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Effect of Light and Ontogenetic Stage on Sink Strength in Bean Leaves[1]

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ABSTRACT

Light (about 3,000 foot-candles) neither increased nor decreased the sink strength of young, rapidly expanding leaves of *Phaseolus vulgaris* L. cv. Black Valentine, as measured by the comparative rates of import of ^{14}C-labeled photosynthates by sink leaves in the light *versus* dark in short term experiments. Although irradiated sink leaves accumulated more ^{14}C activity, the difference was fully accounted for by photosynthetic reabsorption of respiratory CO_2 derived from substrates translocated to the sink leaves.

Maximum sink strength was attained when the sink leaf reached 7 to 8 cm^2 in area (9 to 10% of its fully expanded size). Thereafter sink strength declined rapidly and asymptotically to a near zero value at about 45% final area. During this period, however, the rapid decline in translocation was offset by a rapid rise in the photosynthetic rate of the sink leaf, maintaining a near constant relative rate of dry weight increase until the sink leaf had expanded to about 17% of its final area. Although the increasing photosynthetic capacity was associated with a decreasing import capacity, suggesting that the rate of translocation to the sink leaf was controlled by the developing capacity of the sink leaf for photosynthesis, it was not possible to vary the total (true) translocation rate to the sink leaf by varying the photosynthetic rate of the sink leaf in short term light-dark experiments. Despite a high ratio of source to sink in these experiments, no evidence accrued that translocation into young bean leaves was ever sink-limited.

Factors influencing the partitioning of photosynthates in plants are clearly of economic importance. This paper presents data evaluating two parameters of the translocation system: (*a*) sink strength in relation to light; and (*b*) sink strength in relation to ontogenetic stage or size. Young leaves of bean, usually in the size range of 2 to 18 cm^2, were used as models of sink systems.

"Sink strength" is defined arbitrarily in this paper as the product of sink activity (μg of carbon imported min^{-1} cm^{-2}) and sink size (cm^2), and has, therefore, the units of μg of carbon min^{-1}. The terminology relative to these concepts has been discussed by Wareing and Patrick (13).

MATERIALS AND METHODS

Bean plants (*Phaseolus vulgaris* L., cv. Black Valentine) were grown hydroponically in a growth chamber under a 15-hr photoperiod with a day temperature of 24 C and a night temperature of 18 C. Light intensity at plant level varied from about 1,500 to 2,200 ft-c. Plants were usually used when 12 to 13 days old, at which time the primary leaves were 85% or more fully expanded and the first trifoliate leaf, 2.4 to 18.2%.

On the day before an experiment, the test plants were trimmed to a simplified translocation system by removal of all leaves and buds except for one primary leaf (serving as the source leaf) and the central leaflet of the first trifoliate leaf (serving as the sink). To eliminate the roots as a major competitive sink, the stem was heat-girdled 1 cm below the primary leaf node. The ratio of "total sink" (all plant parts above the stem girdle excluding the source leaf) relative to the sink leaf only, varied with the developmental stage of the sink leaf and the subtending internode, ranging from about 3.1 to 5.1 in most of the plants studied (calculated from the corresponding ratio of ^{14}C in the total sink to leaflet sink determined at the termination of the experiment).

The analytical system used in these studies permitted simultaneous measurements of the net photosynthetic rates of the source leaf and the net translocation rates to the sink leaf, and has been previously described (8). Briefly, the CO_2 in the source leaf cuvette was maintained constant with respect to concentration and specific activity, and the net rate of translocation (in μg of carbon min^{-1}) to the sink leaf was calculated from the rate of accumulation of labeled translocate at steady rate corrected for the counting efficiency.

For purposes of measuring the carbon budget of the sink leaf, the sink leaf was similarly sealed in a cuvette but in an open-loop system as shown schematically in Figure 1. The cuvette incorporated a Geiger-Muller detector hermetically sealed in the base of the cuvette directly subtending the sink leaf. Constant counting geometry was maintained by positioning the leaf between supporting grids of fine nylon monofilament threads held 3 mm above the 1.4-mg cm^{-2} end window. To prevent water vapor from transpiration condensing on the end window (a critical problem, especially when the sink leaf was illuminated), the air distribution manifold in the cuvette was designed so that the air stream passed both above and below the leaf.

Because of the small size of the sink leaves, and particularly because of the rapid extension growth of their petioles, leak-proof seals during the time course of an experiment (about 6–8 hr) were difficult to ensure and high compression mastic seals proved rapidly damaging. Consequently, a "push-pull" flow system was adopted as diagrammed in Figure 1. The cuvette proper was then adjusted to ambient pressure, as read from the Brodie manometers, by adjusting flow resistances (micrometering valves) upstream and downstream from the cuvette. Under these conditions, low compression mastic seals proved adequate.

Rates of air flow, about 75 ml min^{-1}, providing a volume turnover time of about 20 sec, were held constant to ± 0.03 ml min^{-1}. A wet-test meter was used to measure the average integrated flow rates prevailing during the treatment periods (sink leaf in dark *versus* sink leaf in light). Data from the rate meter, the flow-type ion chamber electrometer, and the IR gas analyzer were recorded potentiometrically. From these data the following sink leaf rate parameters were calculated in μg of carbon min^{-1}: rate of

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accumulation of translocate (*i.e.* the net or apparent rate of translocation), rate of net photosynthesis, rate of dark respiration, and rate of $^{14}CO_2$ loss in both light and dark. The ^{14}C content of the plant samples was determined by Van Slyke oxidation (12) and ion chamber electrometry. Leaf areas were measured with a Hamamatsu area meter.

RESULTS AND DISCUSSION

Sink Strength in Relation to Light. In this series of experiments, illumination (about 3,000 ft-c) or darkening of the sink leaf constituted the only major variable, the illuminance of the source leaf and other parts of the plant being held constant at about 3,000 ft-c during the time course of each experiment (about 6–8 hr). Each plant served as its own control, the rate of translocation being first measured to the illuminated sink leaf and then to the darkened sink leaf. In each case transition to a new steady rate was rapid (<15 min).

Table I presents data showing the magnitude of the sink irradiation effect. It is evident that darkening the sink leaf decreased significantly (about 32%) the apparent rate of translocation from source leaf to sink leaf. The primary question is how much of this light effect is simply due to the "conserving" effect of light resulting from photosynthetic refixation of carbon respired by the sink leaf. Respiratory losses of recently imported carbon may be as high as 40 to 50% of total accumulation (7). Photosynthetic recycling of this respired carbon could thus readily account for the apparent promotive effect of light on sink leaf translocation. This argument was tested by measuring the carbon balance of the sink leaves in both light and dark. The data from this series of experiments are given in Table II. Allowing for the mechanical difficulties described above in carrying out these measurements, the conclusion is evident that the total (or true) translocation rate from source leaf to sink leaf (Table II, parameter 4) was independent of light. The average total rate to the illuminated sink leaf was 6.2 μg of carbon min^{-1}, and to the darkened sink leaf, 6.5 μg of carbon min^{-1}.

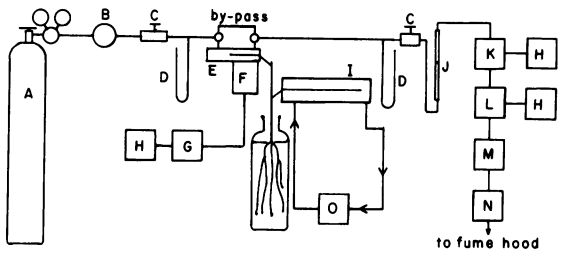

FIG. 1. Schematic of analytical system showing the open-loop and closed-loop systems used for the sink and source leaves, respectively. A: compressed air cylinder; B: low pressure line regulator, Matheson, model 71; C: micrometering valve; D: manometer, Brodie fluid; E: sink leaf cuvette; F: end window Geiger counter; G: rate meter; H: recording potentiometer; I: source leaf cuvette; J: flow meter; K: ion chamber electrometer; L: IR gas analyzer; M: wet-test gas meter; N: pump with variable capacity control; 0: system for maintaining labeled CO_2 at constant concentration and specific radioactivity in the source leaf cuvette loop system. For details of this system, see Swanson *et al.* (8).

Table I. Comparative Net Translocation Rates to Sink Leaf in Light (Period 1, ~ 3000 ft-c, duration 2-3 hr) and Dark (Period 2, duration 2-3 hr).

Source leaf continuously illuminated at ~ 3000 ft-c during both periods. Average of 17 experiments. A part of these data taken from earlier experiments (8).

Treatment	Net Translocation Rate to Sink Leaf	Net Photosynthetic Rate of Source Leaf
Sink leaf in light (Period 1)	100[a]	100[b]
Sink leaf in dark (Period 2)	68 ± 8.8	100.3 ± 5.1

[a]Magnitude of net rate in each experiment equated to 100. Absolute net rate = 4.86 ± 1.86 μg carbon translocated to sink leaf min^{-1} from source leaf.
[b]Magnitude of net rate in each experiment equated to 100. Absolute net rate = 46.7 ± 10.6 μg carbon fixed min^{-1} by source leaf.

The most difficult parameter to assess in this budget is the loss of carbon which escapes refixation after having been transported to the sink leaf and metabolized to CO_2 (Table II, parameter 3). The values given for this parameter have been calculated from the measured efflux of $^{14}CO_2$ from the illuminated sink leaves and corrected to total CO_2 efflux on the assumption that the specific activity of the CO_2 produced in the light is the same as that in the dark. Actually the specific radioactivity of the respiratory substrates may be expected to vary considerably depending on the relative inputs of precursor carbon from translocated carbohydrates (labeled), from photosynthetically refixed CO_2 respired from these translocated substrates (labeled), and from photosynthetically fixed CO_2 derived from the ambient cuvette atmosphere (unlabeled). In addition, the relative inputs from these respective sources will vary depending on the leaf ontogeny. Thus *e.g.* in the very young sink leaves (experiments 1, 2, and 5, Table II, parameter 9), *vis-à-vis* the older sink leaves (experiments 3 and 4), the specific radioactivities of the dark-respired CO_2 averaged respectively 2.61×10^{-5} and 1.53×10^{-5} μg of $^{14}C/\mu$g of ^{12}C. As may be seen from Table II (parameter 5), the net photosynthetic rates in the older sink leaves were considerably higher than in the younger sink leaves. We attribute these ontogenetically related differences in specific radioactivities to the larger input of unlabeled carbon photosynthesized by the older sink leaves during the previous light period.

Fortunately, the actual quantity of carbon escaping recycling in saturating light intensities is small and even a 50% difference in the respective specific radioactivities would not alter the major conclusion that the total (true) rate of translocation to young bean leaves is essentially light-independent at the sink level in short term experiments. Partial effects of sink irradiation attributable to increased transpiration of the sink leaf or to an increase in counting efficiency (8) are evidently very minor at most.

Total substrate availability to the sink leaf was greater in the light than in the dark because of photosynthetic reabsorption and

Table II. Comparative Carbon Budget of Sink Leaves in Light (L, ca 3000 ft-c) and Dark (D).
Source leaves continuously illuminated at ca 3000 ft-c. Rate data calculated as μg carbon/min.

Sink Leaf Parameter Measured	Expt 1		Expt 2		Expt 3		Expt 4		Expt 5	
	L	D	L	D	L	D	L	D	L	D
	μg carbon min^{-1}									
1. Net import rate	3.86	3.28	7.06	4.94	8.56	6.74	4.37	2.95	4.86	3.70
2. Dark respiration rate		1.76		2.23		3.16		2.14		2.43
3. Carbon lost as CO_2 in light[a]	0.24		0.36		0.68		0.86		0.75	
4. Total (true) import rate	4.10	5.04	7.42	7.17	9.24	9.90	5.23	5.09	5.61	6.13
5. Net photosynthesis rate	0.27	0	0.40	0	2.76	0	3.00	0	0.38	0
6. Total carbon gain	4.37	5.04	7.81	7.17	12.00	9.90	8.23	5.09	5.99	6.13
7. Area (cm^2)	4.56		4.29		11.3		10.8		5.90	
8. Area as % final area	5.5		5.2		13.8		13.1		7.2	
9. Spec. Act. of CO_2 produced in dark ($\mu g^{14}C/\mu g^{12}C$)	2.58×10^{-5}		2.67×10^{-5}		1.48×10^{-5}		1.58×10^{-5}		2.51×10^{-5}	

[a]Calculated on the assumption that the specific activity (in $\mu g^{14}C/\mu g^{12}C$) of the CO_2 respired by the sink leaf in the light was the same as that respired in the dark. See text for further discussion.

the net uptake of CO_2 (Table II, parameter 6). Since young leaves at the stage used in these studies are not capable of exporting photosynthates (2, 3, 9, 11), it follows that leaf growth should be faster in the light than in the dark, assuming reasonable constancy in the specific leaf area ($cm^2\ mg^{-1}$). However, for a sink leaf at the 6-cm^2 stage the expected increase in area during a 2-hr interval (the usual treatment period), given a translocation rate of 6.0 μg of carbon/min, a weight conversion efficiency of translocated carbohydrate to leaf dry matter of 0.73 (6), and a specific leaf area of 0.27 cm^2/mg, would be about 0.35 cm^2/2 hr in the light and 0.21 cm^2/2 hr in the dark, a rate difference of only 0.14 cm^2/2 hr. In preliminary experiments, using photographic methods, we have been unable to resolve with certainty differences of this magnitude, though such data as have been compiled do suggest a higher rate in the light, as inferred.

It should be emphasized that the present data apply only to short term experiments (2–3 hr/treatment period) on sink leaves at or near their maximum sink strength. Thrower (10) has reported that prolonged exposure of a young soybean leaf either to dark or to light without CO_2 greatly shortened the life expectancy of the leaf. The data of Habeshaw (5), however, showing significant inhibition of translocation from a source leaf when the whole plant was illuminated, are not readily explained in terms of the present results.

Bünning (1) observed no significant differences in the expansion rates of leaves of several species growing under controlled conditions of 12 hr light-12 hr dark (except for soybean, which expanded faster in the dark than in the light). In the absence of photosynthesis, these results require either a compensating increase in the specific leaf area of the expanding leaves during the dark, or a compensating increase in the rate of mobilization of translocate from various storage areas in the plant (*i.e.* an actual increase in sink strength during the dark). Bünning's data were taken at the time of maximum expansion rates of the leaves; in the present experiments, using plants trimmed to a two-leaf format, stems girdled, the sink activity of the expanding leaves had decayed to a negligible value (<0.00006 $\mu g\ \mu g^{-1}\ min^{-1}$) by the time of their maximum expansion rates.

In summary, the sink strength of young bean leaves, expressed in units of μg of total carbon imported min^{-1}, appears to be independent of light in short term experiments.

Sink Strength in Relation to Ontogenetic Stage. As shown in Figure 2, the sink strength (μg of carbon translocated to the sink leaf min^{-1}) was small during the early developmental stages, rose rapidly to a peak value when the sink leaf was about 7 to 8 cm^2 in area (9–10% of its final expanded area) and then declined rapidly and asymptotically with further increase in size. A similar pattern has been observed for sugar beet, with maximum sink strength occurring at 9 and 7.5% of final expanded areas for leaves respectively attaining final areas of 65 and 225 cm^2 (4; Geiger, Giaquinta, personal communications) and for squash, with the maximum usually occurring at about 10% final area (final area, 130 cm^2; Turgeon, personal communication). In soybeans, however, maximum sink strength was not attained until the leaves had expanded to roughly 25% of their final area (9).

During the early (essentially heterotrophic) stage of leaf development the relative dry weight increase of the sink leaf (dW/dt) 1/W, may be expected to parallel, at least in part, the relative sucrose input rate via translocation. In Table III, data are presented comparing the relative growth rates of the sink leaf and the relative sucrose input rates. The sucrose input rate has been calculated from the carbon input rate read from the smoothed curve in Figure 2, and is taken as equivalent to the dry weight increase contributed by the translocate. The growth rate data (increase in dry weight min^{-1}) have been calcuated from a growth curve (n = 4) plotting area increase of the central leaflet of the first trifoliate leaflet against time. The corresponding dry weight was calculated from a regression equation of dry weight on area

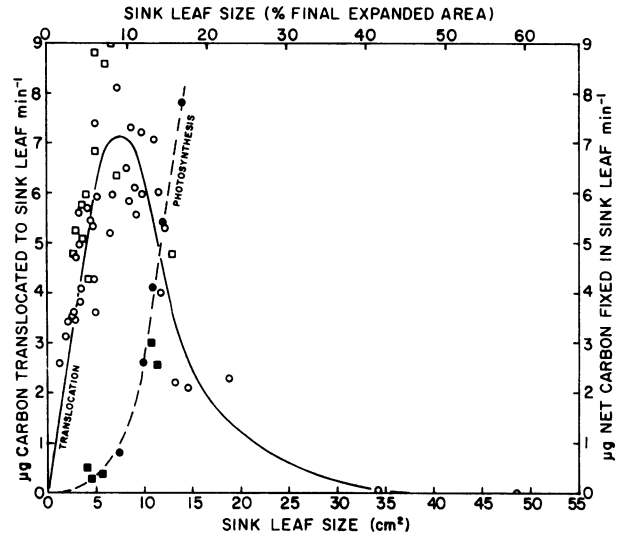

FIG. 2. Translocation (import) and net photosynthetic rates of sink leaf in relation to its developmental stage (area). Translocation curve based on girdled plants trimmed to a one source leaf-one sink leaf format, with sink leaves illuminated (○) or darkened and corrected to the expected value in light (□). Photosynthesis curve (●) represents calculated net photosynthetic rate of sink leaf, in excess of translocation input, required to maintain a constant relative dry weight increase of 0.045% min^{-1} observed during the interval from the 8-cm^2 to the 14-cm^2 stage (Table III, column 5). For this calculation, translocation input rates were corrected for a productive value of 0.73 (6). (■) Represents experimentally determined net photosynthetic rates (Table II, parameter 5).

Table III. Relative Growth Rate of Sink Leaves vs Relative Sucrose Import Rate in Relation to Sink Size

Time[a]	Sink Leaf Area	Sink Leaf Dry Wt	Final Area	Relative Growth Rate[b]	Relative Sucrose Import Rate[c]
hr	cm^2	µgx10^{-4}	"	" min^{-1}	" min^{-1}
30	1.0	0.48	1.21	0.054	0.060
51	2.0	0.90	2.43	0.051	0.064
70.5	4.0	1.69	4.85	0.051	0.066
82	6.0	2.44	7.28	0.048	0.066
90	8.0	3.27	9.71	0.044	0.053
98	10.0	3.90	12.1	0.044	0.039
104	12.0	4.60	14.6	0.043	0.023
110	14.0	5.28	17.0	0.039	0.013
120	18.0	6.65	21.8	0.033	0.006

[a]Time 0 = 10th day after germination.
[b]Percentage by which the dry weight of sink leaf increased per minute (µg dry wt increase · µg dry wt^{-1} · min^{-1} ·100). Average of 4 plants, non-girdled and non-pruned, growing under a 15 hr light-9 hr dark regime.
[c]Computed from the translocation curve in Fig. 2.

(n = 19; r = 0.955). Both the dry weight increase due to sucrose input and dry weight increase associated with growth over a time interval of 1 min were then calculated relative to W, the dry weight of the sink leaf at the time t_1, and the results expressed as % min^{-1}.

The growth rate data were taken from nongirdled, nonpruned plants growing under a regular light-dark regime, and the sucrose input rate data, from girdled plants trimmed to a single source leaf, single sink leaf system in short term experiments. Numerical agreement, therefore, cannot be expected but the relative rates should parallel each other during the period that the leaf is heterotrophically dependent. As may be seen from Table III, this was indeed the case up to the 6-cm^2 stage, and nearly so to the 8-cm^2 stage (maximum sink strength). Following this stage, the time course of these curves diverged sharply, the relative sucrose import rate declining rapidly to near zero, and the relative growth rate of the sink leaf remaining essentially constant until the leaf had expanded to about the 14-cm^2 stage, *i.e.* to 17% of its final area, or 28 hr beyond the 6-cm^2 stage (Table III). A somewhat similar time course for the relative growth rate of *Trifolium subterraneum* L.,

beginning with the exponential growth phase, has been presented by Williams and Bouma (14).

At maximum sink strength, translocation contributed about 7.1 μg of carbon min^{-1} and net photosynthesis about 0.8 μg of carbon min^{-1}, or about 10% of the total (Fig. 2). During the ensuing period, the rapid decline in translocation rate appears to have been matched by a compensating increase in the net photosynthetic rate of the sink leaf, sufficient to maintain the relative rate of increase in the sink leaf constant at approximately 0.045% min^{-1} for an additional 20-hr period beyond the period of maximum import rate (Table III, column 5). Considering the rate of increase in dry weight of the sink leaf to be the sum of its net translocation input rate and net photosynthetic rate, a curve was constructed showing the calculated rate of net photosynthesis in the sink leaf required to maintain the relative rate of dry weight constant at the value noted above, and is given in Figure 2 (●). For this calculation, the translocation rates presented in Figure 2, which approximate to a total or true rate, were corrected for a productive value of 0.73, the ratio of unit weight of dry matter production per unit weight of translocate (6). Experimentally determined values of net photosynthetic rates (from Table II, parameter 5) are also entered in Figure 2 indicated by (■) and are in good agreement with the calculated net photosynthesis data.

These results suggest that the rate of translocation to the sink leaf is sensitively controlled by the developing capacity of the sink leaf for photosynthesis. As indicated above in the sink leaf irradiance studies, however, it was not possible to regulate the rate of translocation to the sink leaf by varying the photosynthetic rate of the sink through short term exposures to light or dark.

Under the conditions of these experiments, substrate input into the sink leaf by photosynthesis (sink leaf photosynthates) did not inhibit the total (true) rate of translocation to the sink leaf. Thus, even in plants in which the ratio of source to sink was considerably accentuated above normal by eliminating competing or alternative sinks, no evidence accrued that translocation was ever sink-limited.

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