Decker (1959) also show no significant qualitative effect of air temperature from 20 to 40°C on the slope of the CO_2 response curve measured on *Mimulus cardinalis* (see also Thomas 1965) while for wheat in 3% oxygen there was similarly a lack of an effect from 13 to 34·3°C (Jolliffe and Tregunna 1968).

Several investigators and reviewers have observed or anticipated that the mesophyll resistance to CO_2 transport is sensitive to changes in the leaf water content (Brilliant 1924; Scarth and Shaw 1951; Gaastra 1959, 1963; Vaadia, Raney, and Hagan 1961; Shimshi 1963; Gale, Kohl, and Hagan 1966; Slatyer 1967). In the experiments described in this paper, with short-term stress in cotton leaves, there was no apparent effect of water stress on the calculated mesophyll resistance in oxygen-free air, even when θ was 55% (−25 bars). This is in general agreement with the conclusions reached previously (Troughton 1969) which were equivocal because of sources of error due to variation in the leaf resistance, changes in the ratio of respiration to photosynthesis, and an increase in the cell wall resistance to water vapour with the reduced water content. The constant mesophyll resistance at all levels of water stress indicates that liquid phase diffusion of CO_2 is unaffected, but is not evidence that the photochemical or biochemical reactions associated with CO_2 fixation are unaffected by water stress, unless these components contribute to r_m.

An influence of light level on the calculated mesophyll resistance has been noted (Bierhuizen and Slatyer 1964; Whiteman 1965) but Brix (1968) found no effect of increasing the light level from 2500 to 3300 f.c. on the CO_2 response curve in air. The apparent effect of light level on r_m could be explained by the variation in photorespiration or by CO_2 not limiting photosynthesis. A threefold variation in light level at low CO_2 levels and in oxygen-free air had no significant effect on r_m in these experiments (Table 2). Even in normal air the CO_2 response curves, at two different light levels, were identical. But the results do suggest a possible prehistory effect which is unlikely to be due to anatomical changes in the plant, as they occurred over relatively short periods of time. If the effect was not due to changes in the characteristics of CO_2 transport in the cells then it may be associated with biochemical changes such as variation in the activity of carbonic anhydrase or ribulose-1,5-diphosphate carboxylase (Björkman 1968*b*).

In contrast to light, water level, and leaf temperature, the oxygen concentration of the air, over the range 0–99%, had a significant effect on r_m (Table 1). In particular the mesophyll resistance was 25% higher in normal than in oxygen-free air. The effect of oxygen levels on r_m may be due to a direct effect of oxygen on photosynthesis (Björkman 1966; Heber and French 1968), although this assumes there is no indirect effect of photorespiration on photosynthesis. Alternatively, in air, there may be a close relationship between photorespiration and the instantaneous rate of photosynthesis.

(*b*) *Photorespiration and Environmental Factors*

CO_2 production in the light in cotton leaves was estimated by extrapolation of the linear portion of the F to C_w relationship to $C_w = 0$ and is referred to as photo-

respiration. Photorespiration was most significantly influenced by the oxygen concentration of the air, being dependent on oxygen over the range from 0 to 99%. As shown in Table 1, oxygen-free air almost completely inhibited photorespiration at 25°C, but photorespiration was doubled when oxygen levels were increased from 21 to 44%. Further increases in oxygen from 44 to 99% hardly affected photorespiration, which is similar to results of Forrester, Krotkov, and Nelson (1966).

The enhancement of photorespiration by increasing leaf temperature in normal air has also been observed by Decker (1959) and Brix (1968), and the results in this paper show that temperature also influences photorespiration in oxygen-free air. A dependence of the CO_2 compensation point on leaf temperature has often been observed (Decker 1959; Brix 1968). As shown in Figure 3 it was seen that the shift in compensation point with temperature was closely related to the effect of temperature on photorespiration. With oxygen levels, however, the shift in CO_2 compensation point was associated with changes in both r_m and photorespiration.

Light level over a limited range did not influence photorespiration, as is also mentioned by Heath and Orchard (1968) and shown by Whiteman and Koller (1967). However, at lower light levels than used in this study it can be shown that photorespiration may be reduced (Holmgren and Jarvis 1967; Brix 1968) and that a reduction may be related to glycolate synthesis (Moss 1968).

(c) *Significance of the Components of the Mesophyll Resistance*

The mesophyll resistance can be thought of as a solubility resistance at the cell walls, resistance to transport in solution, resistance associated with cell membranes, and the activity of enzymes associated with transport or carboxylation (carbonic anhydrase, ribulose-1,5-diphosphate carboxylase, and phosphopyruvate carboxylase). The significance of the enzyme component of r_m is difficult to evaluate but some investigation of its contribution can be made.

It has been shown that ribulose-1,5-diphosphate carboxylase *in vitro* exhibits classical Michaelis–Menten kinetics, and K_m values for bicarbonate for ribulose-1,5-diphosphate carboxylase *in vitro* have been measured at $1{\cdot}1\times10^{-2}$M (Weissback, Horecker, and Hurwitz 1956) and 2×10^{-2}M (Racker 1957). If CO_2 transport in cell solution was independent of the CO_2 concentration, if ribulose-1,5-diphosphate carboxylase *in vivo* had kinetics similar to those determined *in vitro*, and if it were a significant component of r_m, then the relationship of C_w to F would be of a hyperbolic form. The CO_2 concentrations used in these experiments were likely to be low compared with the CO_2 concentration required to produce the maximum velocity, so that r_m would be independent of the CO_2 concentration. Alternatively, if ribulose-1,5-diphosphate carboxylase *in vivo* was an allosteric enzyme (Monod, Changeux, and Jacob 1963), then r_m may not be independent of the CO_2 concentration as the relationship of C_w to F of the enzyme may be, for example, of a sigmoid form. The implication to r_m would be that it would be independent of the CO_2 concentration except at high and low CO_2. At low CO_2, r_m would be higher than over the linear portion of the C_w to F curve.

The mesophyll resistances reported in this paper were determined over a relatively narrow range of CO_2 concentrations from 100 to 200 $\mu g\,l^{-1}$, although a limited number of values of F were determined when C_w was low, that is $< 100\ \mu g\,l^{-1}$.

From experiments where photorespiration was inhibited by oxygen-free air at 25°C and when F was measured for a range of CO_2 concentrations from about 50 to 260 $\mu g\,l^{-1}$ on the same leaf, r_m at different C_w values was obtained from the relationship

$$r_m = C_w/F.$$

The results, as shown in Figure 7, clearly suggest that r_m is not independent of the CO_2 concentration, and for values of $C_w < 100$ $\mu g\,l^{-1}$ there is a substantial increase in r_m. The possibility exists therefore that, if the variation in r_m is due to the enzymatic component of r_m, then *in vivo* regulation of the enzyme or enzymes may occur at low CO_2. Alternatively a transport term may dominate at low CO_2 or, more probably, as F approaches zero the existence of a respiration term will depress F below its true value.

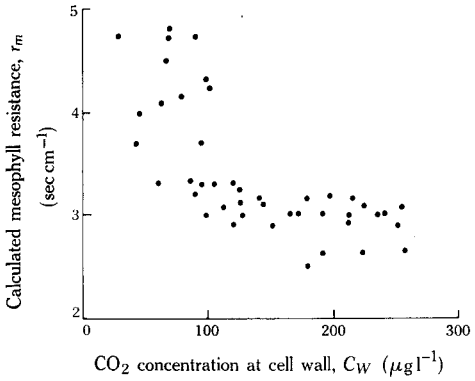


Fig. 7.—Dependence of the calculated mesophyll resistance on the CO_2 concentration at the mesophyll cell wall of cotton leaves at 25°C.

Recently several experimenters have measured changes in level of the carboxylating and associated enzymes in plant species with the Calvin and β-carboxylation pathway of photosynthesis (Björkman 1968*a*, 1968*b*; Cumming and Wagner 1968; Treharne and Stoddart 1968; Wareing, Khalifa, and Treharne 1968; Hatch and Slack, personal communication). Furthermore the suggestion has been made that the change in enzyme level or activity has been large enough to result in a significant effect on the photosynthetic capacity of the plants, independent of associated changes in the resistances to CO_2 diffusion in the liquid or gaseous form, although these latter resistances had not been measured. Björkman (1968*b*), by pretreatment in low or high light, was able to vary the carboxydismutase activity in leaf extracts of a sun ecotype of *Solidago*. Such variation may explain similar light pretreatment effects on r_m observed in our experiments and also by Holmgren (1968). Perhaps a more significant feature of a relationship between enzymes and r_m is the effect of the specific carboxylating enzyme (either ribulose-1,5-diphosphate carboxylase or phosphopyruvate carboxylase). Cotton has the former enzyme with an r_m of about 3 whereas corn has the latter enzyme with an r_m of about 0·8 (Holmgren 1968).

There has been some doubt that the activity of purified enzymes *in vitro* are representative of the same enzymes *in vivo*. A particular source of the doubt has been the low activity of ribulose-1,5-diphosphate carboxylase at CO_2 or bicarbonate

concentrations likely to occur "in nature". Measurements of CO_2 concentrations in plants are normally made outside of the leaf so that CO_2 concentrations at the surface of the enzymes are likely to be considerably lower than outside the leaf due to r_a, r_l, and the resistance associated with CO_2 transport in solution, and may be as low as 5×10^{-7}M.

The liquid phase diffusion component of CO_2 transfer in cotton leaves has a resistance which is less than $2 \cdot 5$ sec cm^{-1}. Estimates, from anatomical data, of the diffusion component of r_m in *Impatiens parviflora* indicates values of $0 \cdot 3$–$4 \cdot 0$ sec cm^{-1}, when it is assumed that CO_2 diffuses as CO_2 and not as bicarbonate (Rackham 1967). It would seem that facilitated CO_2 transport may occur (Enns 1967; Ward and Robb 1967) so that the measured mesophyll resistance in cotton was of the order expected from the measured pathlength of liquid phase diffusion in *Impatiens parviflora*.

In view of the possible chemical and physical nature of r_m it was somewhat surprising to find that r_m was independent of leaf temperature over the limited range which was studied. It is suggested that processes such as the *in vivo* regulation of enzyme activity, or the reduction in CO_2 solubility with increasing temperature, were counteracting other effects which would tend to cause r_m to be lower at higher temperatures.

The relative insensitivity of r_m to the environmental factors studied in these experiments does not preclude an influence of the past history of the plant on the mesophyll resistance, and differences in both the magnitude and effect of the environment on the resistance could be expected between plant species and varieties. At least for cotton leaves similar to those used in this study, the significance of the mesophyll resistance to CO_2 exchange will depend on the excitation resistance and the magnitude of the leaf and boundary layer resistances.

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VI. References

Begg, J. E., and Lake, J. V. (1968).—*Agric. Met.* **5**, 283.
Bierhuizen, J. F., and Slatyer, R. O. (1964).—*Aust. J. biol. Sci.* **17**, 348.
Björkman, O. (1966).—*Physiologia Pl.* **19**, 618.
Björkman, O. (1968*a*).—*Physiologia Pl.* **21**, 1.
Björkman, O. (1968*b*).—*Physiologia Pl.* **21**, 84.
Brilliant, B. (1924).—*C. r. hebd. Séanc. Acad. Sci., Paris* **178**, 2122.
Brix, H. (1968).—*Pl. Physiol., Lancaster* **43**, 389.
Cumming, B. G., and Wagner, E. (1968).—*A. Rev. Pl. Physiol.* **19**, 381.
Decker, J. P. (1959).—*Pl. Physiol., Lancaster* **34**, 103.
Enns, T. (1967).—*Science, N.Y.* **44**, 155.
Gaastra, P. (1959).—*Meded. LandbHoogesch. Wageningen* **59**, 1.
Gaastra, P. (1963).—In "Environmental Control of Plant Growth". (Ed. L. T. Evans.) (Academic Press, Inc.: New York.)
Gale, J., Kohl, H. C., and Hagan, R. M. (1966).—*Israel J. Bot.* **15**, 64.
Forrester, M. L., Krotkov, G., and Nelson, C. D. (1966).—*Pl. Physiol., Lancaster* **41**, 422.

HEATH, O. V. S., and ORCHARD, B. (1968).—*J. exp. Bot.* **19**, 176.
HEBER, U., and FRENCH, C. S. (1968).—*Planta* **79**, 99.
HESKETH, J. (1967).—*Planta* **76**, 371.
HOLMGREN, P. (1968).—*Physiologia Pl.* **21**, 676.
HOLMGREN, P., and JARVIS, P. G. (1967).—*Physiologia Pl.* **20**, 1045.
JARVIS, P. G., and SLATYER, R. O. (1966).—Tech. Pap. Div. Land Res. CSIRO, Aust. No. 29.
JOLLIFFE, P. A., and TREGUNNA, E. B. (1968).—*Pl. Physiol., Lancaster* **43**, 902.
LAKE, J. V. (1967).—*Aust. J. biol. Sci.* **10**, 495.
MCALLISTER, E. D., and MYERS, J. (1940).—Smithson. misc. Collns. N.S. Publ. No. 3591, p. 1.
MONOD, J., CHANGEUX, J. P., and JACOB, F. (1963).—*J. molec. Biol.* **6**, 306.
MOSS, D. M. (1968).—*Crop Sci.* **8**, 71.
RABINOWITCH, E. I. (1951).—"Photosynthesis and Related Processes." Vol. II, Pt. I. (Interscience Publishers, Inc.: New York.)
RACKER, E. (1957).—*Archs Biochem. Biophys.* **69**, 300.
RACKHAM, O. (1967).—In "Light as an Ecological Factor". (Ed. A. P. Hughes.) (Blackwell Press: Oxford.)
SCARTH, G. W., and SHAW, M. (1951).—*Pl. Physiol., Lancaster* **26**, 581.
SHIMSHI, D. (1963).—*Pl. Physiol., Lancaster* **38**, 713.
SLATYER, R. O. (1967).—"Plant–Water Relationships." (Academic Press, Inc.: London and New York.)
THOMAS, M. D. (1965).—In "Plant Physiology". (Ed. F. C. Steward.) Vol. IV. (Academic Press, Inc.: London and New York.)
TREHARNE, K. J., and STODDART, J. L. (1968).—*Nature, Lond.* **220**, 457.
TROUGHTON, J. H. (1969).—*Aust. J. biol. Sci.* **22**, 289.
VAADIA, Y., RANEY, F. D., and HAGAN, R. M. (1961).—*A. Rev. Pl. Physiol.* **12**, 265.
WAREING, P. F., KHALIFA, M. M., and TREHARNE, K. J. (1968).—*Nature, Lond.* **220**, 453.
WARD, W. J., and ROBB, W. L. (1967).—*Science, N. Y.* **156**, 1481.
WEISSBACH, A., HORECKER, B. L., and HURWITZ, J. (1956).—*J. biol. Chem.* **218**, 795.
WHITEMAN, P. C. (1965).—Ph.D. Thesis, Hebrew University of Jerusalem.
WHITEMAN, P. C., and KOLLER, D. (1967).—*New Phytol.* **66**, 663.
ZELITCH, I. (1967).—In "Harvesting the Sun". (Eds. A. S. Pietro, F. A. Greer, and T. J. Army.) (Academic Press, Inc.: New York.)