Photosynthesis and Environmental Stress Interactions in Sugarbeet Leaves*

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Received for Publication October 24, 1983

INTRODUCTION

One of the greatest needs in both research and practical crop production is a method for measuring instantaneous plant growth. If we could go out in the field and make simple rapid measurements of plant growth rates, the application and benefits would be immediate and far reaching. The instantaneous growth rate is characterized by the carbon balance of a plant or crop. Some years ago Terry (9) and some of his associates made detailed studies of CO_2 exchange parameters of beet leaves effected by nutrient deficiencies. Under carefully controlled conditions he found changes that developed in the very early stages of nutrient stress. Following this lead I attempted to make a practical application on sugarbeets (*Beta vulgaris* L.) growing under real field conditions (1). The objective was not accomplished because variation in CO_2 exchange was greater from leaf to leaf than the changes brought on by the initiation of stress.

The data and results reported here come from additional studies using field-grown sugarbeet leaves. The objective was to pinpoint the fundamental differences in the leaves that lead to the large variability in CO_2 assimilation from leaf to leaf.

Since these differences may be selectively effected by various types of plant stress, nitrogen and water variables were applied on the filed plots. Temperatures were also monitored with particular attention to the cool periods that occurred.

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Four steps occur during the course of CO_2 fixation, any of which can individually limit the rate of assimilation.

1. The CO_2 must diffuse through the stomata into the leaf interior.
2. The CO_2 must diffuse through the gas phase of the mesophyll tissue to the cells with chloroplasts.
3. Transport of CO_2 in the liquid phase through cell walls into the chloroplasts must occur.
4. A sufficient supply of both RuBP (ribulose-1,5 biphosphate) and active carboxylating enzyme sites must be present in the chloroplasts.

These four factors may be expressed as individual resistances to CO_2 fixation. The size of each resistance may be estimated from gas exhcange measurements made under carefully controlled conditions. A fifth factor, leaf respiration, must also be considered in this type of analysis, for when the four resistances are low allowing rapid CO_2 fixation, a high respiration rate can negate the net result.

Calculation of the values of these five factors was made for individual leaves. Steps in the CO_2 assimilation path that led to the large differences in photosynthesis among field grown sugarbeet leaves are discussed in light of the results.

MATERIALS AND METHODS

Sugarbeets were grown in the field in Southcentral Idaho on the portneuf silt loam soil (*Durixerollic calciorthid*) which has been described in detail (2). The control treatment was fertilized with N and P and irrigated from furrows in accord with prevailing best management recommendations. Other areas were managed to create either N or water stress by not fertilizing and discontinuing irrigation after July 7.

Throughout the growing season leaves were removed with their petioles submerged in water and brought into the laboratory for detailed gas exchange measurements in the chamber described previously (3). Gas exchange measure-

ments were made using both sides of the leaf with a flow rate of 1 ℓ min^{-1} over 24.5 cm^2 of leaf surface except during August when a connection was inadvertently plugged during repairs causing the gas to flow only across the underside of the leaf surface. Tests showed this reduced net CO_2 assimilation by 15 or 20 percent, but had little effect on the relative differences between the resistances being studied.

Measurements of CO_2 diffusion resistance in the gas phase of the mesophyll tissue were made by varying the ambient pressure and applying the analysis developed previously (3). The assimilation of CO_2 was measured at two light intensities, 715 and 260 $\mu e\ m^{-2}s^{-1}$, and at least three ambient CO_2 levels ranging from 220 to 800 mg m^{-3}. Values of the physical part of the liquid phase mesophyll CO_2 transfer resistance across the cell walls and membranes were calculated from linear regression of the CO_2 response curves using Jones and Slatyer's equation with ambient O_2 held at one percent (7). The stomatal and cuticular resistance, r_s, was obtained from transpiration rates and included 110 s m^{-1} of boundary layer resistance resulting from the leaf chamber geometry and the gas flow rate (3).

RESULTS

A total of 63 leaves were taken into the laboratory and their CO_2 exchange properties studied in detail. Typical results including the more interesting data are presented in Table 1. The table includes four sections with the leaves in each section ranked according to the amount of carbon they were able to fix under high light and CO_2 conditions. The measurements made on these leaves were not intended to mimic photosynthetic rates in the field, but rather to search for differences in gas exchange parameters that may have been imprinted by previous conditions as the leaves grew in the field.

The dark respiration was measured the first minute or so after the light was turned off. In general the values were little different, falling in the range of 0.06 to

Table 1. Net CO_2 fixation rates of field grown sugarbeet leaves as affected by a variety of conditions. The symbols are defined as H, high light 715 μe $m^{-2}s^{-1}$; L, low light 260 μe $m^{-2}s^{-1}$; h, high ambient CO_2 concentrations of 800 mg m^{-3}; l, low CO_2 at 220 mg m^{-3}; P net CO_2 uptake mg $s^{-1}m^{-2}$; r_s, stomatal and boundary resistance to CO_2 diffusion s m^{-1}; r, CO_2 resistance through the mesophyll cell walls and membranes into the chloroplasts, s m^{-1}; and r_c apparent carboxylation resistance, s m^{-1}.

Leaf	Date	Light	CO_2	P	r_s	r	r_c	Leaf Description
	Leaves from the control treatment							
1	09-13	H	h	1.08	190	249	281	Young, large sink, optimum
	PM	H	l	0.43	160		53	conditions
2	06-27	H	h	0.92	330	211	315	Expanding, optimum conditions
	PM	H	l	0.27	450		60	
		L	h	0.49	450	474	764	
		L	l	0.27	290		37	
3	07-16	H	h	0.83	390	193	425	Mature, a bit lighter green
	AM	H	l	0.43	570		177	but N fertilized
4	08-16	H	h	0.70	230	564	378	Expanding, optimum conditions
	AM	H	l	0.29	250		(-66)	
5	08-15	H	h	0.69	240	313	604	Mature, partly shaded
	PM	H	l	0.38	180		111	
	Leaves from nitrogen stressed plants							
6	08-13	H	h	0.92	200	217	417	Expanding, a bit chlorotic
	PM	H	l	0.45	200		28	
7	07-18	H	h	0.81	320	192	514	Mature, light green
	PM	H	l	0.37	240		138	

Table 1 - Continued

Leaf	Date	Light	CO_2	P	r_s	r	r_c	Leaf Description
8	08-28	H	h	0.81	340	517	116	Expanding, light green
	PM	H	l	0.24	350		2	
		L	h	0.30	1020	435	1244	
		L	l	.022	360		132	
9	09-13	H	h	0.64	190	338	432	Young, still dark green
	AM	H	l	0.36	210		23	
10	09-11	H	h	0.29	310	1343	976	Mature, somewhat chlorotic
	AM	H	l	0.10	550		457	
	Leaves from water stressed plants							
11	08-21	H	h	0.98	240	267	314	Mature, flaccid on the
	AM	H	l	0.27	320		168	previous afternoon
12	08-28	H	h	0.79	480	552	(-14)	Expanded, flaccid on the
	AM	H	l	0.19	540		43	previous afternoon
13	09-11	H	h	0.49	510	830	268	Mature, flaccid on the
	PM	H	l	0.19	320		(-116)	previous afternoon
		L	h	0.41	390	3175	(-1573)	
		L	l	0.11	360		(-1539)	
14	08-30	H	h	0.48	830	957	(-127)	Expanded, a bit flaccid
	PM	H	l	0.16	520		(-150)	
	Leaves from cold stressed plants in the control treatment							
15	08-20	H	h	0.73	470	1087	(-453)	Expanded, weather unseason-
	PM	H	l	0.16	560	1087	(-339)	ably cool

Table 1 - Continued

Leaf	Date	Light	CO_2	P	r_s	r	r_c	Leaf Description
16	08-22	H	h	0.73	430	1010	(-308)	Expanded, weather unseason-
	PM	H	l	0.14	460		(-469)	ably cool
17	09-10	H	h	0.59	210	375	743	Expanding, cold following
	AM	H	l	0.31	240		21	warm
18	11-02	H	h	0.59	290	299	382	Expanding, freeze hardy
	AM	H	l	0.11	420		721	
19	11-01	H	h	0.12	1140	2345	4200	Exapnding, not freeze hardy
	AM	H	l	0.10	870		740	

0.08 mg s^{-1} m^{-2}. Younger leaves tended to show larger values as did several of the cold and water stressed plants. Leaf 1 had highest respiration rate of 0.19. It was from a plant in a group in the optimum fertility and water area that had all leaves removed the third week in August. The young leaves that grew from these plants showed high rates of CO_2 fixation and large initial dark respiration. This may have been associated with their large root to leaf ratios providing unusally large sinks for the newly fixed carbon.

The resistances to CO_2 diffusion in the gas phase of the mesophyll tissue did not show any striking differences due to the treatments, falling generally in a range less than 200 m s^{-1}.

The measurements at low light levels were not as interesting as those at saturating light because the experimental errors are larger by comparison than those at high light were the CO_2 flux is greater. The results at low light did show the same general trends at those under high light, though the resistances were generally larger. Results from low light observations are included in Table 1 for leaves 2, 8, and 13, to illustrate the range of numbers that occurred.

The values for the resistance to CO_2 transport from the cell wall into the chloroplast, r, and the residual "carboxylation" resistance, r_c, are the most interesting parameters. Values for r_c were calculated from the classical analogue resistance relation, which defines r_c as the apparent residual, i.e.,

$$P = \frac{C_a - C}{r_s + r} = \frac{C}{r_c} \qquad (1)$$

where P is the gross rate of CO_2 fixation, Ca the concentration of CO_2 in the air outside the leaf, r_s the combined stomatal and boundary resistance to CO_2 transport, and C is the average concentration of CO_2 in the chloroplasts. Assuming second order kinetics one may also express P as

$$P = kC[E\text{-}RuBP] \qquad (2)$$

where k is the rate constant and [E-RuBP] is the concentration of RuBP attached to active carboxylating enzyme sites and thus ready to react with CO_2 to form PGA. Combining eqs. (1) and (2) to eliminate P/C gives

$$r_c = \frac{1}{k} \frac{1}{[E\text{-}RuBP]}. \quad (3)$$

While values of r_c are calculated from experimental data with eq. 1, the values are more meaningful when considered in terms of eq. 3 which shows that values of r_c are inversely proportional to the concentration of RuBP adsorbed on active carboxylating sites in the chloroplasts. Thus, when values of r_c are large, it follows that the concentration of RuBP and/or the activity of the carboxylating sites in the chloroplasts are low and may limit photosynthesis.

At high levels of ambient CO_2, r_c becomes large because the amount of CO_2 in the chloroplasts begins to saturate the reaction making the number of active carboxylating sites or the amount of RuBP the limiting factor. On the other hand, under low CO_2 the carboxylating resistance may be low because carboxylase activity and RuBP is high with respect to the amount of CO_2 in the chloroplasts, eq. 2. When r_c remains large at low CO_2 values the inherent ability of the chloroplasts to fix carbon is surely impaired.

As the rate of carbon fixation decreases the results in Table 1 show that either the liquid phase transfer resistance to CO_2 flow into the chloroplasts increases, or the carboxylation resistance increases, or both. For example, leaf 4 fixed less carbon than leaf 2 because of a larger resistance to CO_2 transport into the chloroplasts though there was no obvious reason why this should have occurred. Leaf 5 fixed less carbon than 3 because of higher resistances to both CO_2 transport into the chloroplast and to carboxylation. It may be noted that the experimental error involved in measuring the CO_2 assimilation rates was no more than $\pm$ 0.03 mg $m^{-2}s^{-1}$. Thus the difference was real in the leaves' abilities to fix CO_2

at high light and ambient CO_2 levels.

Nitrogen deficient leaves that showed the higher rates of CO_2 assimilation tended to show some limitations due to carboxylating activity, i.e., leaves 6 and 7, compared to 1 and 2. At lower rates however, liquid phase transport resistances were large. Water stressed leaves fixing carbon at lower rates did show predominantly high resistances to CO_2 transfer into the chloroplasts (leaves 12-14). This has also been observed in water stressed cotton leaves (6). In leaves 12-14, resistances were so large they forced negative values for r_c which is not in keeping with the physical model on which r and r_c are based. The same problem is evident for leaves 15 and 16. The weather had been unseasonably cool, over cast and damp for several days beginning August 17. After being conditioned to this type of weather in the field sugarbeets may typically show some wilting on the first warm day with full sun, even though soil water is adquate; so perhaps the large values of r for leaves 15 and 16 resulted from the same phenomena manifest in leaves 12-14. Other leaves studied during the period August 18-24 that were from low nitrogen or soil water areas did not show the high r values, for example, leaf 11.

Leaf 17 exhibited a different type of cold response than leaves 15 and 16. In this case the weather had been unseasonably warm for several days, maximum over 30°C and minimum only 11°C. On the 10th of September the low was 7° and the high 23°C. That afternoon analysis of leaf 17 showed an unusually high carboxylating resistance. High carboxylating resistances following leaf desiccation had been reported (4), but other studies may be cited suggesting the carboxylating system can recover during prolonged water stress (4).

Measurements were continued into early November after the leaves had been freezing for several nights with lows of -4°C. While the leaves thawed and looked healthy during the day their stomata were sluggish and slow to open in the mornings. Leaves 18 and 19 visually appeared

to be identical but leaf 19 had obviously been injured by freezing for its stomata resisted opening even in the laboratory. Its r and r_c values, as well as its respiration, were large. The leaf was from one of the plants that had been defoliated in August while leaf 18 was from a nearby plant that had not had its leaves removed.

DISCUSSION AND CONCLUSION

My confidence in the values of r is no more than $\pm$ 100 s m^{-1} based on variation of the measurements that made up the CO_2 response curves. The values were obviously too large when they forced r_c to be negative. This is a serious limitation in the analysis of stepwise resistances to CO_2 assimilation. It probably arises from some of the assumptions made in the Jones and Slatyer derivation which are not adequate for all types of stressed leaves grown in the field, i.e., r may sometimes be a function of internal leaf CO_2 levels.

One may ask what effect removing the leaf from the plant has on CO_2 responses. In the case of sugarbeets, removal evidently does not cause much change for several hours provided the petiole is kept submerged in water. In preliminary trials, leaves attached to potted plants were placed in the chamber and allowed to come to steady state under the high light and CO_2 conditions. The petioles were then cut and after a minute or so the CO_2 assimilation returned to its previous steady level and remained near there for about five hours before beginning to gradually decrease. The data reported here were obtained within at least 3.5 hours following leaf removal.

While the experiment described here was of an exploratory nature, the four types of resistances did not generally indicate sharp individual correlations with differently stressed leaves that had obviously developed different inherent abilities to fix CO_2. The inherent differences themselves did seem to transcend the change form field plants to excised leaves fixing CO_2 under controlled laboratory conditions. In general, the data indicated that the resistance to CO_2 transport form the cell wall

into the chloroplast may increase following water stress and some types of chilling. In other cases, the CO_2 fixation rate is limited more by the carboxylation resistance which maybe interrupted as less than optimum amounts of RuBP attached to active carboxylation sites in the chloroplasts. Differences in stomatal resistance, gas phase mesophyll resistance, and dark respiration were generally small.

Unfortunately, it is still not apparent how one might make a simple CO_2 exchange measurement on a few leaves in the field that would signal the onset of plant stress before any visual signs occur. We must better understand and model the kinetics of CO_2 assimilation to reach that important goal. Nevertheless, it is obvious that differences do exist and, because they do, the potential for progress is real.

LITERATURE CITED

(1) Cary, J. W. 1977. Photosynthesis of sugarbeets under N and P stress: Field measurements and carbon balance. Agron. J. 69:739-744.

(2) Cary, J. W., and W. W. Rasmussen. 1979. Response of three irrigated crops to deep tillage of a semi-arid silt loam. Soil Sci. Soc. of Am. J. 43:574-577.

(3) Cary, J. W. 1981. Calculation of CO_2 gas phase diffusion in leaves and its relation to stomatal resistance. Photosynthesis Research 2:185-194.

(4) Heuer, B., and Z. Plaut. 1981. Carbon dioxide fixation and ribilose-1, 5-biphosphate carboxylase activity in intact leaves of sugarbeet plants exposed to salinity and water stress. Ann. Bot. 48:261-268.

(5) Hodanova, D. 1979. Sugarbeet canopy photosynthesis as limited by leaf age and irradiance: Estimation by models. Photosynthetica 13:376-385.

(6) Jones, H. G. 1973. Moderate term water stress and associated changes in some photosynthetic parameters in cotton. New Phytol. 72:1095-1105.

(7) Jones, H. G. and Slatyer, R. O. 1972. Estimation of the transport and carboxylation components of intracellular limitation to leaf photosynthesis. Plant Physiol. 50:283-287.

(8) Loomis, R. S., and E. Ng. 1977. Influences of climate on photosynthetic productivity of sugarbeet. Proc. 4th Int. Congr. on Photosynthesis, p. 259-268. Printed in Great Britian.

(9) Terry, N., and A. Ulrich. 1973. Effects of phosphorus deficiency on the photosynthesis and respiration of leaves of sugarbeet. Plant Physiol. 51:43-47.