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Effect of Translocation-Hindering Procedures on Source Leaf Photosynthesis in Cucumber

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ABSTRACT

Three treatments which altered translocation rate were applied to cucumber plants: Girdling of source leaf petiole; removal of all aerial sinks; removal of all source leaves except one. Two different effects were observed, one short-term (during the initial 6 hours), and one long-term (detected after several days).

The short-term effect was observed exclusively in girdled leaves and involved a reduction in $^{14}CO_2$ fixation rate paralleled by an increase in stomatal resistance. The effects were maximal after 3 hours with subsequent recovery. Stomatal closure apparently resulted from the 5 to 10% water deficit temporarily detected in girdled leaves which probably induced the observed temporary increases in abscisic acid content. Kinetin counteracted the effects of girdling.

The long-term effect was detected 3 days after girdling and 3 to 5 days after sink manipulation. An increase or decrease in $^{14}CO_2$ fixation rate was observed when the sink-source ratio was increased or decreased respectively, accompanied by a respective decrease or increase in starch content. Changes in the relative amount of $^{14}CO_2$ incorporated into various photosynthetic products were also observed. Stomatal closure was not involved, and the decrease in CO_2 fixation was not counteracted by kinetin.

There is controversy in the literature as to whether the rate of photosynthesis of source leaves is influenced by translocation-hindering manipulations such as decreasing the sink-to-source ratio or phloem girdling. In certain plants it has been possible to show that partial or complete removal of fruits, buds, young leaves, or storage organs (*i.e.* decrease in sink-to-source ratio) decreases the photosynthetic rate of the well developed mature leaves (8, 10, 11, 16). Moreover, concomitant increases in carbohydrate level was observed in the source leaf, as predicted by the hypothesis that photosynthesis is inhibited by the accumulation of its end-products (23). However, there are also reports that photosynthesis in intact leaves is independent of the starch levels in the chloroplasts (7, 18, 26). Some workers have attributed the decrease in CO_2 fixation rate observed after sink removal or girdling to stomatal closure (10, 17, 27).

The evidence is conflicting not only with regard to the type of response to sink manipulation and girdling but also the time required to observe an effect, if any. For instance, King *et al.* (16) reported a decrease in photosynthesis of source leaves 3 h after ear removal in wheat plants; Austin and Edrich (1), however, detected no effect of similar treatment in the same plant until several weeks after sink removal. Some of the differences between reported results may have arisen because growing conditions and handling techniques varied between laboratories.

We have conducted an investigation into the short- and long-term effects of decreased sink-to-source ratio and petiole girdling on the metabolic activities of source leaves in several plant species, grown under similar controlled environmental conditions. We have endeavored to separate stomatal from nonstomatal effects; and, further, to investigate the role of photosynthetic intermediary compounds in the possible control of photosynthesis by translocation. We have observed two separate and distinguishable effects, one short-term (during the 6 h following treatment) and one detectable only after several days. In this communication we report on the short-term and long-term effects of translocation-hindering procedures in cucumber plants.

MATERIALS AND METHODS

Cucumber (*Cucumis sativus* cv Dalila) plants were grown from seeds in 2-L cylindrical plastic containers filled with vermiculite, grade 3. Seedlings were thinned to one plant per container 4 d after emergence. Plants were irrigated in excess daily with deionized H_2O and twice a week with full strength Hoagland solution. Excess water and solution drained through the bottom of the containers. Plants were grown in a growth chamber at 25 ± 1° C air temperature and 50 ± 5% RH. Maximal quantum flux density of 450 $\mu E\ m^{-2}\ s^{-1}$ (400–700 nm) was provided by fluorescent tubes for a photoperiod of 13 h. A special section in the growth chamber, in which environmental conditions were identical, was enclosed for atmospheric CO_2 enrichment. CO_2 was released from condensed cylinders and maintained at 1500 $\mu l/l$. Plants were transferred to this enclosure 7 d prior to girdling.

Plants were used in the experiments at the age of 10 to 12 weeks. Girdling was effected by running a fine stream of boiling water over the petiole of the third or fourth oldest leaf for 2 to 3 s. Measurements were made on the girdled leaf at various intervals after treatments as indicated in the text.

The rate of CO_2 fixation was determined on 1.5 cm^2 areas of attached leaves according to the method earlier described (14). The method involves enclosing the area in a microchamber and flushing it for 20 s with an airstream containing 300 $\mu l/l$ $^{12}CO_2$ + $^{14}CO_2$ at a rate of 60 $cm^3\ min^{-1}$.

The evolution of CO_2 by leaves was determined on two leaf discs which were enclosed in a divided plexiglass chamber (total volume 15 cm^3) illuminated from above with a light source at an intensity of 300 $\mu E\ m^{-2}\ s^{-1}$ (400–700 nm). Prehumidified air containing a mixture of $^{14}CO_2$ and $^{12}CO_2$ (300 $\mu l/l$) was passed over the enclosed leaf discs at a rate of 250 $cm^3\ min^{-1}$ for 45 min. The chamber was then flushed with humidified CO_2-free air for 30 s before collection of the CO_2 evolved by the leaf discs, and subsequently for 60 min during which the CO_2 evolved was trapped in a series of tubes containing cold ethanolamine solu-

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tion. $^{14}CO_2$ trapped in the ethanolamine was determined by liquid scintillation counting. For the estimation of dark CO_2 from one leaf disc, one side of the chamber was covered with aluminum foil.

Determination of fixed ^{14}C and its distribution among intermediates and end products of photosynthesis was determined after standard extraction of 1.0 cm^2 leaf discs by 80% ethanol followed by 20% hot ethanol and water. The extracts were combined and concentrated to 0.5 ml under vacuum. Compounds were separated on TLC plates according to Platt and Rand (25). After location of the labeled spots by exposure to x-ray film, the cellulose containing the various spots were removed and counted by liquid scintillation. Spots were identified by co-chromatography with the various cold substances.

Total starch content of leaves was determined on the remaining parts of the same leaves which had been used for photosynthesis measurements. Leaf material was dried, ground, and subsamples of 200 mg dry weight were extracted with ethanol and water as above and then extracted with 20% $HClO_4$ which was analyzed by the anthrone method (19).

ABA was determined on 5-g samples collected from girdled and nongirdled leaves. The plant material was ground in 100 ml methanol-acetic acid (8:2) mixture and incubated for 4 d at 4°C. After filtration the solution was concentrated to about 10 ml in a rotovapor at 37°C, made up to 50 ml with H_2O, and brought to pH 8.5 with NaOH. The fraction containing ABA was separated first with petroleum ether and then with ethyl acetate, evaporated to dryness (at 40°C), and redissolved in 2 ml ethyl acetate. ABA was determined by GC after methylation according to Mizrahy *et al.* (21).

Leaf diffusive resistance was determined on the lower leaf surface by a Li-Cor diffusion porometer. Relative leaf water content was estimated according to Barrs and Weatherley (2).

Girdled and nongirdled plants were treated with kinetin in several experiments. Kinetin was applied by foliar spraying at a concentration of 10^{-5} M in aqueous solution containing 0.1% Tween-20 as surfactant.

RESULTS

Short-Term Effect. Photosynthesis of source leaves, calculated following a 20-s $^{14}CO_2$ labeling period, was depressed by girdling (Fig. 1a). The effect appeared to be temporary. The maximal effect was observed about 3 h after treatment, and after 4 h the CO_2 fixation rate started to increase again. Control values were regained 6 to 7 h after girdling (Fig. 1a). A progressive increase in stomatal resistance was also noted in girdled leaves, with the maximum value again being observed 3 h after translocation had been impaired (Fig. 1b). Subsequently, the stomata reopened.

Stomatal closure as a result of girdling was also evidenced by the decrease in the amount of CO_2 evolved from girdled leaves. In the light, the quantity of CO_2 released will depend both on the magnitude of photorespiration, and on the proportion of the CO_2 evolved which diffuses to the atmosphere through the stomata, relative to that refixed in the chloroplasts. In these experiments CO_2-free air was passed over the discs during assessment of $^{14}CO_2$ evolution in order to avoid dilution of fixed ^{14}C by the newly fixed nonlabeled C with consequent alteration of specific activity. The ratio of the $^{14}CO_2$ released under these conditions in the light to that given off in the dark over the same period has been calculated (Table I). This assay gives a measure of photorespiration and the latter appears to be 3 times greater than dark respiration under present experimental conditions.

Since the flow rate of Co_2-free air was high (to keep CO_2 concentration low) and identical for girdled and nongirdled plants, the change in ratio can be attributed to the increase in stomatal resistance or to a decrease in photorespiration. The two factors are probably connected as an increase in internal CO_2 due to higher photorespiration would lead to stomatal closure. Table I shows that girdling, though it increased the diffusive resistance of the leaves, did not decrease the quantity of $^{14}CO_2$ evolved in the dark. The reduction of the $^{14}CO_2$ light/$^{14}CO_2$ dark ratio from 3 (control) to 1.27 (3 h after girdling) is indicative of the effect of the treatment on the amount of CO_2 evolved in the light.

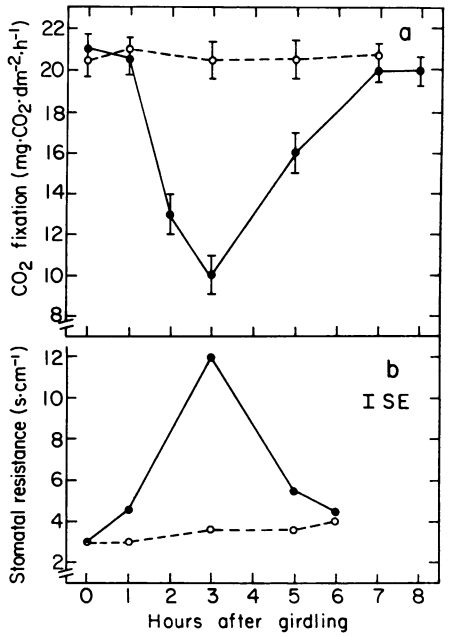

FIG. 1. Short-term effect of girdling on rate of photosynthesis (a) and stomatal resistance (b) of source leaf of cucumber plants. The points give the average of 10 replicates ± SE (a) or of 20 individual measurements ± SE (b). (○), control; (●), girdled.

Table I. *Determination of the $^{14}CO_2$ Evolved (Light)/$^{14}CO_2$ Evolved (Dark) Ratio in Control and Girdled Cucumber Leaves*

Leaf discs were labeled for 45 min. The chamber was then flushed with CO_2-free air for 30 s before collection of the CO_2 evolved and subsequently for an additional 60 min. The $^{14}CO_2$ evolved was collected in a trapping solution.

Treatment	Time after Treatment	Radioactivity		CO_2 Light/ $^{14}CO_2$ Dark
		Collected in the light	Collected in the dark	
	h	*dpm*		*ratio*
Control		5.73×10^6	1.91×10^6	3.00
Girdled	3	2.35×10^6	1.85×10^6	1.27
Girdled	6	5.20×10^6	1.90×10^6	2.73

Starch did not accumulate significantly in girdled leaves during the 6 h following girdling (not shown). Investigation of the distribution of ^{14}C between various photosynthetic products after 20 s or after 1 min labeling with $^{14}CO_2$, also revealed no significant effect of the treatment. The photosynthetic products examined are listed for another experiment in Table IV. Thus, the treatment did not affect either the pool sizes of intermediate metabolites or the relative rate of synthesis of end-products during the first 6 h.

Girdling caused a temporary water deficit in the leaves. The relative water content fell from 92% ± 0.3 to 88% ± 0.5 at 3 h after treatment. After 6 h, however, the leaves had recovered and returned to control water status (92% ± 0.7).

Free ABA content was observed to rise sharply in girdled leaves after a lag period of about 1 h, and reached a value almost 10 times the control after 3 h (Fig. 2). An equally sharp decline

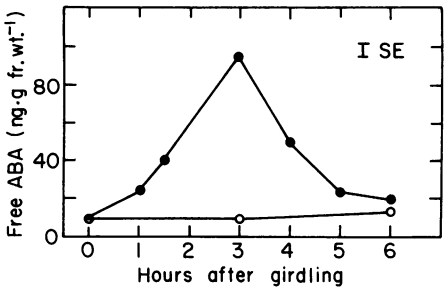

FIG. 2. Short-term effect of girdling on the free ABA content of the source leaf of cucumber plants. The points give the average of three individual extractions. The SE is indicated. (○), control; (●), girdled.

Table II. *Effect of Kinetin on the Rate of Photosynthesis and Stomatal Resistance of Girdled and Control Cucumber Leaves (Short Term)*

An aqueous kinetin solution (10^{-5} M) containing 0.1% Tween 20 was sprayed on the leaves immediately after girdling. Values are means of 10 replicates ± SE.

Treatment	Time after Girdling	Gross Photosynthesis	Stomatal Resistance
	h	*mg CO_2/dm² h*	*s/cm*
Control		20 ± 0.5	4 ± 0.3
Control + kinetin		20 ± 0.5	4 ± 0.3
Girdled	3	8 ± 0.6	12 ± 1.0
Girdled + kinetin	3	21 ± 1.0	4 ± 1.0

followed and after 6 h free ABA content was close to the control value again.

Foliar application of 10^{-5} M kinetin completely reversed the effect of girdling on photosynthetic rate and stomatal resistance (Table II). Treatment of control leaves with the hormone affected neither the photosynthesis rate nor the stomatal resistance (Table II).

In contrast to girdling, sink removal had no detectable short-term effect on either photosynthetic rate or on stomatal resistance in source leaves. Similarly, no changes in starch content of the source leaves was detected as a result of such treatment (data not shown).

Long-Term Effects of Girdling and Source-Sink Manipulations. Both girdling (Fig. 3) and sink removal (Fig. 4) led to a decrease in the photosynthetic rate of source leaves. This long-term effect of girdling became apparent 3 d after the treatment (Fig. 3) earlier than that of sink removal (5 d, Fig. 4).

If the ambient CO_2 concentration was raised to 1500 μl/l, the rate of photosynthesis of control plants doubled, and its sensitivity to girdling increased (Fig. 3b). A 40% drop in CO_2 fixation rate was already apparent after 1 d, while after 3 d the rate had fallen by 60%, as compared with 28% at 300 μl/l ambient CO_2.

The starch content of source leaves was increased by girdling both at 300 or 1500 μl/l ambient CO_2 (Table III). This effect on photosynthesis was also visible after 1 d at 1500 μl/l but only after 3 d at 300 μl/l. The relative increase in starch content brought about by girdling was similar in the two cases. The absolute values for starch were substantially higher at 1500 μl/l CO_2.

When the aerial sinks (buds, flowers, and fruits) were removed daily, a new steady state CO_2 fixation rate was attained after 16 d which was about 50% of the control value (Fig. 4). By contrast, when the sinks were removed only once (at day 0) a recovery in the rate of CO_2 fixation was observed starting 14 d after treatment. The development of new skins (young leaves) restored the photosynthetic rate to its original value after about 20 d. Sink removal also led to the accumulation of starch in the source leaves (Fig. 4). A level 4.5 times higher than that in control leaves

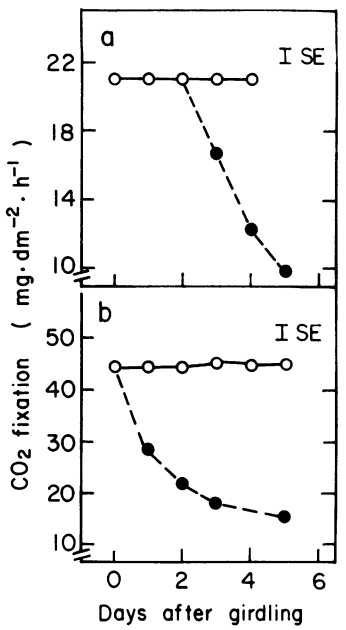

FIG. 3. Long-term effect of girdling on rate of photosynthesis of source leaf of cucumber plants at 300 μl/l CO_2 (a) or 1500 μl/l CO_2 (b). In the latter case, the plants were subjected to high CO_2 concentration for a week prior to girdling. Points give the average of 10 replicates. The SE is indicated. (○), control; (●), girdled.

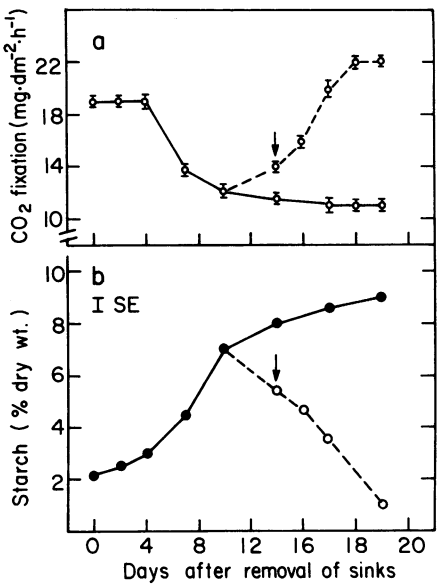

FIG. 4. Long-term effect of sink removal on rate of photosynthesis (a) and starch content (b) of source leaf of cucumber plants. Sinks (young leaves, buds, fruits, and flowers) were removed continuously (continuous curve) or exclusively at the beginning of the experiment (dashed curve). Arrows indicate the time at which newly-formed leaves became visible on the plants. The points give the average of 10 replicates; the SE is indicated.

was reached in the case where sinks were removed daily. Where sinks were removed only once, progressive mobilization of the accumulated starch commenced after 14 d in parallel with the recovery of photosynthesis, indicating the complete reversibility of the effects of decreasing sink demands. By the 20th d, the development of new buds and leaves had brought about an increase in CO_2 fixation rate to 16% above the initial value. This effect correlated with a decrease in starch content to a level below

Table III. *Starch Content of Girdled and Control Source Leaves of Plants Grown at Two CO_2 Concentrations*

Results are averages of five measurements ± SE.

Treatment	Time after Girdling	Starch Content at Following CO_2 Concn. (μl/l)	
		300	1500
	d	*% of dry wt*	
Control		3.5 ± 0.9	8.5 ± 0.3
Girdled	1	3.5 ± 0.9	10.3 ± 0.3
Girdled	3	6.0 ± 0.8	20.7 ± 0.4
Girdled	6	12.0 ± 0.9	30.0 ± 0.9

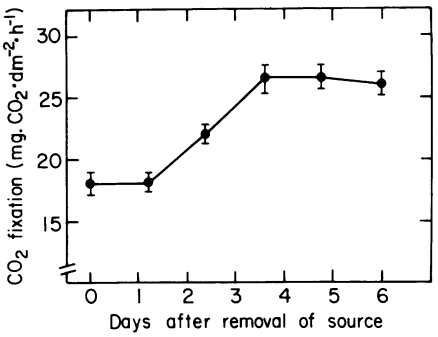

FIG. 5. Long-term effect of source removal on the rate of photosynthesis of remaining source leaf of cucumber plants. All source leaves except one were removed. Points give the average of 10 replicates ± SE.

Table IV. *Effect of Girdling on the ^{14}C Distribution among Photosynthetic Products in the Cucumber Leaves*

The results give the ^{14}C fixed in each individual compound as percentage of the total ^{14}C recovered in the TLC plates. Intact leaves were labeled for 20 s with $^{14}CO_2$ (300 μl/l; 80 × 10^{-4} dpm/ml gas) discs of 1 cm^2 were then removed and extracted twice in hot ethanol (80%, 20%) and once in H_2O. The extracts were combined and concentrated to 0.5 ml. Aliquots of 10 μl were used for chromatography.

Compound	Control	Days after Treatment	
		3	5
	% of total cpm		
PGA	12.9	3.5	2.5
F6P	1.0	1.6	3.0
G6P + S7P	2.3	2.5	4.0
PEP	8.5	4.9	2.5
Glycolate	1.2	1.3	2.0
Fructose	5.7	3.1	3.1
Maltose	1.6	2.5	2.5
Sucrose	15.0	10.2	7.0
Stachyose	14.4	7.1	7.7
Serine + glucose + glycine	9.2	13.0	15.0
Alanine	9.4	17.0	22.8
UDP-glucose		2.1	3.0
Glycerate	8.4	17.2	9.1
Malate	2.7	3.0	5.0
Unknown	7.7	10.9	10.8

the initial value.

When the source-sink ratio was manipulated by removing sources, *i.e.* when the sink-source ratio was increased rather than decreased, opposite effects to those were obtained as shown in Figure 5. Removal of all source leaves except one brought about a 39% increment in CO_2 fixation rate 3 d after treatment. Such removal of source leaves also accelerated the development of leaf photosynthetic capacity. Whereas in a control plant photosynthetic capacity increased gradually from 5 to 20 mg CO_2/dm^{-2} h^{-1} as leaf area increased from 20 to 50% of its final value, in a plant retaining only one source leaf photosynthetic capacity had already attained its maximum value when the leaf was only 30% of its final area.

Table V. *Effect of Kinetin on the Rate of Photosynthesis and Stomatal Resistance of Girdled and Control Cucumber Leaves (Long Term)*

An aqueous kinetin solution (10^{-5} M) containing 0.1% Tween 20 was sprayed on the leaves immediately after girdling and then once every 24 h. Values are means of 10 replicates ± SE.

Treatment	Days after Girdling	Photosynthesis	Stomatal Resistance
		mg CO_2/dm^2 h	*s/cm*
Control		20.0 ± 0.5	3 ± 0.5
Control + H_2O + 0.1% Tween 20		20.8 ± 0.5	3 ± 0.5
Control + kinetin		20.0 ± 0.5	3 ± 0.5
Control + kinetin	5	20.0 ± 0.5	3 ± 0.5
Girdled	2	19.0 ± 0.5	3 ± 0.7
Girdled + kinetin	2	19.5 ± 0.5	3 ± 0.7
Girdled	3	10.0 ± 1.0	3 ± 0.4
Girdled + kinetin	3	10.0 ± 1.0	3 ± 0.5
Girdled	5	7.3 ± 0.7	3 ± 0.6
Girdled + kinetin	5	8.1 ± 0.7	3 ± 0.7

The long-term effects of girdling and sink removal (in contrast to the short-term) included an altered pattern of incorporation of ^{14}C into various metabolic end products and photosynthetic intermediates during 20 s of $^{14}CO_2$ supply. The results were consistent in successive experiments, and a typical example is given in Table IV. It was repeatedly observed that the treatments decreased the relative amount of ^{14}C incorporated into PGA, PEP, sucrose, stachyose, and fructose; and increased that incorporated into G6P+S7P, F6P, UDPG, alanine, aspartate, and probably glycine.

We have investigated whether the long-term effect of girdling, like the short-term effect, could be related to stomatal closure. Table V shows that stomatal resistance did not rise during the first 5 d after girdling, a period during which the effect on CO_2 fixation manifested itself. The Table shows, further, that in this case kinetin treatment did not counteract the effect of girdling on photosynthesis.

At 1500 μl/l ambient CO_2 stomatal resistance was higher—approximately 4.2 s/cm, but here too girdling produced no effect on resistance.

DISCUSSION

There are a number of strong indications that the depression in rate of photosynthesis visible during the first few hours after girdling (the 'short term' effect) was the result of stomatal closure. Stomatal resistance rose temporarily after girdling, and the time course for rise and fall of resistance accorded with that for the fall and subsequent recovery of photosynthetic rate. The fall in the ratio of $^{14}CO_2$ evolved in the light to that evolved in the dark (Table I), attributable to an effect of girdling on CO_2 evolution in the light, also implicates stomatal closure.

The closure of the stomates was almost certainly related to the raised levels of free ABA in the girdled leaves. Free ABA content rose almost 10-fold (Fig. 3) and the time course of its rise and subsequent fall accords with the curves showing the effect of girdling on stomatal resistance and rate of photosynthesis. The fact that foliar application of kinetin (which is known to antagonize the action of ABA on stomata) completely countered the girdling effects both on CO_2 fixation and on stomatal resistance is strong confirmatory evidence that ABA-mediated stomatal closure was the underlying mechanism for the short-term effect.

We suggest that the ABA accumulation and stomatal closure were the result of mild water stress induced in the leaf by the application of hot water to the petioles. The drastic and instantaneous increase in tissue temperature may have brought about transient impairment of water transport, possibly because of damage to the younger xylem vessels. Measurements of the relative water content, in fact, showed a temporary reduction of 4 to 5% in girdled leaves. The decrease in relative water content and probably in leaf water potential, will have brought about a steeper gradient in water potential through the plant. This will have resulted in higher rates of water flow and eventual recovery of plant water status and photosynthesis. Our results stand in contrast to those of Setter *et al.* (27) and Wiebe and Prosser (30) who were unable to detect an effect of girdling (in the former case) or heating a portion of the leaf blades (in the latter) on leaf water status. Hsiao (15) regards a 5% reduction in leaf water content as a mild water stress and Stalfelt (28) has shown that a 2% water deficit is sufficient to alter the degree of stomatal opening. In addition, a more direct inhibition of photosynthesis by water stress, not via the stomata, cannot be excluded.

Bengston *et al.* (4) reported that a reduction of 5% in the relative water content of wheat plants brought about a 5-fold increase in ABA content of the leaves. Settler *et al.* (27) have attributed ABA accumulation after girdling to interference with its normal translocation from the leaves. The transitory character of the rise in ABA level in the present investigation, however, and the fact that both ABA content and relative water content regained normal values 5 to 6 h after treatment, support the proposal that the leaves underwent mild water stress in the present instance.

In this investigation, in contrast to those of Setter *et al.* (27) and King *et al.* (16), removal of flowers, fruits, buds, and young leaves unlike girdling did not affect the rate of photosynthesis in source leaves during the first 6 h. Geiger (9) has reported that in bean plants sink removal had no short-term effects on photosynthesis even though a change in translocation rate occurred within 30 min. Borchers-Zampini *et al.* (6) have also demonstrated rapid changes in transport rates from source leaves following sink manipulation. The results reported here thus suggest that decrease in translocation out of source leaves is not directly linked to changes in CO_2 assimilation, at least during the first few hours.

The long-term effects of sink removal and girdling, unlike the short-term effects of the latter, were not mediated by stomatal closure. This was indicated by the lack of a concomitant effect on stomatal resistance, and further, by the fact that kinetin treatment brought about no reversal. Good correlation is seen, by contrast, between the rise and fall in starch levels and inhibition and recovery of CO_2 fixation (Fig. 4, a and b).

Comparison between plants growing at 300 and 1500 μl/l ambient CO_2, respectively, yielded the rather striking finding that leaves of ungirdled plants at high ambient CO_2 concentration were achieving a very high rate of photosynthesis in spite of the fact that their starch content was as high or higher than that apparently depressing photosynthesis in the girdled plants at 300 μl/l. Their rate of photosynthesis was, in fact, more than twice that of the control plants at the low ambient CO_2 concentration. This result seems to indicate rejection of the proposal that starch affects photosynthesis by reducing the free volume of the stroma (22). Neither is it consistent with the suggestion that depression of photosynthesis is due to interference by starch of light transmission within the chloroplast (23) or to binding by starch of Mg^{2+} needed to activate ribulose-1,5-bisphosphate carboxylase. It does not rule out another proposal, however, that starch offers mechanical resistance to CO_2 diffusion toward the carboxylation enzyme. Thus, it is of interest that relatively small additions to the high starch content of the high CO_2 plants during the first day after girdling acompanied immediate and more drastic inhibition of photosynthesis than was observed in the case of the low CO_2 plants. It thus seems that Milford and Pearman's (20) suggestion that starch content needs to exceed a certain threshold to depress photosynthesis cannot be the explanation of these findings.

Even if the reduction in translocation would lead to accumulation of transportable sugars, which are sucrose and stachyose in the case of Cucurbitaceae (3, 29), direct inhibition of photosynthesis is unlikely since the chloroplast membrane is impermeable to sucrose (12), and probably also to stachyose. In fact, galactinol-forming enzymes which are responsible for the formation of stachyose were not found in chloroplast lysates from cucumber leaves (24). However, high levels of these sugars might, by feedback or mass action, bring about accumulation of intermediaries in the chain of synthesis. Such precursors which can cross the chloroplast membrane and might thus cause inhibition of CO_2 fixation include uridine-5-triphosphate glucose (13), and fructose-6-phosphate. Incorporation of ^{14}C into these compounds increased sharply after girdling in our experiments (Table IV). Enzymes likely to be affected by feedback inhibition could thus be sucrose phosphate phosphatase, and galactinol-forming enzymes.

Feedback effects via intermediates of sucrose and stachyose metabolism might also lead to stimulation of the glycolate pathway sequence of glycolate → glycine → serine → glycerate. Table IV indeed indicates a rise in glycerate (after 3 d), alanine, aspartate, and possible glycine + serine and a decrease in both phosphoenolpyruvate and glycerate 3-phosphate which suggest an accelerated rate of conversion of phosphoenolpyruvate to pyruvate.

Alanine is synthesized in the chloroplast from pyruvate and might be used as an alternative pool for carbon accumulation, after saturation of other pools. It has been suggested (5) that alanine, aspartate, and some other amino acids may substitute for sucrose for temporary carbon storage in certain grasses.

LITERATURE CITED

1. AUSTIN RB, J EDRICH 1975 Effects of ear removal on photosynthesis, carbohydrate accumulation and on the distribution of assimilated ^{14}C in wheat. Ann Bot 39: 141–152
2. BARRS HD, PE WEATHERLEY 1962 A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust J Biol Sci 15: 413–428
3. BEITLER GE, JE HENDRIX 1974 Stachyose: an early product of photosynthesis in squash leaves. Plant Physiol 53: 674–676
4. BENGSTON C, SO FALK, S LARSON 1977 The after-effects of water stress on transpiration rate and changes in abscissic acid content of young wheat plants. Physiol Plant 41: 149–154
5. BOLAND RL, GB GARNER, CJ NELSON, KH ASAY 1977 Amino acid and carbohydrate composition of leaves of tall fescue genotypes differing in malic acid accumulation. Crop Sci 17: 543–544
6. BORCHERS-ZAMPINI C, AB GLAMM, J HODDINOTT, CA SWANSON 1980 Alterations in source-sink patterns by modifications of source strength. Plant Physiol 65: 1115–1120
7. CARMI A, I SHOMER 1979 Starch accumulation and photosynthetic activity in primary leaves of bean (*Phaseolus vulgaris* L.). Ann Bot 44: 479–484
8. CLAUSSEN W, E BILLER 1977 Die Bedeutung des Saccharose-und Stärkegehalte der Blätter für die Regulierung der Netto Photosyntheseraten. Z. Pflanzenphysiol 81: 189–198
9. GEIGER DR 1976 Effects of translocation and assimilate demand on photosynthesis. Can J Bot 54: 2337–2345
10. HABESHAW D 1973 Translocation and the control of photosynthesis in sugar beet. Planta 110: 213–226
11. HALL AJ, PL MILTHORPE 1978 Assimilate source-sink relationship in *Capsicum annuum* L. III. The effect of fruit excision on photosynthesis and leaf and stem carbohydrates. Aust J Plant Physiol 5: 1–13
12. HELDT HW, F SAUER 1971 The inner membrane of the chloroplast envelope as the site of specific metabolite transport. Biochim Biophys Acta 234: 83–91
13. HEROLD A 1980 Regulation of photosynthesis by sink activity—the missing link. New Phytol 86: 131–144
14. HEUER B, Z PLAUT 1981 Carbon dioxide fixation and ribulose-1,5-bisphosphate carboxylase activity in intact leaves of sugar beet plants exposed to salinity and water stress. Ann Bot 48: 261–268

15. HSIAO TC 1973 Plant responses to water stress. Annu Rev Plant Physiol 24: 519–570
16. KING RW, IF WARDLAW, LT EVANS 1967 Effect of assimilate utilization on photosynthetic rate in wheat. Planta 77: 261–276
17. KOLLER HR, JH THORNE 1978 Soybean pod removal alters leaf diffusion resistance and leaflet orientation. Crop Sci 18: 305–307
18. LITTLE CHA, K LOACH 1973 Effect of changes in carbohydrate concentration on the rate of net photosynthesis in mature leaves of *Albies balsamea*. Can J Bot 51: 751–758
19. MCCREADY RM, J GUGGOTE, V SILVIERA, AS OWENS 1950 Determination of starch and amylase in vegetables. Anal Chem 22: 1156–1158
20. MILFORD GF, J PEARMAN 1975 The relationship between photosynthesis and the concentrations of carbohydrates in the leaves of sugar beet. Photosynthetica 9: 78–83
21. MISRAHY Y, A BLUMENFIELD, AE RICHMOND 1972 The role of abscisic acid and salination in the adaptive response of plants to reduced root aeration. Plant Cell Physiol 13: 15–21
22. NAFZIGER ED, HR KOLLER 1976 Influence of leaf starch concentration on CO_2 assimilation in soybean. Plant Physiol 57: 560–563
23. NEALES TF, LD INCOLL 1968 The control of leaf photosynthesis rate by the level of assimilate concentration in the leaf: a review of the hypothesis. Bot Rev 34: 107–123
24. PHARR DM, HN SOX, RD LOCY, SC HUBER 1981 Partial characterization of the galactinol forming enzyme from leaves of *Cucumis sativus* L. Plant Sci Lett 23: 25–33
25. PLATT SG, L RAND 1979 Thin layer chromatographic separation of ^{14}C-labelled metabolites from photosynthesis. J Liq Chrom 2: 239–253
26. POTTER JR, PJ BREEN 1980 Maintenance of high photosynthetic rates during the accumulation of high leaf starch levels in sunflower and soybean. Plant Physiol 66: 528–531
27. SETTER TL, NA BRUN, ML BRENNER 1980 Stomatal closure and photosynthetic inhibition in soybean leaves induced by petiole girdling and pod removal. Plant Physiol 65: 884–887
28. STALFELT MG 1955 The stomata as a hydrophotic regulator of the water deficit of the plant. Physiol Plant 8: 572–593
29. WEBB KL, WA BURLEY 1964 Stachyose translocation in plants. Plant Physiol 39: 973–977
30. WIEBE HN, RJ PROSSER 1977 Influence of temperature gradients on leaf water potential. Plant Physiol 59: 256–258