

Effects of Light Intensity and Oxygen on Photosynthesis and Translocation in Sugar Beet¹

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JEROME C. SERVAITES² AND DONALD R. GEIGER

Department of Biology, University of Dayton, Dayton, Ohio 45469

The mass transfer rate of ¹⁴C-sucrose translocation from sugar beet (*Beta vulgaris*, L.) leaves was measured over a range of net photosynthesis rates from 0 to 60 milligrams of CO₂ decimeters⁻² hour⁻¹ under varying conditions of light intensity, CO₂ concentration, and O₂ concentration. The resulting rate of translocation of labeled photosynthate into total sink tissue was a linear function (slope = 0.18) of the net photosynthesis rate of the source leaf regardless of light intensity (2000, 3700, or 7200 foot-candles), O₂ concentration (21% or 1% O₂), or CO₂ concentration (900 microliters/liter of CO₂ to compensation concentration). These data support the theory that the mass transfer rate of translocation under conditions of sufficient sink demand is limited by the net photosynthesis rate or more specifically by sucrose synthesis and this limitation is independent of light intensity *per se*. The rate of translocation was not saturated even at net photosynthesis rates four times greater than the rate occurring at 300 microliters/liter of CO₂, 21% O₂, and saturating light intensity.

controlling productivity (reviewed by Neales and Incoll, 16) is of great agronomic importance, because many efforts are being directed at selecting plant varieties with high rates of net photosynthesis. Hofstra and Nelson (13) found a correlation between photosynthesis and translocation within a number of plant species. The C₄ photosynthetic pathway varieties were found to have higher photosynthesis and translocation rates than the C₃ photosynthetic pathway varieties. Causes for the difference, whether a result of an increased rate of translocate production or a more efficient system for vein loading of translocate, were not determined. When grown under low O₂ atmospheres, C₃ plants show an increased rate of dry matter production (2, 3). Dry matter increases should be reflected in an increased mass transfer rate. In this study, the mass transfer rate of ¹⁴C-sucrose translocation from sugar beet leaves was measured over a range of net photosynthesis rates which were varied by adjusting light intensity, CO₂ concentration, and O₂ concentration in an attempt to determine the independent effects of light and photosynthetic carbon assimilation on translocation.

The individual roles of the source, sink, and path of active translocating systems are currently under investigation. According to the Münch pressure-flow theory of translocation, the source maintains the driving power for translocation through build up of a sucrose concentration gradient between source and sink by photosynthesis. Photosynthesis would be expected to limit the rate of translocation by limiting the availability of sucrose. Evidence for this direct control by photosynthesis in regulating the translocation rate is sparse (4, 10, 18, 24). Much discrepancy exists in the literature over the single and combined effects of light intensity and photosynthesis controlling translocation from the source. Some workers have indicated that light directly promotes translocation, independently of the assimilation of CO₂, by the production of ATP through cyclic photophosphorylation. The ATP would act either by increasing the rate of vein loading (11) or by directing more carbon into sucrose rather than storage compounds (21). Others have shown that the light effect may be caused by some product of increased photosynthetic carbon fixation, *e.g.* sucrose (18, 24), ATP from noncyclic photophosphorylation (12, 19), or some unknown intermediate (10).

The question of whether photosynthesis or translocation is

MATERIALS AND METHODS

Plants. Sugar beet plants (*Beta vulgaris* L., Klein E type of monogerm hybrid) were grown in solution culture under a 16-hr photoperiod with a 24 C day temperature and 17 C night temperature. Light intensity at leaf level was about 1200 ft-c during the 30- to 35-day growth period. Leaves of the experimental plants were removed except for a source leaf with a 10-cm long blade (0.5 dm²) and a sink leaf with a 3- to 4-cm long blade.

Measurement of Net Photosynthesis Rate. The experimental system used permitted the simultaneous measurement of net photosynthesis and translocation rates and has been reported earlier (7, 8). The day before the experiment, the source leaf was sealed in the leaf chamber and the system leak-tested. Before the experiment, compressed air containing 400 μl/l of CO₂ was allowed to flow over the leaf. Just before the experiment the leaf was allowed to photosynthesize for at least 2 hr at a light intensity of 7200 ft-c. The experiment was started by allowing labeled CO₂ of known specific radioactivity (40 moles/Ci) to be taken up by the leaf. The CO₂ concentration of the system was regulated between set high and low levels (a 100-μl/l span) on the recorder monitoring the infrared gas analyzer output. As the CO₂ concentration of the system was reduced by photosynthesis to the low level, a solenoid valve opened allowing labeled CO₂ to enter the system and return the CO₂ concentration to the set high level. Net photosynthesis was calculated as the change in μg of C divided by the product of the time required to reduce the CO₂ concentration by 100 μl/l and the leaf area. By this method, numerous photosynthesis rate measurements were made during the course of a single

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² Present address: Department of Agronomy, The University of Illinois, Urbana, Ill. 61801.

experiment and the CO₂ concentration of the system was kept within $\pm 50 \mu\text{l/l}$ of the mean set level.

Measurement of Translocation Rate. The accumulation of ¹⁴C-sucrose was monitored with a GM tube held against the sink leaf. This accumulation rate was normalized to total accumulated in the sink per unit source leaf area and converted to a total carbon basis as described previously (8).

Light Intensity. Light intensity was altered by varying the number of lamps (300-w, tungsten filament) used. Light reaching the leaf was filtered through a 2-cm layer of water and a 3-cm layer of 5% (w/v) CuSO₄ solution. Light intensity was measured by an illumination meter (Weston, model 756), fitted with a small aperture. A light intensity of 600 ft-c at the leaf surface measured 1.52×10^4 ergs sec⁻¹ cm⁻² on a radiometer (YSI, model 65). Leaf temperature was monitored by a thermistor touching the underside of the leaf. The source leaf temperature in the plant chamber was maintained at 30 ± 1 C by a temperature regulator (Haake-Brinkman, model KT-62).

Low Oxygen Atmosphere. A low O₂ atmosphere was produced by circulating N₂ gas through the system while momentarily bypassing the leaf chamber. Labeled CO₂ was added to the system and the system opened to the leaf chamber. This procedure was repeated resulting in a lowered O₂ concentration of the entire system to about 1% as measured with an oxygen electrode (YSI, model 55).

Varying Photosynthesis Rate. In order to vary net photosynthesis over a large range, sugar beet leaves were subjected to a wide variety of experimental conditions. Photosynthesis was measured under three light intensities: 2000, 3700, and 7200 ft-c. Preliminary studies showed that photosynthesis saturated at a light intensity of about 3500 ft-c at 300 $\mu\text{l/l}$ of CO₂. CO₂ concentration was varied from the compensation concentration to 900 $\mu\text{l/l}$. Photosynthesis was measured under these levels of light intensity and CO₂ concentration in both air (21% O₂) and low O₂ (1% O₂). In this study, the average net photosynthesis rate under 21% O₂, 300 $\mu\text{l/l}$ CO₂, and saturating light intensity measured 16.0 ± 1.3 mg of CO₂ dm⁻² hr⁻¹. Under 1% O₂, this rate enhanced about 38% to 21.9 ± 1.9 mg of CO₂ dm⁻² hr⁻¹. Experiments were designed so that by varying CO₂ and O₂ concentrations nearly equal net photosynthesis rates obtained under different light intensities.

RESULTS

The results of a typical experiment are seen in Figure 1. When the source leaf was photosynthesizing at a steady rate, the accumulation of ¹⁴C-sucrose by the sink leaf eventually

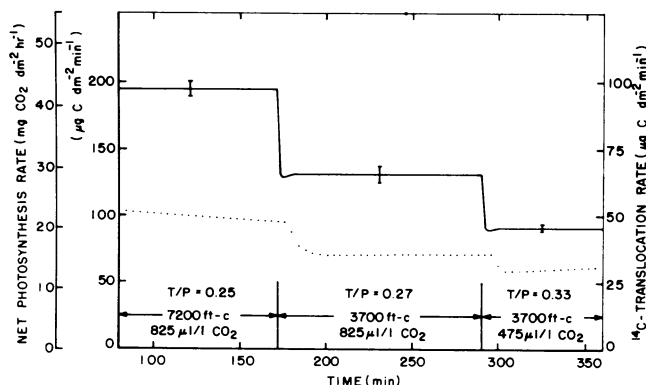


FIG. 1. Time course of net photosynthesis (—) and ¹⁴C-translocation (····) during a single 6-hr experiment. Photosynthesis was controlled by first varying the light intensity and then the CO₂ concentration at the source leaf.

Table I. *Translocation Rate Experiments*

Results are shown of two experiments in which translocation rates were measured while net photosynthesis rates were kept constant by increasing CO₂ concentration (No. 14) or lowering O₂ concentration (No. 8) while light intensity was varied.

Exp.	Measurement	CO ₂ Concn	Light Intensity	O ₂ Concn	Net Photosynthesis	Translocation	T/P Ratio
		$\mu\text{l/l}$	ft-c	%	$\mu\text{g C dm}^{-2} \text{ min}^{-1}$	$\mu\text{g C dm}^{-2} \text{ min}^{-1}$	
8	1	600	7200	21	94.9 ± 1.0	24.9 ± 0.1	0.26
	2	600	3700	1	98.9 ± 1.4	23.8 ± 0.4	0.24
14	1	300	7200	21	73.8 ± 2.1	21.3 ± 2.0	0.29
	2	350	3700	21	70.4 ± 1.6	16.3 ± 0.3	0.23
	3	850	2000	21	67.0 ± 2.3	15.3 ± 1.1	0.23

reached a constant steady rate. Upon lowering the light intensity or CO₂ concentration (or both) the photosynthesis rate quickly declined reaching a new steady rate. Translocation rate remained unchanged for a few minutes, presumably the time required for velocity and path-length adjustment of sucrose reaching the sink leaf, and then the translocation rate dropped exponentially until it again approached a new steady rate. In other experiments an increase in O₂ concentration resulted in similar changes. Usually three different rate measurements of translocation and photosynthesis could be made on one plant during the course of a 6-hr experiment.

The manipulation of the net photosynthesis rates under different light intensities was accomplished easily at lower CO₂ concentrations but become more difficult as the CO₂ concentration was increased, because light intensity became limiting. As seen in Table I, for experiment 14, net photosynthesis rates overlap for the differing light intensities, but the T/P ratios are nearly the same. Since some variation exists among the T/P ratios, a large number of measurements were pooled and analyzed statistically to see if this variation was significant. Combined measurements of individual experiments indicate that translocation and photosynthesis are directly and linearly correlated (Fig. 2). Correlation coefficient of combined points in Figure 2 is 0.91 ($P < 0.01$). Regression analysis of the data points for the three light intensities show the regression lines are not significantly different ($P < 0.05$).

Net photosynthesis in low O₂ was about 40% higher than in air at the same light intensity. Increased photosynthesis in low O₂ was reflected in a proportionate increase in translocation rate. This effect was used to advantage to manipulate the net photosynthesis rate in order to obtain similar rates at different light intensities. As seen in Table I, for experiment 8, when light intensity was reduced from 7200 to 3700 ft-c, but net photosynthesis was kept constant by decreasing the O₂ concentration, the T/P ratios remained unchanged. Linear regression analysis of combined measurements of translocation and net photosynthesis under 1% O₂ (Fig. 3) gave a regression line not significantly different from the regression line of measurements under 21% O₂ ($P < 0.05$).

The regression lines of Figures 2 and 3 are very similar and intercept a translocation rate of about $5.5 \mu\text{g of C dm}^{-2} \text{ min}^{-1}$ at zero net photosynthesis rate. This extrapolation of translocation rate to zero net photosynthesis rate appears valid since in one experiment the CO₂ concentration of the system was allowed to deplete to the CO₂ compensation concentration and after 2 hr, sufficient time for the attainment of a steady level of translocation, a translocation rate of $6.6 \mu\text{g of C dm}^{-2} \text{ min}^{-1}$

³ Abbreviation: T/P: translocation rate/photosynthesis rate.

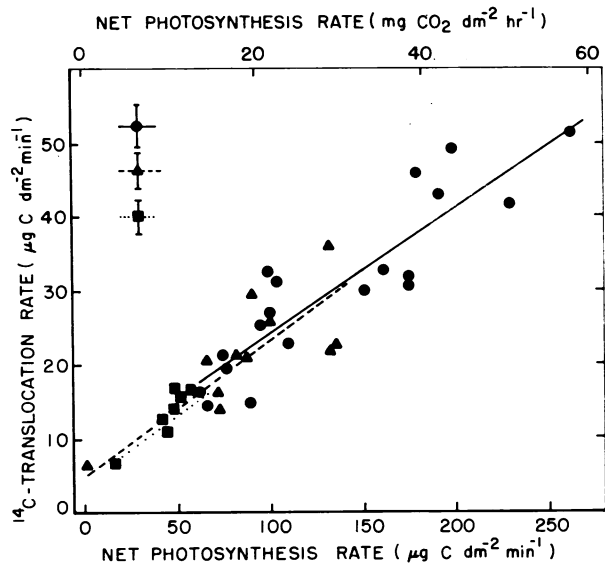


FIG. 2. Plot of individual measurements from 19 separate experiments of translocation and net photosynthesis rates in 21% O_2 and light intensities 2000 ft-c (\cdots , \blacksquare), 3700 ft-c ($---$, \blacktriangle) and 7200 ft-c ($---$, \bullet). Net photosynthesis was controlled by varying CO_2 concentration at the source leaf.

was measured. Geiger and Batey (5) found that upon darkening the source leaf, the translocation rate dropped in about 150 min to a minimum rate of about $6 \mu g$ of C $dm^{-2} min^{-1}$, a rate similar to the minimum rate measured here. After about 90 min, starchlike reserves were converted to sucrose and contributed to the translocate. Such a situation could be occurring here when the photosynthesis rate is reduced to zero by reducing the CO_2 level instead of darkening the source leaf. Habeshaw (10) also measured a basal rate of translocation upon darkening sugar beet source leaves. The basal rate was found to represent a constant demand made on the source leaf by the meristem and root (nonphotosynthetic sink tissue) and was found to increase with age of the plant and size of the root. It seems likely that the high sink demand in this plant, which has all but one source leaf removed, promotes translocation of stored material. The basal rate measured here appears to be a constant rate and additive to the contribution of the source. This import may be a contribution of other sink tissue (beet roots or petiole) to sink leaf accumulation independent of the source leaf contribution. Previous experiments indicate that the extra sink contribution only becomes a significant source of translocate to the sink leaf when either the source leaf is darkened (5) or excised (22).

DISCUSSION

Hartt (11) proposed the theory of phototranslocation, *i.e.* light-driven translocation, based on the finding that basipetal translocation, in detached sugarcane leaves, saturated at very low light intensities (100 ft-c) whereas photosynthesis saturated at higher light intensities (6000 ft-c). Cyclic photophosphorylation was postulated to be the mechanism producing ATP necessary for active phloem loading. Because in this study light intensity *per se* was not found to influence translocation, cyclic photophosphorylation probably does not directly limit translocation at these light intensities. It is possible that cyclic photophosphorylation saturates at light intensities lower than those used here (1).

Decreasing net photosynthesis rate either by decreasing light

intensity or CO_2 concentration (Fig. 1) or by increasing O_2 concentration results in a decrease in translocation rate indicating a direct cause and effect relationship between photosynthesis and translocation. The reduction in translocation rate is proportional to the decrease in net photosynthesis rate. Evidently some product of photosynthetic carbon fixation is limiting translocation. This product has been suggested to be sucrose (18, 24), ATP from noncyclic photophosphorylation (12, 19), or some unknown product such as a Calvin cycle intermediate having a regulatory influence on phloem loading (10). Noncyclic photophosphorylation is probably not limiting translocation because translocation at the same net photosynthetic rate is the same in air (21% O_2) and low (1% O_2) oxygen. Low O_2 does not have a stimulatory effect on NADPH or ATP production (9), but greatly reduces the direct inhibition of CO_2 fixation by O_2 and the subsequent loss of carbon from the leaf by photorespiration (17). Under the condition of high sink demand, present in the trimmed plant, it appears that the amount of carbon fixed into translocate species is limiting the translocation rate. It may be that with very low sink demand, this factor may limit translocation.

These data are consistent with the earlier work of Geiger and Swanson (8), which showed that the source leaf sucrose pool was rapidly turning over; most of the sucrose produced (96%) was translocated with the remainder accumulating in the leaf. They concluded that the entire leaf sucrose pool was in a near steady state of production and translocation. Geiger and Batey (5) have shown that in darkened leaves the rate of translocation and the source leaf sucrose level dropped simultaneously. Recently, Christy and Swanson (4) determined that under steady state conditions, the rate and velocity of translocation were dependent on both the source leaf sucrose concentration and photosynthesis rate.

Although many factors are known to influence the translocation rate, *e.g.* sieve tube area (6), leaf age (23), and size of sink (5), the photosynthesis rate appears to exert considerable control as well. From these data, it is clear that when compar-

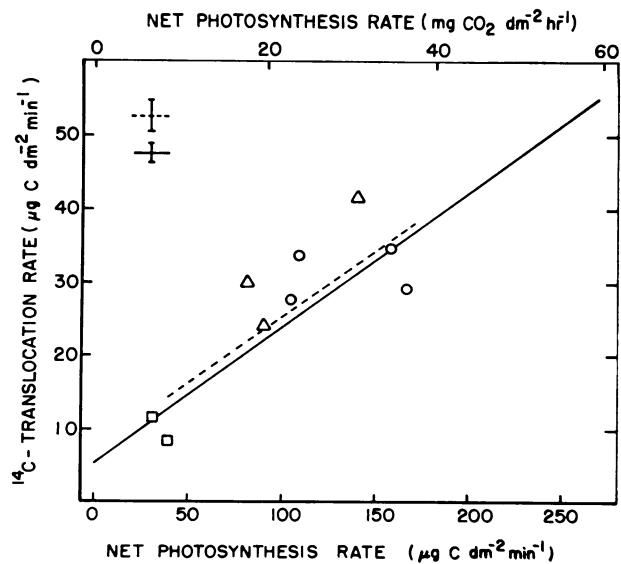


FIG. 3. Plot of individual measurements from eight separate experiments of translocation and net photosynthesis rates in 1% O_2 ($---$) and light intensities of 2000 ft-c (\square), 3700 ft-c (\triangle) and 7200 ft-c (\circ). Net photosynthesis was controlled by varying the CO_2 concentration at the source leaf. The solid line is a regression line of all points in Figure 2, which are from measurements made in 21% O_2 .

ing translocation rates for a single plant or between plants, the photosynthesis variable must be held constant. Liu *et al.* (14) partially attributed a higher photosynthesis rate in a cultivar of *Phaseolus* to a higher translocation rate. The T/P ratios of the two cultivars, calculated from the data given, are nearly identical and do indeed show a correlation between photosynthesis and translocation. Since the photosynthesis variable was not kept constant during the translocation measurement, the cultivar with the higher photosynthesis rate had a proportionally higher translocation rate. It is not clear from their data whether the increased photosynthesis rate was a result of increased translocation or vice versa.

Translocation was not found to reach a saturating level in this study, even at photosynthesis rates four times greater than rates under normal conditions (300 $\mu\text{l/l}$ of CO_2 , 21% O_2 , and saturating light intensity). Apparently the simplified source-sink system used here, in which all source leaves but one were removed, resulted in a sink demand for sucrose greater than the single source leaf could supply. When such conditions occur, it seems unlikely that sucrose will accumulate and result in a feedback inhibition of photosynthesis (16). Such an inhibition occurring in agricultural crops in the field is likewise doubtful since crowded plants have much more sink than source capacity (15). Unfortunately in this study, translocation was only a small percentage of net photosynthesis (20–25%) probably a result of the relatively immature stage of the source leaves (23). Others (10, 23) have found translocation to increase in more mature leaves to 90 to 100% of net photosynthesis rate. In one case (10) translocation was found to saturate at a rate of 0.2 mg of hexose $\text{cm}^{-2} \text{hr}^{-1}$ (136 μg of C $\text{dm}^{-2} \text{min}^{-1}$). Reasons for the increase in T/P ratio with leaf age is not known, but most likely may be caused by an increased partitioning of carbon into sucrose in older leaves. Source leaves used in this study were found to incorporate about one half the label into ethanol-insoluble compounds and only about one-third into sucrose (20). The manipulation of a translocating system to a saturated condition could be of experimental use in studying the problems of translocation kinetics, sink control of translocation, and feedback inhibition of photosynthesis.

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