PLANT WATER STATUS AND CARBON DIOXIDE EXCHANGE OF COTTON LEAVES

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Summary

The effect of plant water status on the diffusion of CO_2 in the gas and liquid phase in leaves of cotton plants was studied in a single leaf chamber under conditions of constant light level and temperature and when photosynthesis was limited by the CO_2 supply. A controlled range of relative leaf water contents from 56 to 96% was obtained by varying root temperature from 6 to 30°C while the tops of the plants were at a constant temperature. Decreasing water content resulted in an increase in the calculated leaf diffusive resistance and a decrease in CO_2 exchange. Under the environmental conditions used, plant water status primarily affects CO_2 exchange by regulating stomatal aperture. The mesophyll resistance, which was estimated in air and in an oxygen-free atmosphere, did not vary with the relative leaf water content down to 75% but increased progressively as relative water content dropped from 75 to 56%.

I. INTRODUCTION

Carbon balance models have been used to describe plant growth in relation to light and CO_2 levels (e.g. Monteith 1965; de Wit 1965; Duncan *et al.* 1967) and experiments under controlled environment conditions have established that the relationship between light and CO_2 exchange is adequate for predicting plant growth at different light levels from measurements of CO_2 exchange (McCree and Troughton 1966*a*, 1966*b*). To extend these models to describe the influence of plant water status on plant growth rate, it is initially necessary to understand the processes involved in the reaction between plant water status and CO_2 exchange. The general relationship is that a reduction in plant water content reduces net photosynthesis (Vaadia, Raney, and Hagan 1961; Brix 1962; Slatyer 1967). The effect is generally explained by an increase in the diffusive resistance of the stomata which limits the supply of CO_2 to the chloroplasts, although specific evidence for this relationship is lacking.

It would be surprising if changes in CO_2 exchange caused by a range of plant water contents could be solely attributed to changes in stomatal diffusive resistance. Gaastra (1959) has identified resistances other than stomatal diffusive resistance which may limit CO_2 exchange in non-water-stressed plants and has suggested that low plant water contents may increase the mesophyll resistance (see also Slatyer 1967). Also, McCree and Troughton (1966*a*) have shown that the rate of respiration varies widely and others have shown that it is dependent on plant water status (Schneider and Childers 1941; Vaadia, Raney, and Hagan 1961; Greenway and Hiller 1967).

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Using the relationship between CO_2 exchange and diffusive resistance, this paper describes the effect of water stress on CO_2 exchange of single leaves of cotton. To develop the CO_2 exchange–leaf resistance relationship at high plant water contents, use was made of the occurrence of oscillations in stomatal aperture, and therefore in leaf resistance, in leaves (Howe 1964; Barrs and Klepper 1968; Troughton and Cowan 1968). The mesophyll resistance to CO_2 was estimated from CO_2 response curves in air and an oxygen-free atmosphere over a range of leaf water contents.

II. MATERIALS AND METHODS

(a) Plant Material

Uniform experimental plant material was obtained by growing Deltapine smooth leaf cotton plants from seed for about 2 months in a controlled environment. Day temperature was 30°C, night temperature 25°C, day length $12\frac{1}{2}$ hr, and plants were kept at a light level of about 100 W m^{-2} ($0 \cdot 4$–$0 \cdot 7 \mu$) produced by fluorescent tubes and incandescent lamps. The plants were grown in modified Hoaglands nutrient solution which was aerated and kept near cabinet temperature. Metasystox was occasionally used to control red spider infestations. Experiments were conducted on single leaves about 130–150 cm^2 in area, and from the same insertion level on the stem of plants of similar age and appearance.

(b) Leaf Chamber and Environmental Conditions

The leaf chamber was of the type described by Jarvis and Slatyer (1966), but without the inner chamber so that measurements were made on the whole leaf. Conditions round the leaf in the chamber were closely controlled, but the stem and other leaves on the plant were in an air-conditioned laboratory at a temperature of 25°C and with normal room lighting. The roots were kept in aerated Hoaglands nutrient solution and controlled to any temperature necessary to produce water stress in the plants.

Air, drawn by a compressor from outside the building, had an initial CO_2 concentration of 590 ± 10 μg l^{-1} (320 ± 5 v.p.m.) during the experiments. This air was heated by a water-bath and a heater in the air inlet line before entering the chamber. A boundary layer resistance to CO_2 of $0 \cdot 67$ sec cm^{-1} during the experiments was achieved by using flow rates of about 15 litres min^{-1} through the chamber, by having fans on both sides of the leaf, and by passing air at 15 litres min^{-1} in a loop through a temperature-controlled water-bath back into the chamber. When required, the O_2 or CO_2 concentration was controlled by adding or removing O_2 or CO_2 from the incoming air or by using air stored in polyvinyl chloride balloons but premixed to the required gas concentration.

Light from a mercury vapour H.P.L.R. lamp was passed through a K.G.I. Schott glass filter and $1 \cdot 5$ cm of water before reaching the leaf. Photosynthetically active radiation ($0 \cdot 4$–$0 \cdot 7 \mu$) was measured with a Kipp thermopile with appropriate filters, and is expressed as light absorbed by the leaf in W m^{-2}. Light absorbed by the leaf was measured as $0 \cdot 8$ of the light incident on the leaf, in position in the chamber and from measurements made in an Ulbricht integrating sphere. Absorbed light was assumed to be constant throughout the experiments at 100 W m^{-2}. It was thought that light absorbed by water-stressed leaves would be significantly different from that by non-stressed leaves, but incident and transmitted light monitored with a silicon solar cell throughout the experiments showed that the absorbed light never varied by more than 5%.

Leaf temperature was measured by pressing three thermocouples to different areas on the underside of the leaf and leaf temperature was kept at 30 ± 1°C during all the experiments. CO_2 exchange was monitored with a Grubb–Parsons differential infrared gas analyser and the flow rates of air were measured with flow-type rotameters. The specific humidity of the ingoing air and the air in the chamber was determined by a differential psychrometer (Slatyer and Bierhuizen 1964).

The relative leaf water content (R.L.W.C.) of the leaf in position in the chamber was derived from measurements obtained by positioning a promethium source above and a Geiger

tube below the leaf so that β-rays transmitted by the leaf were continuously monitored, and, when required, the water content of the leaf was derived from these records by the method described by Jarvis and Slatyer (1967). Direct measurements of the R.L.W.C. and plant water potential were made by sampling the leaf in the chamber, immediately the experiment was finished, by methods outlined by Slatyer (1967).

(c) Calculation of the Diffusive Resistances to CO₂ Transfer in Leaves

Free diffusion of CO_2 from the bulk air to the chloroplasts in leaves is restricted in the vapour phase by the boundary layer (r_a), stomatal (r_s), and cuticular (r_c) resistances and in the liquid phase by the mesophyll resistance (r_m) (Penman and Schofield 1951; Gaastra 1959). Derivation of r_a (from measurements with blotting paper) and the leaf resistance r_l (where $1/r_l = 1/r_s + 1/r_c$) was from the analagous calculated resistances to water vapour (Slatyer and Bierhuizen 1964), and all resistances refer to the area circumscribed by the leaf outline.

The additional resistance encountered by a flux of CO_2 into a leaf compared with the efflux of water vapour is termed the mesophyll resistance and involves a diffusion and a carboxylation term. The mesophyll resistance in this experiment was measured when the rate of CO_2 exchange was light-saturated and limited by the CO_2 supply. If respiration is ignored then

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

where C_a is the concentration of CO_2 in the ambient air ($\mu g\,l^{-1}$), C_c is the concentration of CO_2 at the chloroplasts ($\mu g\,l^{-1}$), and F is the net flux of CO_2 into the leaf ($g\,cm^{-2}\,sec^{-1}$) and is positive with respect to the plant when the leaf is gaining CO_2.

Invariably the measured F for plants like cotton contains a term due to respiration and therefore is an underestimate of the gross photosynthesis, but estimates of r_m can be made from CO_2 response curves in air or zero O_2, whereby the respiration term can be partly or wholly avoided (Lake 1967).

(d) Sequence of Measurements

Plants without a known prehistory of water stress were chosen for experiments. A leaf was allowed to equilibrate with leaf chamber conditions by maintaining a normal photoperiod and leaving the leaf overnight in the chamber prior to an experimental sequence. Measurements were made on the non-water-stressed leaf the following day, after leaf temperature and leaf diffusive resistance had been steady for at least an hour. Root temperature was then lowered over a period of about half an hour to the new desired temperature and a transient period, often lasting an hour, followed, during which time the water content of the leaf decreased and diffusive resistance increased. Measurements at the new steady state of plant water content were taken as soon as β-ray transmission by the leaf and CO_2 exchange had remained constant for an hour. During experiments necessitating longer periods of water stress the same sequence of measurements were maintained.

Experiments often had to be discontinued because stomata would not open fully in the morning, or they would respond rapidly to changes in root temperature and thus prevent a reduction in leaf water content, or the stomata would cycle (Troughton and Cowan 1968).

III. Results

(a) Root Temperature, Plant Water Status, and Leaf Diffusive Resistance

In these experiments plant water content was altered by varying the temperature of the nutrient solution surrounding the roots, while the leaf being studied remained in a constant environment in the leaf chamber. A change in the β-ray transmission of the leaf was noted within minutes of a sudden change in root temperature. With the plant material and environmental conditions used in this study the R.L.W.C. was

independent of root temperature from 25 to 35°C but was significantly affected by temperatures below 25°C, so that in general the lower the root temperature the lower the R.L.W.C. (Fig. 1), although there was not a close relationship.

The increase in leaf diffusive resistance accompanying the increase in R.L.W.C. with change in root temperature was probably due to an effect of the R.L.W.C. on the stomatal aperture. R.L.W.C. and leaf diffusive resistance were closely related over a wide range of water contents (Fig. 2), although this relationship only refers to steady states and to the specific environmental conditions of this experiment.
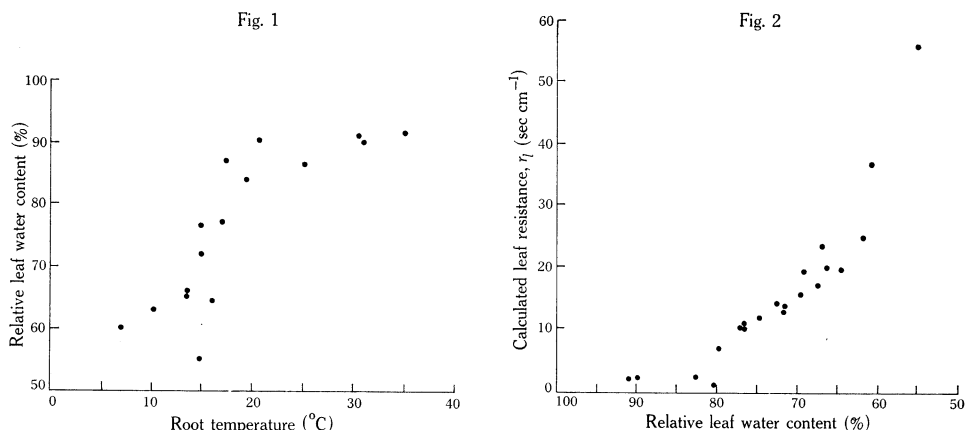

Fig. 1.—Influence of root temperature on the relative leaf water content of cotton leaves.

Fig. 2.—Relationship between the leaf diffusive resistance and the relative leaf water content of cotton leaves under steady state conditions.

(b) Oscillations in the Leaf Diffusive Resistance and the Rate of CO_2 Exchange

Steady-state plant conditions were not always maintained during the experiments and often oscillations in the rate of transpiration, CO_2 exchange, leaf water content, and leaf temperature occurred. These oscillations, as illustrated in Figure 3, were often of a simple harmonic form with a period which varied from 16 to 44 min and in general were similar to those previously reported (Howe 1964; Ehrler, Nakayama, and van Bavel 1965; Karmanov, Meleschenko, and Savin 1965; Barrs and Klepper 1968).

The oscillations were sometimes initiated when root temperature was reduced, although on two occasions initiation appeared to be spontaneous. Subsequently oscillations were more reliably produced when required by reducing the radiation load for 3–5 min or rapidly changing leaf temperature.

Under these experimental conditions, when CO_2 supply was limiting CO_2 exchange and at 30°C, oscillations in the rate of transpiration and CO_2 exchange were closely related (see Fig. 3). This suggests that CO_2 exchange was mainly under the

control of the leaf resistance rather than a mesophyll resistance, even at high rates of transpiration. However, transpiration can change independently of leaf resistance, for example when changes in the difference in specific humidity between the leaf and air occur. Accordingly, CO_2 exchange was expressed as a function of leaf resistance rather than transpiration. A relationship, as shown in Figure 4, between CO_2 exchange and leaf diffusive resistance has been developed from oscillations in two leaves and the average from several cycles when the R.L.W.C. was high and leaf temperature was 30°C. This relationship was then used to predict CO_2 exchange in leaves subject to water stress under steady-state conditions.
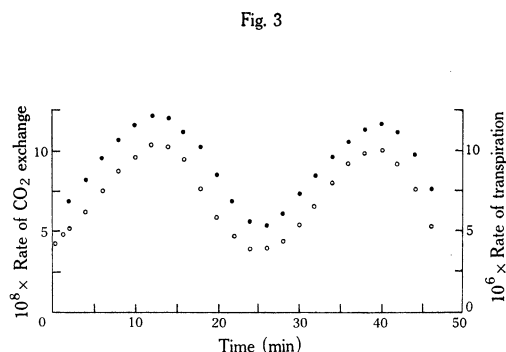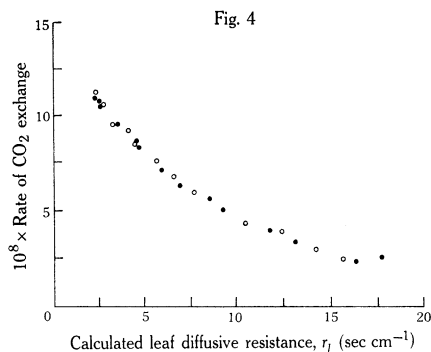

Fig. 3.—Characteristic oscillations in the rate of CO_2 exchange (●) and the rate of transpiration (○) in cotton leaves (both expressed as $g\ cm^{-2}\ sec^{-1}$).

Fig. 4.—Relationship between leaf diffusive resistance and CO_2 exchange ($g\ cm^{-2}\ sec^{-1}$) derived from several oscillations on two cotton leaves. ● Leaf 1. ○ Leaf 2.

(c) Prediction of CO₂ Exchange in Leaves Water-stressed for Short Periods

To test the influence of short-term water stress on CO_2 exchange, the leaves were stressed for about 2 hr. The results are presented in Table 1 where each value of the actual CO_2 exchange is derived from a different leaf and is the average value obtained over 15 min. The rate of CO_2 exchange predicted from the leaf diffusive resistance–CO_2 exchange relationship was approximately equal to the actual CO_2 exchange over a wide range of R.L.W.C. values. The ratio of the predicted to actual CO_2 exchange closely approached unity which suggested that, in spite of the wide range of R.L.W.C. values, CO_2 exchange could be reasonably predicted from knowing the leaf diffusive resistance under known conditions of light, leaf temperature, and CO_2 concentration.

The most severe level of water stress imposed during these experiments was 56% R.L.W.C. This resulted in a calculated leaf diffusive resistance to CO_2 of 56·0 sec cm⁻¹ which could be regarded as the cuticular component of the leaf resistance. This value is close to that of 53·7 sec cm⁻¹ derived by Bierhuizen and Slatyer (1964) for cotton leaves, but obtained from measurements of water vapour exchange in the dark.

Although cuticular transfer of CO_2 during the severe water stress was very low at about $0\cdot8\times10^{-8}$ g cm^{-2} sec^{-1}, uptake by the leaves was still positive. This result only applies to the conditions of this experiment, i.e. short-term stress and when the light absorbed by the leaf remained constant due to the leaf being maintained in a position horizontal to the radiation input. Normally this degree of stress would produce a characteristic drooping of the leaves thereby reducing the radiation absorbed by the leaf.

TABLE 1

PREDICTED AND ACTUAL CO_2 EXCHANGE IN WATER-STRESSED LEAVES

Leaf No.	R.L.W.C. (%)	Calculated Leaf Diffusive Resistance (sec cm^{-1})	$10^8 \times CO_2$ Exchange (g cm^{-2} sec^{-1})*		Ratio of Predicted/ Actual CO_2 Exchange
			Predicted	Actual	
1	92·0	1·7	14·0	13·9	0·99
2	88·0	2·4	10·7	10·2	1·05
3	77·4	10·4	4·3	4·4	0·98
4	77·0	11·2	4·2	4·4	0·95
5	72·0	13·7	3·2	3·0	1·06
6	67·7	17·2	2·36	2·5	0·94
7	67·5	23·6	1·86	1·8	1·03
8	65·0	19·7	2·2	2·0	1·10
9	61·1	36·8	1·2	1·2	1·00
10	56·0	56·0	0·9	0·8	1·13

* At an initial CO_2 concentration of 548 μg l^{-1}.

(d) CO_2 Exchange in Leaves Water-stressed for 8 hr

Since the only changes in CO_2 exchange in leaves stressed for short periods could be explained by changes in leaf resistance, the period of stress was increased to 8 hr. Furthermore, the plants were allowed to recover from stress overnight and their ability to recover measured by the rate of CO_2 exchange the following day.

Initially the leaves were stressed to a R.L.W.C. of 75–80% and the results were closely comparable to the 2-hr stress. Furthermore, recovery of CO_2 exchange was within 15% of pre-stress levels once the leaf resistance was steady the day after stress.

The intensity of stress was then increased on four further plants to produce a R.L.W.C. of less than 75%. During stress, the CO_2 exchange throughout the 8 hr was at a rate expected from the leaf diffusive resistance (Table 2), but recovery from stress was incomplete. However, the CO_2 exchange during recovery was that expected from the leaf diffusive resistance, so that the inability to recover was a function of the stomata rather than a direct effect on the photosynthesis or respiration of the leaf.

This effect can be illustrated by leaf 4 (Table 2) which suffered the most severe stress of 62% R.L.W.C. The non-stressed leaf, attached to the plant, was left overnight in the chamber and on the following morning measurements were taken in the non-stressed condition as soon as the stomata were considered to be fully open. The leaf resistance was $1\cdot8$ sec cm^{-1} and CO_2 exchange $12\cdot6\times10^{-8}$ g cm^{-2} sec^{-1} as shown in Figure 5. The roots of the plant were cooled and stress developed. The

stomata began closing, and during this phase the relationship between CO_2 exchange and the leaf diffusive resistance was obtained. The plant was left severely stressed for 8 hr during which time the rate of CO_2 exchange was that expected from the CO_2 exchange–leaf diffusive resistance relationship. Late in the day the stomata were

TABLE 2

CO_2 EXCHANGE IN LEAVES WATER-STRESSED FOR 8 HR

Leaf resistances (r_l) are expressed as $cm\,sec^{-1}$, net fluxes of CO_2 into leaves (F) as $g\,cm^{-2}\,sec^{-1}$, and R.L.W.C. as a percentage

Leaf No.	Pre-stress		During Stress			Post-stress	
	r_l	$10^8 \times F$	R.L.W.C.	r_l	$10^8 \times F$	r_l	$10^8 \times F$
1	2·0	12·2	80	6·8	6·2	2·1	12·0
2	2·22	11·7	72	13·0	3·5	2·2	11·6
3	1·85	12·8	77	10·1	4·6	2·1	11·5
4	1·8	12·6	62	25·0	1·3	2·2	11·5
5	2·5	10·0	70	16·0	2·5	2·5	9·9
6	2·3	12·0	73	14·1	2·95	2·8	10·6

closed further than they had been during the previous 7 hr, resulting in a further reduction in CO_2 exchange and an increase in the R.L.W.C. The leaf was allowed to recover overnight and the CO_2 exchange the following day was lower than before stress but was at a rate expected from the leaf diffusive resistance (Fig. 5). Leaf water content had fully recovered. On this day oscillations in the leaf resistance
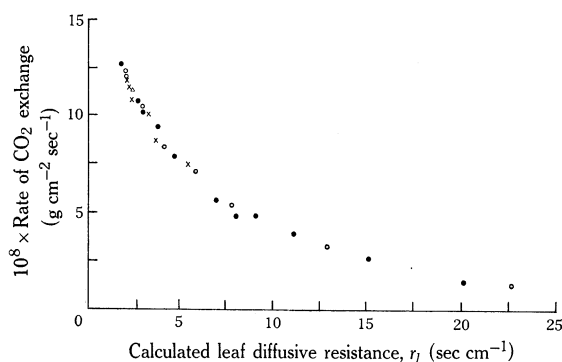

Fig. 5.—The CO_2 exchange and leaf diffusive relationship on four successive days for cotton leaves.
● Day 1: stress developing.
× Day 2: recovery from stress and during oscillations.
○ Day 3: stress developing.
△ Day 4: recovery from stress.

developed and, when the leaf resistance during part of the cycle was lower than in the morning, CO_2 exchange increased as expected, which provided further evidence that photosynthesis had been unaffected. Leaving the plant unstressed for a third day resulted in a further recovery of the stomata. The plant was then stressed again in a similar pattern but once more any change in CO_2 exchange was related to the leaf diffusive resistance.

(e) CO_2 Exchange in Leaves Stressed for Three Consecutive Days

Experiments were conducted on three leaves which were severely stressed during light hours (12 hr) for three consecutive days. Overnight there was partial recovery of the R.L.W.C. of the leaves, but the level was soon reduced when the radiation load increased in the morning. Again, in all leaves and on each day the CO_2 exchange was that expected from the leaf diffusive resistance. On release from water stress the leaf diffusive resistance was greater than pre-stress levels (Table 3) but there were no other apparent after-effects on the CO_2 exchange of the leaves.

TABLE 3

CO_2 EXCHANGE IN LEAVES WATER-STRESSED FOR THREE CONSECUTIVE DAYS

Units for r_l and F are as for Table 2

Leaf No.	Pre-stress		During Stress		Post-stress (day 1*)		Post-stress (day 2*)		A/B
	r_l	$10^8 \times F$	r_l	$10^8 \times F$	r_l (A)	$10^8 \times F$	r_l (B)	$10^8 \times F$	
1	2·3	11·6	25	1·4	2·8	10·0	2·4	11·2	0·82
2	2·35	12·0	31	0·95	2·6	11·1	2·4	11·1	0·90
3	2·1	13·0	29	1·2	2·5	11·0	2·3	11·4	0·84

* Days after cessation of water-stress conditions.

(f) Calculated Mesophyll Resistances

The previous experiments show that the over-riding effect of water stress on CO_2 exchange is mediated through changes in the leaf diffusive resistance and therefore, most probably, the stomatal resistance. This implies that, under the limited range of conditions imposed in these experiments, the rate of photosynthesis was limited by the CO_2 supply so that the stomata effectively controlled the CO_2 concentration at the mesophyll cell wall. Consequently the mesophyll resistance would appear to have been unaffected by the R.L.W.C.

At normal R.L.W.C. values the mesophyll resistance was estimated by a linear extrapolation to $r_l = 0$ of the CO_2 exchange–leaf resistance relationship obtained over a range of leaf resistances of 2–5 sec cm^{-1} (Whiteman and Koller 1967), and from the relationship of the CO_2 concentration at the mesophyll cell wall (C_w) to F for a range of C_w values and oxygen-free conditions. C_w was derived from knowing C_a and correcting for the concentration reduction across r_a and r_l. In water-stressed leaves the response curve methods alone could be used, but only a limited number of results were obtained because of the difficulty of maintaining a constant r_l under stress while the CO_2 concentration was being varied.

Initially a wide range of CO_2 concentrations were used from 150 to 900 $\mu g\,l^{-1}$ but only results derived from a narrow range of the linear part of the CO_2 concentration–CO_2 exchange relationship (from 250 to 500 $\mu g\,l^{-1}$) are presented. The limited number of results from response curves was supported by calculations from

instantaneous CO_2 fluxes over a range of known leaf resistances. These calculations required knowing the CO_2 concentration at or near the chloroplasts and it was assumed to be zero (or small) with respect to the levels of CO_2 used in the experiment (although logically it must be some positive value).

The calculated mesophyll resistances under non-water-stressed conditions was between $2 \cdot 0$ and $2 \cdot 9$ sec cm^{-1} (Table 4). The variation may have been due to

TABLE 4

SUMMARY OF CALCULATED MESOPHYLL RESISTANCES

Method	No. of Observations	R.L.W.C. (%)	r_l (sec cm^{-1})	r_m (sec cm^{-1})	r_m/r_l
From F where $r_l = 0$			0	$2 \cdot 9$	
From F where $r_l = 0$, but corrected for respiration (30%)			0	$2 \cdot 01$	
From slope of CO_2 response curve* in air	2	88–92	$2 \cdot 3$	$2 \cdot 3$	$1 \cdot 0$
	1	75	$12 \cdot 0$	$2 \cdot 5$	$0 \cdot 21$
	1	67	$19 \cdot 8$	$4 \cdot 0$	$0 \cdot 20$
	3	60–65	$25 \cdot 0$	$6 \cdot 2$	$0 \cdot 25$
From slope of CO_2 response curve* in an oxygen-free atmosphere	3	85–90	$2 \cdot 7$	$2 \cdot 0$	$0 \cdot 74$
	2	70	$15 \cdot 2$	$2 \cdot 2$	$0 \cdot 14$
From equation (1) in water-stressed plants. No correction for respiration		$92 \cdot 0$	$1 \cdot 7$	$2 \cdot 2$	$1 \cdot 32$
		$88 \cdot 0$	$2 \cdot 4$	$2 \cdot 9$	$1 \cdot 20$
		$77 \cdot 4$	$10 \cdot 4$	$2 \cdot 0$	$0 \cdot 20$
		$77 \cdot 0$	$11 \cdot 2$	$1 \cdot 4$	$0 \cdot 12$
		$72 \cdot 0$	$13 \cdot 7$	$4 \cdot 6$	$0 \cdot 36$
		$67 \cdot 7$	$17 \cdot 2$	$4 \cdot 5$	$0 \cdot 26$
		$67 \cdot 5$	$23 \cdot 6$	$6 \cdot 9$	$0 \cdot 29$
		$65 \cdot 0$	$19 \cdot 7$	$8 \cdot 0$	$0 \cdot 4$
		$61 \cdot 1$	$36 \cdot 8$	$7 \cdot 7$	$0 \cdot 21$
		$56 \cdot 0$	$56 \cdot 0$	$12 \cdot 5$	$0 \cdot 22$

* $\Delta F/\Delta CO_2$.

variation between plants or the extent to which the different methods corrected for respiration. Variation in the R.L.W.C. between 75 and 92% did not produce a measurable influence on r_m but the decrease in the R.L.W.C. from 75 to 56% resulted in an apparent increase in r_m from about $2 \cdot 5$ to $12 \cdot 0$ sec cm^{-1} (Fig. 6). The significance of this change in r_m on CO_2 exchange is small because F had changed from $13 \cdot 9$ to $3 \cdot 0 \times 10^{-8}$ g cm^{-2} sec^{-1} before there was any change in r_m. It had previously been shown that there was a good relationship between CO_2 exchange over a range of leaf resistances in stressed and non-stressed leaves, so that the observed variation in r_m may not be directly associated with changes in the leaf water content but rather the variation in the leaf resistance.

IV. DISCUSSION

The experiments reported here illustrate the perturbations in a plant system that can result from disturbing a single factor in an otherwise steady environment. The environment on the top of the plant was kept constant but reducing root temperatures below 22°C resulted in a reduction in the relative leaf water content and thereby the leaf diffusive resistance through closure of the stomatal aperture. This increase in the leaf diffusive resistance with increasing water stress resulted in a reduction in the gaseous exchange of water vapour and CO_2, and the reduction in water vapour exchange increased leaf temperature.

Fig. 6.—Influence of the relative leaf water content on the calculated mesophyll resistance of cotton leaves (as shown in Table 4).

(a) Root Temperature and the Relative Leaf Water Content

Varying root temperature was found to be a useful and successful method of obtaining a range of plant water contents, as can be seen from Figure 1. The treatment can be easily and quickly applied, it can maintain a constant plant water content over a long period under controlled environment conditions, and as yet no detrimental side effects have been noticed even after 3 consecutive days of severe stress.

Arndt (1937) noted wilting in young cotton plants when the roots were cooled, even when the roots were in nutrient solution. This influence of root temperature on leaf water content is well established (Kramer 1949; Ehrler 1963; Cox and Boersma 1967), and a reason suggested by Kramer (1949) for this effect is an influence on cell permeability. Kuiper (1963, 1964) has shown that low root temperatures reduce the uptake of water but that the Q_{10} for water uptake of $3 \cdot 8$ can be reduced to $1 \cdot 18$ by the application of alkenyl succinic acid which is suggested to influence membrane permeability. Clearly the actual leaf water content at a given root temperature will depend on the relationship between the rate of absorption and rate of loss of water by the plant, and on the rate of change of these processes relative to each other at the time of imposition of the root temperature treatment.

(b) Relative Leaf Water Content and Stomatal Aperture

Several observers have associated a reduced transpiration rate with low root temperature (Kramer 1949; Locher and Brouwer 1965; Cox and Boersma 1967;

Nelson 1967), and Nelson (1967) extended his observations to show that the reduction in transpiration was associated with changes in stomatal aperture. The results in this paper show conclusively an influence of root temperature on the R.L.W.C. and thereby on the calculated leaf diffusive resistance. During these experiments any change in the R.L.W.C. lower than 85% produced an immediate response in the stomata and, although there was a tendency for leaf diffusive resistance to be dependent on leaf water content (Fig. 2), the equilibrium relationship took some time to develop.

This equilibrium relationship is specific to the conditions and material of this experiment and the exact resistance offered by stomata at different R.L.W.C. values will depend on other environmental and plant factors affecting stomatal aperture. For example, it has been noted (Barrs and Klepper 1968; Cowan and Troughton, unpublished results) that in leaves exhibiting oscillations the R.L.W.C. and leaf diffusive resistance can be out of phase by as much as 15 min, so that transpiration was highest when the R.L.W.C. was least. However, this influence of the R.L.W.C. on stomatal aperture through its control of the turgor of the guard cells, or subsidiary or other epidermal cells (Milthorpe and Spencer 1957; Heath 1959; Ketellapper 1963), could be the reason for initiating or sustaining the oscillations.

The inability of the stomata to completely recover overnight from water stress was not directly a function of the R.L.W.C., as the R.L.W.C. attained normal levels (about 90%) within an hour of release of the low root-temperature treatment. Failure of stomata to fully recover from water stress has often been noted and, by using epidermal strips from leaves, Fischer (1967) was able to associate the failure with the guard cells, not with the mesophyll tissue, and suggested that this failure might be due to a loss of metabolic activity.

(c) Plant Water Status and the Resistances to CO_2 Transport

The results clearly support the contention that stomata are of primary significance in controlling CO_2 exchange in plants that are water-stressed (Piesk and Winkler 1956; Brix 1962; El-Sharkawy and Hesketh 1964; Slatyer 1967) particularly when CO_2 supply is limiting photosynthesis. That the leaf diffusive resistance (r_l) exerts the main control on CO_2 exchange can be seen from the close relationship between the rate of CO_2 exchange and transpiration in leaves exhibiting oscillations and the degree to which CO_2 exchange could be predicted, in water-stressed leaves, from data on environmental conditions and r_l. It may then be thought that cotton stomata are more sensitive to water stress than other plants and, by closing at higher R.L.W.C. values, may "protect" the photosynthetic reactions. But low R.L.W.C. values were obtained, and data of Ehlig and Gardner (1964) and El-Sharkawy and Hesketh (1964) imply that cotton stomata are at least less sensitive to water stress than sorghum, pepper, trefoil, and sunflower.

The conclusion that stomata offer the main control to CO_2 exchange suggests that the mesophyll resistance is of secondary importance. At high R.L.W.C. values the calculated mesophyll resistance (r_m) for the cotton leaves in this experiment of between 2 and 3 sec cm^{-1} were essentially similar to minimum values previously reported for cotton (Bierhuizen and Slatyer 1964) and for similar plants (Gaastra 1959). In general there was good agreement between the methods used to derive r_m,

particularly if account is taken of possible differences between methods of correction for the influence of CO_2 from respiration or CO_2 exchange.

Oxygen-free air was used to inhibit photorespiration, and CO_2 exchange was enhanced by 30–35% in low-oxygen atmospheres compared with air at 30°C and CO_2 concentrations of 550 μg l^{-1}, which is similar to values reported by Hesketh (1967). This same level of enhancement occurred in water-stressed leaves, which suggests that the significance of photorespiration to productivity (Forrester, Krotkov, and Nelson 1966) would also apply to plants at low water contents. Of particular interest was that the slope of the CO_2 response curve in air was 15% lower than in low O_2 which is similar to the result of Forrester, Krotkov, and Nelson (1966). This may be interpreted as an influence of O_2 on photosynthesis (Björkman 1966; Forrester, Krotkov, and Nelson 1966) although it may, in part, be due to a respiration component which changed rapidly with the rate of CO_2 exchange (Lake 1967).

When water stress was imposed, mesophyll resistances calculated from CO_2 response curves in air and from F at a given CO_2 concentration were unaffected until the R.L.W.C. was less than 75%. Although the change in r_m from 2·5 to 12·0 sec cm^{-1} when the R.L.W.C. was reduced from 75 to 56% may have been an effect of water stress on the photosynthetic process other than through the CO_2 diffusion resistances, it seems that the effect may have been associated with stomatal closure. A possible explanation would be that the mesophyll resistance has, in effect, two components, one of which refers to CO_2 transfer in solution from cells surrounding the stomates to the chloroplast, and the other associated with CO_2 which diffuses via the cuticle. If the latter path is longer, then the r_m associated with it will be larger, so that as the stomata close the proportion of the total CO_2 taken up by the cuticle increases, and r_m will be greater the higher the leaf resistance.

Several investigators have suggested non-stomatal control of photosynthesis in water-stressed plants (Brilliant 1924; Scarth and Shaw 1951; Gaastra 1959; Shimshi 1963; Gale, Kohe, and Hagan 1966), and it is readily conceded that at some stage and under some conditions other factors will influence photosynthesis. Certainly water stress depressed some processes associated with photosynthesis in isolated chloroplasts (Todd and Blaser 1965; Nir and Poljakoff-Mayber 1967). The degree to which non-stomatal factors can influence CO_2 exchange were highlighted by Troughton and Cowan (1968) when, under conditions when stomata were expected to control CO_2 exchange, exchange was observed to be independent of the leaf diffusive resistance for part of the period of the oscillation.

(d) Root Temperature, Plant Water Content, and Plant Growth

Low root temperatures reduce plant growth and it has been suggested that the effect is due to an influence on the water status of the plants (Nelson 1967; Unger and Danielson 1967). Clearly the results in this paper show that the relationship between root temperature and net photosynthesis operates via the leaf water content and leaf diffusive resistance. However, root temperature may influence stomatal action or other growth processes through controlling the rate of production or translocation of substances formed in the roots (Oritani 1963; Weiss and Vaadia 1965).

Discussion has often centered round the possibility of increasing the water-use efficiency of plants by utilizing the extra resistance in the CO_2 compared with the

water-vapour pathway (Slatyer and Bierhuizen 1964). Increasing r_l without changing r_m should cause a proportionally greater reduction in transpiration than in photosynthesis. While this would apply to natural conditions where the water supply to the plants is limited, the results in this paper suggest that, should water be unlimited and the CO_2 supply limiting photosynthesis, then reducing r_l would enhance CO_2 uptake. The results indicate increases in CO_2 uptake down to an r_l of $1\cdot4$ sec cm^{-1}, and estimate a potential CO_2 uptake of 19×10^{-8} g cm^{-2} sec^{-1} when $r_l = 0$ and at normal air concentrations of CO_2 of 580 μg l^{-1}. Rates approaching this have been measured in young cotton leaves $(16\cdot7 \times 10^{-8}$ g cm^{-2} sec^{-1}; Elmore, Hesketh, and Muramoto 1967), although in older leaves the average value was about 10×10^{-8} g cm^{-2} sec^{-1}, which may be due to a change in stomatal or mesophyll resistance with age. It would seem that under a favourable environment, with irrigation and when nutrients, insects, and disease were not limiting photosynthesis, then productivity may be increased by reducing the leaf resistance to a minimum by genetic or artificial means.

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VI. References

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