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# Effect of Altered Sink:Source Ratio on Photosynthetic Metabolism of Source Leaves[1]

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ZVI PLAUT*, MARIA LUISA MAYORAL[2], AND LEONORA REINHOLD
*Institute of Soils and Water Agricultural Research Organization P.O. Box 6, Bet Dagan 50-250, Israel* (Z.P., M.L.M.); *and Department of Botany, The Hebrew University of Jerusalem, Jerusalem 91904, Israel* (L.R.)

## ABSTRACT

**When seven crop species were grown under identical environmental conditions, decreased sink:source ratio led to a decreased photosynthetic rate within 1 to 3 days in *Cucumis sativus* L., *Gossypium hirsutum* L., and *Raphanus sativus* L., but not in *Capsicum annuum* L., *Solanum melongena* L., *Phaseolus vulgaris* L., or *Ricinus communis* L. The decrease was not associated with stomatal closure. In cotton and cucumber, sink removal led to an increase in starch and sugar content, in glucose 6-phosphate and fructose 6-phosphate pools, and in the proportion of $^{14}C$ detected in sugar phosphates and UDPglucose following $^{14}CO_2$ supply. When mannose was supplied to leaf discs to sequester cytoplasmic inorganic phosphate, promotion of starch synthesis, and inhibition of $CO_2$ fixation, were observed in control discs, but not in discs from treated plants. Phosphate buffer reduced starch synthesis in the latter, but not the former discs. The findings suggest that sink removal led to a decreased ratio inorganic phosphate:phosphorylated compounds. In beans $^{14}C$ in sugar phosphates increased following sink removal, but without sucrose accumulation, suggesting tighter feedback control of sugar level. Starch accumulated to higher levels than in the other plants, but $CO_2$ fixation rate was constant for several days.**

Altered sink demand has been observed to lead to a change in the photosynthetic rate of the source leaf in a number of species (6). The mechanism by means of which the requirements of the sink are transmitted to the source is still far from clear (10). Moreover, in some investigations no such sink/source interaction has been detectable. Some of the contrasting results in the literature may have resulted from differences between growing conditions and experimental procedure in different laboratories; others, to differences in response between different species. We have been conducting an investigation into the effect of procedures which alter translocation rates on the metabolic activities of source leaves in several plant species grown under the same controlled environmental conditions. We recently reported (17) on two effects observable in cucumber following steam-girdling or sink removal. A short-term effect was maximal after 3 h with subsequent recovery, and involved stomatal closure probably induced by temporary increases in leaf water stress and ABA level, with no detectable change in the pattern of $^{14}C$ incorporation into metabolites. A long-term effect was detected after 3 d and involved changes in the relative amount of $^{14}C$ incorporated into various photosynthetic products and intermediary compounds, but was not associated with stomatal responses. Herein we report on the long-term effects of sink metabolism in a number of other species and discuss the findings in relation to the possible nature of the signal transmitting sink demand to source activity.

## MATERIALS AND METHODS

Cotton (*Gossypium hirsutum* L. cv Acala Sj-2), bean (*Phaseolus vulgaris* L. cv Bulgarian), cucumber (*Cucumis sativus* L. cv Dalila), radish (*Raphanus sativus* L. cv Munchner Bierrettich), bell pepper (*Capsicum annuum* L. cv California Wonder), eggplant (*Solanum melongena* L. cv Black Beauty), and castor bean (*Ricinus communis* L.) were grown in 2-L cylindrical plastic containers filled with vermiculite. Plants were thinned to one plant per pot shortly after emergence and irrigated twice a week with deionized water and once a week with half-strength Hoagland solution. Excess water and solution were drained through the bottom of the container. All species were grown in a chamber maintained at 25°C, at a quantum flux density of 450 $\mu$mol $m^{-2}$ $s^{-1}$ (400–700 nm) and a 13 h/11 h light/dark photoperiod.

Experiments with cotton were conducted about 10 d after the appearance of the first flower (11–12 weeks after emergence). Bean plants were used at the stage of the fully expanded first trifoliate leaf (22–25 d after emergence).

One experiment with cotton (Fig. 1) was conducted on field grown plants, Cotton was planted on May 25 in rows 1 m apart and thinned to 10 plants/$m^2$ after emergence. Following light sprinkling to ensure emergence, a trickle irrigation system was installed. Laterals were located next to plant rows and emitters were 40 cm apart. The plants were irrigated once a day and the quantity of water supplied was based on evaporation from a Class A pan. Nutrients containing urea, $H_3PO_4$ and KCl at N:P:K ratio of 4:1:2 were predissolved in the irrigation water at a concentration of 80 g × $m^{-3}$. Plants were used for this experiment on August 12, when there were about 10 bolls or flowers per plant.

The sink removed in the case of the growth-chamber grown cotton was the developing first boll, and measurements were conducted on the mature leaf adjacent to this boll peduncle. In the field-grown cotton, various numbers of bolls or flowers were removed, and measurements were conducted on four leaves of different ages along the plant axis. The extracts of these were combined. In the case of bean plants, the sink removed included the young developing second trifoliate leaf and developing buds. The first trifoliate leaf was considered as the source leaf.

$CO_2$ fixation rate was measured in 1.5 $cm^2$ circular areas of attached leaves according to a procedure described earlier (12).

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[2] Present address: Centro de Ecologia, IVIC, Apartado 1827, Caracas, 1010H, Venezuela.

These areas were enclosed in a microchamber and flushed with an air stream containing 300 $\mu l\ L^{-1}$ $^{14}CO_2$ + $^{12}CO_2$ for 60 s. Ethanol soluble substances and $HClO_4$-soluble substances were determined as in Mayoral *et al.* (17). The former comprised mainly sugars and amino acids, the latter mainly starch. Distribution of $^{14}C$ among intermediates and end products of photosynthesis were determined by the calvin-Bassham method as described earlier (17). Sugars were analyzed in the combined ethanol and $H_2O$ extracts. Glucose was estimated using glucose oxidase and O-dianizidine dehydrochloride.

Sugar phosphates were determined on 3 g fresh leaf material sampled at midday and immediately homogenized in 12 ml ice-cold medium containing 50 mM MeS-NaOH (pH 6.0), 0.5 g PVPP, 150 mg BSA, and 10 mM DTT. Following centrifugation at 15,000*g* the supernatant was deproteinized with $HClO_4$ (final concentration 15%) and neutralized with KOH. The extract was then shaken for 2 min with activated carbon.

We have compared this method on our material with that of Seeman and Sharkey (25) and Lilley *et al.* (15). The leaf was frozen in liquid $N_2$ while still attached to the plant, then ground in liquid $N_2$ and extracted with $HClO_4$ while still in the frozen state. After subsequent analysis, the sugar phosphates were expressed on the basis of leaf area. In a series of tests the values obtained for the two methods were very close, those obtained by the second method being consistently very slightly lower.

After centrifugation the supernatant was used for determination of the two hexose phosphates. The assay was conducted sequentially with glucose 6-P dehydrogenase and P-glucose isomerase (13).

Sucrose was estimated as reducing sugar following KOH hydrolysis according to Handel (8). We have calibrated this method for our material against the standard glucose oxidase method, and have found a linear relationship between the values obtained by the two techniques. The equation expressing this proportionality is $y = 0.036 + 1.6x$ (regression coefficient 0.86), where $y$ is the sucrose concentration as assayed by the anthrone method (8) and $x$ is that assayed by glucose oxidase. Starch was analyzed as outlined earlier (17).

Sugar feeding experiments were conducted on excised 2.0 $cm^2$ leaf discs floated on sucrose or mannitol solutions for 3 h in the dark, following by 30 min in the light. Phosphate feeding experiments were carried out on discs floated on various solutions (see "Results") for 16 h in the dark followed by 2 h in the light. $CO_2$ fixation by the discs was determined in a manner similar to that used for intact leaves after blotting on filter paper.

## RESULTS

A survey of seven crop species showed that even when all the plants were grown under identical environmental conditions and subjected to similar treatments, their response to sink manipulation varied. The plants could be assigned to two groups (Table I). In cucumber, cotton, and radish, decreasing sink demand brought about a highly significant decrease in the rate of photosynthesis in the source leaf. No such decrease could be detected in pepper, eggplant, bean, or castor bean. On the other hand, a significant increase in starch content was observed not only in the case of members of the first group, but also in bean, a member of the second. One member of each group (cotton and bean, respectively) was studied in greater detail as reported below.

Table II shows that, in the case of cotton, a 33% decrease in the $CO_2$ fixation rate of the source leaf was already observable 1 d after removal of sinks. In addition, a 3.3-fold increase in the starch content of the source leaf was also visible within 1 d. Starch accumulation continued to increase, and $CO_2$ fixation rate to decrease, over the subsequent days of the experiment and the value for starch content after 8 d was over five times that of the control.

Table I. *Effect of Altering the Sink/Source Ratio on Photosynthesis and Starch Content of Source Leaves in Various Plant Species*

Sinks (young leaves, fruits, flowers, or buds) were removed and measurements were made several days after treatment as indicated. Results are expressed as the mean of 10 replicates ± SE.

| Species | Sink/Source | Time after Treatment | $CO_2$ Fixation | Starch |
|---|---|---|---|---|
| | *ratio* | *d* | *mg·dm$^{-2}$ h$^{-1}$* | *% dry wt.* |
| A. Species in which photosynthesis was decreased by sink removal | | | | |
| Cucumber | Control | | 15 ± 1 | 11 ± 1 |
| | Decreased | 6 | 5 ± 1 | 20 ± 1 |
| Cotton | Control | | 28 ± 1 | ND[a] |
| | Decreased | 5 | 10 ± 1 | ND |
| Radish | Control | | 14 ± 1 | 4 ± 1 |
| | Decreased | 9 | 7 ± 1 | 8 ± 1 |
| B. Species in which photosynthesis was not affected by sink removal | | | | |
| Pepper | Control | | 30 ± 3 | ND |
| | Decreased | 6 | 30 ± 3 | ND |
| Eggplant | Control | | 22 ± 3 | ND |
| | Decreased | 6 | 21 ± 3 | ND |
| Bean | Control | | 14 ± 0 | 10 ± 1 |
| | Decreased | 4 | 14 ± 4 | 40 ± 1 |
| Castor bean | Control | | 36 ± 4 | ND |
| | Decreased | 4 | 30 ± 4 | ND |

[a] Not determined.

Table II. *Effect of Sink Removal on the Photosynthetic Rate, Stomatal Resistance, and Starch Content of Source Leaves of Cotton Plants ± SE*

| Sink/Source | Time after Sink/Source Manipulation | $CO_2$ Fixation Rate | Stomatal Conductance | Starch |
|---|---|---|---|---|
| *ratio* | *d* | *mg·dm$^{-2}$·h$^{-1}$* | *cm·s$^{-1}$* | *% of dry wt* |
| Not changed | | 25.5 ± 0.5 | 0.26 ± 0.02 | 4.0 ± 0.9 |
| Decreased | 1 | 16.9 ± 0.4 | 0.26 ± 0.02 | 12.6 ± 1.0 |
| Decreased | 3 | 6.4 ± 0.8 | 0.28 ± 0.03 | 15.0 ± 1.0 |
| Decreased | 6 | 6.4 ± 0.8 | 0.27 ± 0.02 | 18.0 ± 1.2 |
| Decreased | 8 | 3.5 ± 0.6 | 0.19 ± 0.02 | 22.0 ± 1.0 |
| Increased | 3 | 26.5 ± 1.0 | 0.34 ± 0.05 | 3.5 ± 0.5 |

The decrease in $CO_2$ fixation rate could not be attributed to stomatal closure, as in the case of the short-term effect on photosynthesis outlined earlier (17), since no effect on stomatal resistance could be detected until the 8th d (Table II). Increasing the sink:source ratio produced no significant effects on the source leaf parameters measured, though there was a slight tendency toward a higher $CO_2$ fixation rate and a lowered starch content.

Figure 1 shows that the rate of photosynthesis in the source leaf (as measured 4 d after treatment) was an inverse function of the number of bolls in an active state of development which had been removed. While glucose content did not change, the accumulation of both starch and sucrose was also a function of the percentage of bolls removed (Fig. 1B). Stomatal resistance, by contrast, was unaffected by removal of up to 60% of the bolls, and showed only a slight rise if more than that number were removed.

Not only was the starch pool larger following sink removal (Fig. 1B), but an effect of sink removal on the distribution of $^{14}C$ between starch and ethanol-soluble substances was visible within 5 min of supply of $^{14}CO_2$ to the source leaf. In one experiment 25 ± 3% of the total cpm was found in the ethanol-insoluble fraction in the control, as compared with 40 ± 2% in the case of plants with bolls removed.

A more detailed investigation into the distribution of $^{14}C$ among various metabolic end-products and photosynthetic intermediates after 1 min of $^{14}CO_2$ supply is summarized in Table

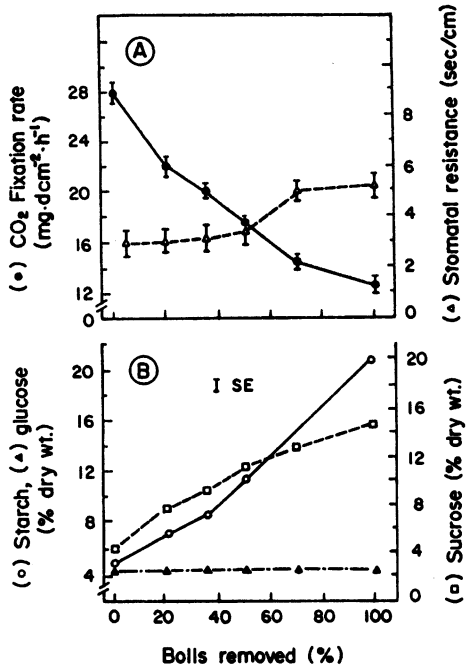


FIG. 1. A, Effect of boll removal from cotton plants on photosynthesis and stomatal resistance of source leaves; B, effect of boll removal on sucrose, glucose and starch contents of source leaves. Cotton plants were grown in the field with trickle irrigation. The experiment was conducted on 80-d-old plants with at least 10 bolls and flowers per plant. Measurements were made on four source leaves along the plant axis 3 d after removal of various numbers of bolls or flowers.

IIIA. Qualitatively, the effect of sink removal in young and old leaves was very similar. Moreover, it closely resembled the pattern previously reported for cucumber (17). In all three cases sink removal brought about a decrease in the amounts of $^{14}C$ incorporated into PGA, P-enolpyruvate and sucrose. On the other hand, in all three cases the treatment increased $^{14}C$ incorporation into glucose 6-P plus sedoheptulose 7-P, fructose 6-P, UDP glucose, alanine, aspartate (and in the case of young leaves, also into maltose).

The $^{14}C$ incorporation pattern suggested the possibility that sugar phosphates might accumulate in leaves following sink removal. This possibility was checked by direct analyses of the pool sizes for glucose 6-P and fructose 6-P. Table IV shows that a statistically significant increase in the size of these pools was already manifest 3 d after boll removal.

The increase in the amount of phosphate bound in the form of organic phosphates indicated in Table IV suggested that there might have been a corresponding fall in cytoplasmic free phosphate content, with consequences for sucrose synthesis and starch formation. Determination of cytoplasmic $P_i$, as distinct from that in other cellular compartments, is beyond the scope of techniques commonly in use, though an approach using NMR is now being pioneered (23). We have, however, investigated the effect of modulating internal $P_i$ concentration in cucumber as well as in cotton. Discs removed from control or girdled leaves were floated on 50 mM phosphate buffer, 50 mM tris-maleate buffer (as a control), or on 10 mM mannose, known to sequester cytoplasmic $P_i$ as mannose phosphate (9, 11). Table V shows the interesting findings that the effect of mannose treatment was substantial in the case of control plants, but very slight in that of girdled plants. Both in cotton and in cucumber mannose supply raised the proportion of $^{14}C$ which was incorporated into starch in the control discs at the expense of that detected in ethanol-soluble metabolites. It also depressed the rate of $CO_2$ fixation. In

Table III. *Effect of Sink Removal on $^{14}C$ Distribution among Photosynthetic Products in Source Leaves of Cotton and Bean*

Experiments were performed 3 d after sink removal in the case of field-grown cotton and after 3 or 6 d in that of bean. Circular areas (1.5 $cm^2$) on attached leaves were labeled for 1 m with $^{14}CO_2$. $^{14}C$ detected is expressed as percent of total $^{14}C$ recovered.

| | Young Leaves | | Mature Leaves | |
|---|---|---|---|---|
| | Control | Sink removed | Control | Sink removed |
| A. Cotton | | | | |
| PGA | 8.4 | 6.0 | 9.7 | 2.2 |
| Fructose 6-P | 1.0 | 2.5 | 1.4 | 3.0 |
| Glucose 6-P + sedoheptulose 7-P | 1.3 | 3.0 | 1.9 | 2.8 |
| P-enolpyruvate | 11.5 | 4.7 | 16.7 | 10.0 |
| Fructose | 5.9 | 6.1 | 5.0 | 4.4 |
| Maltose | 1.4 | 2.3 | 1.0 | 1.1 |
| Sucrose | 20.0 | 11.3 | 22.0 | 7.3 |
| Serine + glucose + glycine | 10.3 | 9.5 | 7.5 | 6.0 |
| Alanine | 23.6 | 32.0 | 10.6 | 26.5 |
| UDP-Glucose | 1.3 | 3.0 | 0.5 | 3.3 |
| Glycerate | 9.3 | 11.2 | 16.5 | 15.8 |
| Malate | 1.7 | 1.5 | 2.7 | 0.7 |
| Citrate | 0.2 | 0.6 | 0.2 | 0.1 |
| Unknown | 4.1 | 6.3 | 4.3 | 6.8 |

| | Control | Days after Sink Removal | |
|---|---|---|---|
| | | 3 | 6 |
| B. Beans | | | |
| PGA | 12.6 | 12.8 | 5.4 |
| Fructose 6-P | 1.7 | 1.5 | 3.8 |
| Glucose 6-P + sedoheptulose + 7-P | 2.3 | 2.4 | 14.5 |
| P-enolpyruvate | 2.6 | 2.8 | 1.0 |
| Fructose | 7.5 | 7.4 | 9.0 |
| Maltose | 3.0 | 3.1 | 8.5 |
| Sucrose | 43.3 | 42.9 | 16.7 |
| Serine + glucose + glycine | 1.9 | 1.8 | 4.4 |
| Alanine | 10.7 | 10.8 | 18.1 |
| UDP-Glucose | 1.0 | 1.0 | 1.5 |
| Glycerate | 9.6 | 9.7 | 12.0 |
| Malate | 1.8 | 1.7 | 0.8 |
| Unknown | 2.0 | 2.1 | 4.3 |

Table IV. *Effect of Sink Removal on Glucose 6-P and Fructose 6-P Concentrations in Source Leaves of Cotton Plants*

Cotton plants were grown in the field with trickle irrigation. About 65% of the bolls and flowers were removed from 95-d-old plants. Leaves at the internodes where sinks had been removed were sampled during the subsequent 9 d, frozen in the field in dry ice, and extracted within 15 min.

| Time after Sink Removal | Glucose 6-P | | Fructose 6-P | |
|---|---|---|---|---|
| | Control | Treated | Control | Treated |
| *d* | *nmol/g fresh wt ± SE* | | | |
| 0 | 318 ± 20 | | 50 ± 7 | |
| 3 | 350 ± 10 | 514 ± 32 | 41 ± 5 | 91 ± 10 |
| 5 | 380 ± 20 | 544 ± 40 | 61 ± 5 | 103 ± 6 |
| 7 | 390 ± 52 | 556 ± 31 | 82 ± 8 | 122 ± 5 |
| 9 | 328 ± 41 | 529 ± 39 | 51 ± 10 | 108 ± 19 |

Table V. *$CO_2$ Fixation Rates and Distribution of $^{14}C$ between Ethanol-Soluble and $HClO_4$-Soluble Photosynthate in Discs Floating on Phosphate, Mannose, or Tris-Maleate Buffers following their Removal from Girdled or Control Cotton or Cucumber Leaves*

Discs were removed 3 d after girdling and floated on tris maleate (50 mM, pH 6.8); phosphate (50 mM, pH 6.8), or 10 mM mannose for 16 h in the dark followed by 2 h in the light. Discs were exposed to $^{14}CO_2$ for 1 min as in Table I. Results are means of 10 measurements ± SE.

| | Control | | | Girdled | | |
|---|---|---|---|---|---|---|
| | $CO_2$ Fixation rate | Ethanol-soluble | $HClO_4$-soluble | $CO_2$ Fixation rate | Ethanol-soluble | $HClO_4$-soluble |
| | $mg \cdot dm^{-2} \cdot h^{-1}$ | *$^{14}C$ as % total $^{14}C$ recovered* | | $mg \cdot dm^{-2} \cdot h^{-1}$ | *$^{14}C$ as % total $^{14}C$ recovered* | |
| A. Cotton | | | | | | |
| Tris-maleate | 29 ± 1 | 83 ± 2 | 17 ± 2 | 19 ± 2 | 76 ± 1 | 24 ± 1 |
| Phosphate | 29 ± 1 | 87 ± 1 | 13 ± 2 | 16 ± 1 | 83 ± 1 | 17 ± 1 |
| Mannose | 19 ± 1 | 54 ± 1 | 46 ± 1 | 16 ± 1 | 76 ± 1 | 24 ± 1 |
| B. Cucumber | | | | | | |
| Tris-maleate | 20 ± 2 | 76 ± 2 | 24 ± 2 | 9 ± 1 | 40 ± 1 | 60 ± 1 |
| Phosphate | 15 ± 1 | 82 ± 1 | 18 ± 1 | 8 ± 1 | 76 ± 1 | 24 ± 1 |
| Mannose | 15 ± 1 | 55 ± 2 | 45 ± 1 | 6 ± 1 | 40 ± 1 | 60 ± 1 |

the discs from girdled leaves, by contrast, it had no detectable effect on $^{14}C$ incorporation into starch in either plant and a much smaller effect on $CO_2$ fixation. Treatment with phosphate buffer, on the other hand, affected girdled leaves more strongly than it did the controls. In the case of both species, the percentage $^{14}C$ incorporated into starch was depressed to the level of that in the discs from ungirdled leaves. However, phosphate supply failed to raise the rate of $CO_2$ fixation to that in the ungirdled leaves.

The role of sucrose in mediating the control of $CO_2$ fixation and $^{14}C$ distribution among photosynthetic products was also studied with isolated leaf discs. When sucrose was fed to such discs, the incorporation of $^{14}C$ into starch was greatly enhanced, up to a concentration of 75 mM sucrose when a plateau was obtained (Fig. 2). $CO_2$ fixation dropped over the entire concentration range tested. The effect of sucrose appeared to be metabolic rather than osmotic, as indicated by the fact that when mannitol was substituted for sucrose, $CO_2$ fixation was not affected up to an external mannitol concentration of 100 mM

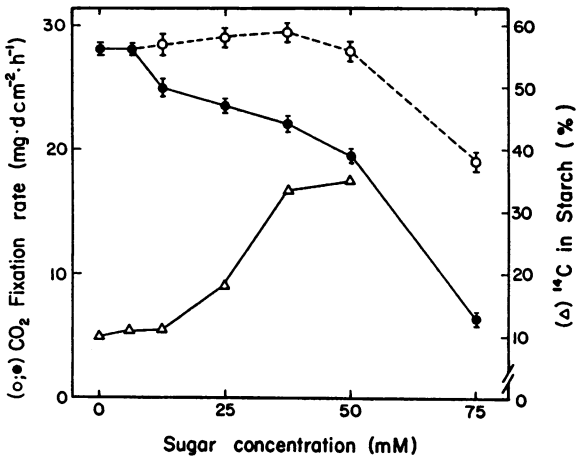


FIG. 2. Rates of $^{14}CO_2$ fixation and $^{14}C$ incorporation into starch in cotton leaf discs floated on various sucrose or mannitol concentrations. Discs containing no major veins (2.0 $cm^2$) were removed from cotton leaf blades and floated on sucrose or mannitol solutions for 3 h in the dark followed by 30 min in the light. The rate of $CO_2$ fixation was determined in a microchamber flushed with $^{14}CO_2$ as for intact leaves. Circles, $^{14}CO_2$ fixation for discs in mannitol (O) or sucrose (●) medium. Triangles, $^{14}C$ incorporated into starch for discs in sucrose medium. Vertical bar denotes standard error of the means.

Table VI. *Distribution of $^{14}C$ among Photosynthetic Products of Cotton Leaf Discs Floated on Sucrose Solutions of Various Concentrations and Exposed to $^{14}CO_2$ for 1 Min*

Discs (2 $cm^2$) were removed from leaves and floated on increasing concentrations of sucrose for 3 h in the dark followed by 30 min in the light. They were then exposed to $^{14}CO_2$ and extracted as in Table III. The figures give $^{14}C$ detected as percent of total $^{14}C$ recovered.

| | Sucrose Concentration in Medium (mM) | | | |
|---|---|---|---|---|
| | *0* | *50* | *100* | *150* |
| PGA | 6.0 | 2.8 | 2.8 | 1.0 |
| Glucose 6-P + sedoheptulose 7-P | 5.0 | 5.1 | 5.0 | 5.0 |
| P-enolpyruvate | 13.1 | 4.3 | 3.1 | 1.4 |
| Fructose | 4.7 | 3.6 | 3.6 | 2.5 |
| Maltose | 2.5 | 2.5 | 2.5 | 2.5 |
| Sucrose | 13.1 | 9.8 | 6.8 | 3.0 |
| Glucose + serine + glycine | 13.8 | 12.8 | 12.4 | 12.8 |
| Alanine | 30.0 | 47.4 | 52.0 | 60.0 |
| Glycerate | 8.8 | 7.7 | 8.3 | 7.8 |
| Unknown | 3.0 | 4.0 | 3.5 | 4.0 |

(Fig. 2). In a number of respects sugar feeding affected the percentage incorporation of $^{14}C$ into metabolic products in a manner similar to sink removal (Table VI). Incorporation into alanine increased, while that into sucrose, fructose, PGA, and P-enolpyruvate was decreased, most markedly in the latter case, where even at an external sucrose concentration of 50 mM $^{14}C$ incorporation was reduced by about two-thirds (Table VI).

In the case of bean, a member of the nonresponsive group of plants (Table I), removal of the stem and young trifoliate leaves did not affect the rate of $CO_2$ fixation of the source leaves (Table VII). A marked increase in the starch—but not sugar—content of the source leaves was nevertheless detectable. Corroboration that the rate of starch synthesis was strongly affected by sink removal may be seen in the fact that, 3 d after treatment, 68% and 28% of the $^{14}CO_2$ fixed during a 60 s period had been incorporated into starch in the case of girdling and sink removal, respectively, as compared with 15% in control leaves.

Changes in the $^{14}C$ distribution among photosynthetic products were detectable in source leaves only 6 d after sink removal (Table IIIB). Then, as in the case of cotton (Table IIIA) and cucumber (17), the treatment decreased percentage incorporation into PGA, P-enolpyruvate, sucrose and malate and increased incorporation into glucose 6-P, fructose 6-P, maltose, alanine,

Table VII. *Effect of Sink Removal on the Rate of $CO_2$ Fixation, Leaf Area, Leaf Dry Weight, Chl, Starch, and Sugar Content of Bean Leaves*

Results are averages of 10 plants ± SE.

| Time after Treatment | Rate of $CO_2$ Fixation | Leaf Area | Leaf Dry Wt | Chl Content | Starch Content | Total Sugars |
|---|---|---|---|---|---|---|
| *d* | $mg \cdot dm^{-2}\ h^{-1}$ | $cm^2$ | $mg \cdot dm^{-2}$ | $mg \cdot dm^{-2}$ | % dry wt | |
| 0 | 14.5 ± 0.5 | 91 ± 2 | 254 ± 4 | 3.0 ± 0.5 | 10 ± 2 | 5.0 ± 1.3 |
| 3 | 14.3 ± 0.8 | 100 ± 3 | 270 ± 9 | 4.0 ± 0.5 | 15 ± 1 | 5.5 ± 2.0 |
| 6 | 14.7 ± 0.6 | 108 ± 3 | 360 ± 12 | 6.0 ± 0.5 | 26 ± 2 | 6.0 ± 1.5 |
| 8 | 14.8 ± 0.4 | 113 ± 2 | 456 ± 12 | 7.5 ± 0.4 | 30 ± 4 | 5.0 ± 1.0 |

glycerate, and aspartate.

The high percentage of label detected in alanine in the case of all the plants examined (cucumber, cotton, and bean) after sink removal commanded attention. Alanine is synthesized in chloroplasts from pyruvate, which in turn originates in the cytoplasm from the reaction of P-enolpyruvate and ADP, catalyzed by pyruvate kinase. Examination of the activity of pyruvate kinase in cucumber leaves indicated that the enzyme was apparently activated following girdling and sink removal (data not shown).

## DISCUSSION

Even when experimental plants are grown under identical environmental conditions, response to sink manipulation varies between species (Table I). In one group of plants which includes cotton and cucumber, sink removal brought about a reduction to $CO_2$ fixation within 1 to 3 d. Porometer measurements showed that in contrast to the shorter term inhibition reported previously (17), this effect was not associated with stomatal closure (Table II). The size of the effect on $CO_2$ fixation, and of the effects on sugar and starch accumulation which accompanied it, bore a quantitative relationship to the number of sinks removed (Fig. 1).

Herold (10) has pointed out that the chloroplast barrier can be regarded as the ultimate barrier between source and sink. Changes in sink requirement may be reflected in altered rates of the reactions concerned with synthesis of sucrose, associated, probably with changes in the rate of synthesis of fructose 2,6-bisP (27), which is recognized as playing a key role in regulating sucrose synthesis (3). However, it is at the level of the chloroplast envelope that sink requirement is ultimately linked with the source. Since transport across this barrier is highly selective, only a very few metabolites could act as messengers. $P_i$, triose-P, and PGA which are ferried across this barrier by the $P_i$-triose phosphate antiporter, should therefore be regarded as likely candidates for the latter role (10).

The present investigation has provided evidence consistent with Herold's proposal (11) that the signal linking sink removal to source activity might be a lowered $P_i$ concentration in the cytoplasm. This situation would result from feedback inhibition by accumulated sucrose of sucrose phosphate synthetase and/or sucrose phosphate phosphatase with resultant accumulation of sugar phosphates (7) and thus a lowered $P_i$/phosphorylated compound ratio. Huber (14) has reported that the activity of sucrose-P synthetase from several plant species is inhibited by sucrose *in vitro* and that leaf starch content is negatively correlated with the activity of this enzyme (4, 14). The $P_i$/phosphorylated compound ratio will be further decreased as a result of inhibition of cytosolic FBPase by Fru 2, 6-$P_2$, the level of which rises under these conditions, particularly because of the rise in Fru-6-P concentration (27). During unimpeded sucrose synthesis four $P_i$ molecules are released along the pathway four triose-P molecules to one sucrose, and are transported into the chloroplast in exchange for triose-P by the phosphate antiporter. Lowered [$P_i$] is likely to inhibit photosynthesis by diminishing triose-P/$P_i$ exchange across the chloroplast envelope, with a resultant decrease in the ATP/ADP ratio (1); this would lead to a high PGA/$P_i$ ratio in the chloroplasts which would favor starch synthesis (22).

A technique for quantitative determination of $P_i$ concentration in the cytoplasm of the leaf cells, as distinct from that in the other cellular compartments, is not at present available, though one based on NMR may become available in the near future (23). However, the following of our findings would be in accord with the picture just outlined:

(a) Sink removal led to an increase in sucrose concentration (Fig. 1). It also led to increase in size of the $G_6P$ and $F_6P$ pools, as was shown by direct determinations (Table IV) and further indicated by the consistent increase in the proportion of $^{14}C$ detected in sugar phosphates and UDP-glucose (Table III, A and B; [17]). The increase in the sugar phosphate pool suggests a decreased ratio $P_i$/phosphorylated compounds.

(b) Heldt *et al.* (9) have demonstrated that supply of mannose to leaf discs lowers cytoplasmic [$P_i$] by sequestering the latter as mannose phosphate. The treatment greatly enhances starch synthesis, although C from mannose is not incorporated into the starch skeleton. In our experiments mannose feeding strongly promoted $^{14}C$ incorporation into starch in the case of leaf discs from control plants, as expected (9, 11), and $CO_2$ fixation was depressed (Table V, A and B). The striking lack of a comparable effect in plants treated by girdling (which affects metabolic activities of source leaves in a manner similar to sink removal [17]) accords with the suggestion that cytoplasmic $P_i$ was in any case already low in these source leaves.

(c) Supply of phosphate buffer to leaf discs reduced the high level of incorporation of $^{14}C$ into starch in discs from girdled plants, restoring it to the level observed in control plants (Table V, A and B). Phosphate supply had little or no effect on starch synthesis in the control plants. These results support the suggestion that cytoplasmic $P_i$ was low in leaves from treated plants. In both sets of discs supply of phosphate had a slightly inhibitory effect on photosynthesis and this may perhaps be explained as the effect of suprooptimal $P_i$, as shown by several investigators (5, 29).

When control leaf discs were fed progressively with higher concentrations of sucrose, $^{14}C$-starch formation increased while $^{14}CO_2$ fixation was progressively decreased (Fig. 2). In an interesting experiment Natr *et al.* (19) observed that the accumulation of photosynthates by floating leaf segments had a relatively small inhibitory effect on photosynthesis, but that dry matter accumulation which resulted from previous absorption of glucose had a much stronger inhibitory effect. Starch synthesis was somewhat greater in the former case. We now suggest that this puzzling finding could be explained on the basis of cytoplasmic [$P_i$]. The latter would be expected to be maintained at a lower level where feedback inhibition by absorbed sugar results in accumulation of sugar phosphates, than in the case of photosynthate accumulation, which involves continuous turnover of $P_i$ and other metabolites.

In all our experimental species the proportion of $^{14}C$ entering the amino acid fraction was higher in leaves of treated plants than in those of controls. Specifically, alanine accumulated as a result of sink manipulations. Pyruvate kinase, the enzyme involved in alanine synthesis, is a regulatory enzyme in glycolysis. A rise in fructose 2,6-bisP as a result of sink removal (27) would increase the flux of metabolites in the direction of glycolysis (26); by controlling the rate of pyruvate formation from PEP, pyruvate kinase would also be expected to have a regulatory effect on the subsequent formation of alanine in the chloroplasts. Apparent *in vivo* activation of pyruvate kinase was observed in leaves starting 5 d after removal of the sinks. Activation has also been reported under conditions of carbohydrate accumulation due to K deficiency (28). Alanine may serve as an alternative pool for temporary storage of carbon after saturation of the sucrose pool.

The increase in alanine (which is not completely accounted for by the decrease in P-enolpyruvate) thus suggests that FBPase played an important role in our experiments. On the other hand, the increase in label in UDPglucose, fructose 6-P, and glucose 6-P suggests control at the sucrose-P synthetase site. Perhaps both enzymes were responsible for the observed decrease in photosynthesis. The activity of these two enzymes was found to be enhanced when the demand for sucrose was increased by partial defoliation (24).

The failure of sink removal to depress source leaf photosynthesis in the bean plants was not due to intermediate storage of carbohydrates or to increased activity of alternative sinks, as evidenced by the fact that starch accumulated markedly in the source leaves. Although an increase in $^{14}C$-labeled phosphorylated sugars was observed as in cotton, no accumulation of sucrose was detectable. This suggests that the feedback control of sucrose synthesis by the sucrose level must be tighter in this plant, and a slight rise in sucrose above a threshold level suppresses sucrose-P synthetase and/or fructose bisphosphatase more completely. A more drastic decrease in the amount of $P_i$ shuttled into the chloroplast apparently leads, in this plant, to greater autonomy in chloroplast carbon metabolism. Bean leaves can accumulate starch to much higher levels than were seen in plants of the cotton group (Table I).

Our previous observation (17) that high ambient $CO_2$ concentrations enabled cucumber plants to achieve a very high rate of photosynthesis in spite of their high starch content, led us to question the proposal that starch accumulation depresses photosynthesis, and that the basis for starch inhibition of photosynthesis was a reduction of the volume of free stroma (18), reduction of light transmission within the chloroplast (20), or the binding by starch of $Mg^{2+}$ needed to activate ribulose 1,5-bisP carboxylase. Our present evidence strengthens the likelihood that the rise in starch levels observed when the sink-source ratio is decreased is not the cause of the lower $CO_2$ fixation rate, but the result of other mechanisms associated with it. The lack of detectable inhibition in bean plants following sink removal may, in addition, be related to changes in mesophyll thickness, Chl, and dry weight per unit area of the source leaves (2). These modifications would provide a greater internal leaf area across which $CO_2$ could diffuse to the carboxylating enzyme (21).

The enhanced incorporation of $^{14}CO_2$ into maltose observed in bean and the other species after sink removal (Table III, A and B) may also be due to lowered ($P_i$), since the reaction between glucose 1-P and glucose to form maltose and $P_i$ would be driven in the forward direction under these conditions (11, 22). Maltose may serve as a primer for starch synthesis (16).

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