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Effect of Sink Region Cooling on Translocation of Photosynthate[1]

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Summary. The effect of metabolic inhibition of the sink tissues on translocation of ^{14}C-labeled photosynthate was studied by cooling part or all of the sink region in a translocating sugar beet plant (*Beta vulgaris* L. var Klein Wanzleben).

When the sink region was cooled, 4 phases were observed: a temporary decline, a period of translocation at the pre-treatment rate, a period of decline, and a new steady rate at 35 to 45 % of the original rate. The new rate persisted throughout 26 hours of cooling.

Cooling half the blade of a developing leaf caused a decline in translocation to the uncooled half. When a portion of the beet was cooled, translocation to a developing leaf located above the supply leaf node increased 30 %.

Translocation into the treated region recovered rapidly and completely when cooling ceased indicating that cooling had not caused serious damage to tissues. Enhancement of the proportion of ^{14}C as sucrose in the cooled portion of the sink leaf as compared with the corresponding warm side indicated that sucrose is the chief species of translocate molecule arriving in the sink.

The data suggest that the translocation process includes active uptake into storage and growing areas.

A number of mechanisms have been proposed for generating the pressure gradients postulated in the pressure-flow translocation model (1, 4, 6, 8, 11, 13, 17). In this model system translocate is conceived as moving from a site of supply (the source) to a place of utilization or storage (the sink). Attention has recently focused on the parenchyma cells associated with sieve elements in the source and sink regions (2, 6, 7, 13). These cells (the pumps) were proposed to be the site of an active process by which sucrose is secreted into sieve elements in the source and removed in the sink. If this proposed mechanism actually operates in a plant, it appears likely that the source and the sink rather than the path would be the site of low temperature inhibition of translocation.

Following the finding of Curtis and Herty (5) that low temperature inhibited translocation of photosynthate from leaves, there appeared numerous apparently contradictory reports on the effects of temperature on translocation (cf. reviews in 10, 15, 18). Conflicting results may be due to differences in the type and severity of response to low temperature in various portions of the translocation system and in different species of plants. In another paper (15) it was shown that translocation in the sugar beet occurs at an undiminished rate along a translocation path maintained a 2° for periods of up to 24 hours while the source and sinks were at 28 to 30°. In contrast, translocation in bean was greatly reduced under these conditions.

To determine the site of low temperature inhibition of translocation and to test the proposed active transport mechanism postulated for sink regions, I undertook a series of experiments using localized cooling of part or all of the sink.

Materials and Methods

Experimental material consisted of 5- to 7-week-old sugar beet plants (*Beta vulgaris* L. var Klein Wanzleben) pruned to a simplified translocation system. Culture methods and preparation of plant material were carried out as described previously (9, 10).

The sink leaf, crown and top of the beet were cooled with a Haake model KT 62 controlled temperature bath. Coolant was circulated through a 9.5 mm inside diameter copper coil lining an acrylic plastic chamber 10 cm in diameter and 15 cm high (fig 1). Temperature was measured with a 1.1 mm diameter thermistor positioned against the surface of the sink leaf. The temperature at this point showed short-term changes of less than 0.2° and could be held within ± 1° for at least 24 hours. The roots and lower part of the beet were main-

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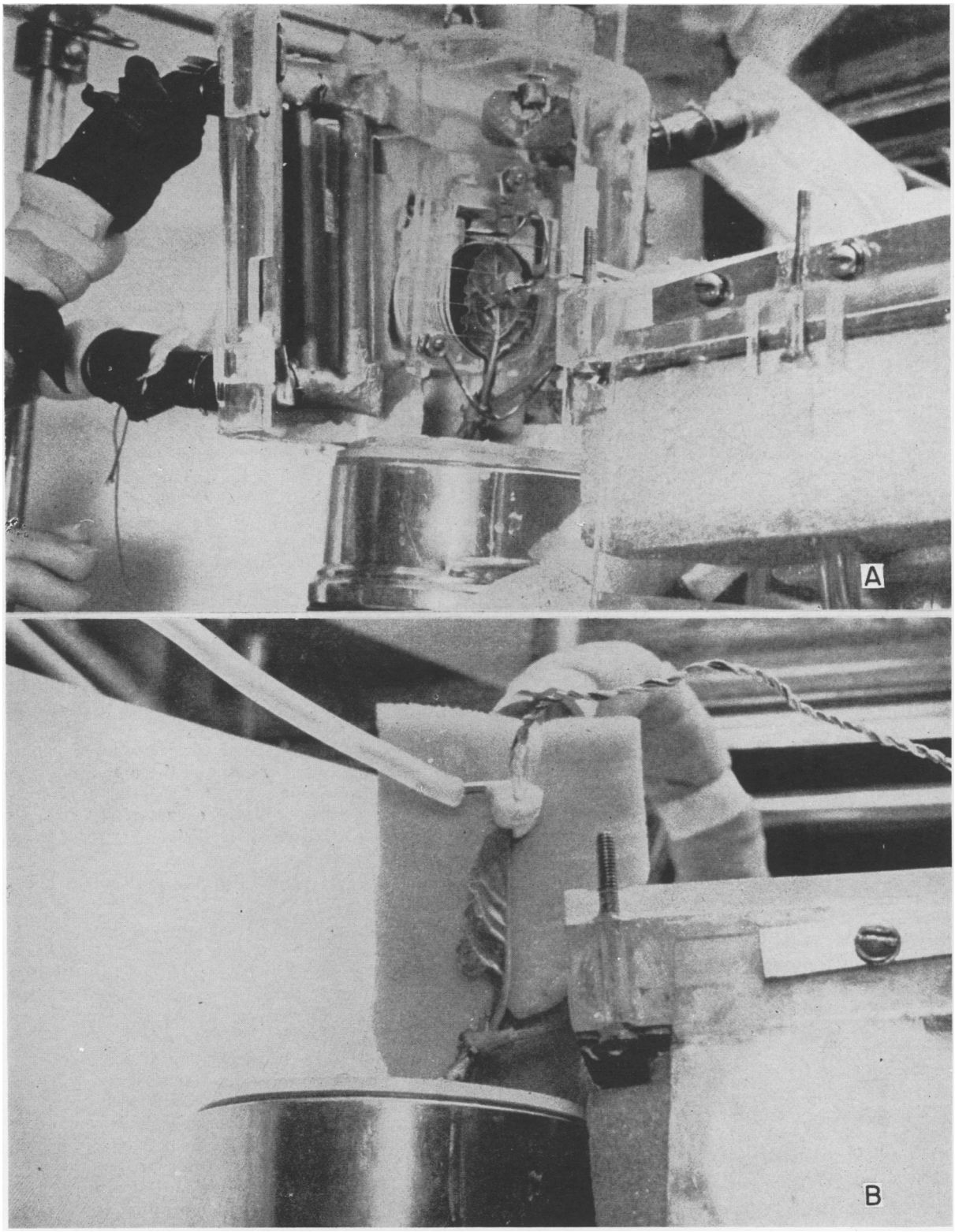


FIG. 1. Cooling jackets. A) Chamber for cooling sink leaf, crown, beet and base of supply leaf petiole. Insulation has been removed, and one wall swung out to show GM monitored sink leaf. Note fan and thermistor. B) Jacket for cooling half of sink leaf. One side of the blade projects into air space of cooling jacket.

tained at the desired temperature by a cooling coil immersed in the mineral solution bathing the roots. A GM-detector projected approximately 5 cm into the cooling chamber and was unaffected by temperature changes in the 0 to 30° range employed.

The sink leaf lamina on only 1 side of the midrib was cooled by inserting that portion of the blade into the chamber of a cooling jacket through a slit in a sheet of plastic foam which closed one side of the chamber (fig 1). A slowly moving stream of air was circulated through the inner chamber containing the leaf to provide even cooling. Cold ethanol from a cooling bath was circulated through the outer chamber of the copper jacket.

In experiments in which cooling was restricted to a 4-cm portion of the beet, a 0 to 1° temperature was maintained by immersing the part of the beet to be cooled in crushed ice.

Carbon dioxide of measured specific activity was supplied at a concentration of approximately 0.05 % (v/v) during the entire labeling period. The method yields translocate of constant specific activity and permits monitoring the translocation rate in a developing leaf that is a known fraction of the total sink (10). Accumulation of translocate in the sink leaf was followed by a GM-ratemeter; the output was processed by means of a computer curve-fit program. Total ^{14}C translocated was determined by oxidizing the sink organs to CO_2 and measuring the activity present with an ion chamber.

Translocation rate was calculated by multiplying the first derivative of curve-fitted GM-ratemeter data by efficiency and specific activity factors as described previously (10). In those experiments in which the entire sink region was cooled, the distribution pattern between sink organs (sink leaf, crown, beet, roots, base of supply leaf petiole) was presumed to remain unchanged during cooling, and translocation rates could be calculated from the ratemeter data. Where only a portion of the sink region was cooled, rates were calculated for delivery into the treated portion of the sink because of possible change in relative distribution between sink organs caused by localized cooling. In cases where the sink leaf was covered with the sink leaf cooling jacket an average translocation rate was calculated from the form of the typical curve for approach to isotopic equilibrium (10) and the ^{14}C content of the sink. Details of methods for ion chamber assay of total ^{14}C and chromatographic analysis of ^{14}C-sucrose were given previously (10).

Results

Effect of Cooling. When the roots, beet, crown and developing leaf that together constitute the translocation sink were cooled to 2 to 5°, 4 phases were seen in the translocation rate monitored in the sink leaf (fig 2). In all 4 experiments of this type there was an immediate decline in rate followed by complete or nearly complete recovery. This phase, which lasted 30 to 60 minutes, was similar to the temporary decrease noted in previous experiments in which a 2-cm portion of petiole was cooled (15). In the present study a 10- to 12 cm length of translocation path was cooled along with the sink region and this temporary decline was interpreted to be due to cooling of the translocation path. During the second phase, which followed recovery, the translocation rate remained steady for 30 to 60 minutes. The third phase began 60 to 90 minutes after the start of cooling and lasted about 100 minutes. During this phase the rate of translocation declined to 35 to 45 % of the rate prior to cooling (table I, rows 1–5). The rate became nearly steady during the fourth phase, and showed minor, long-period oscillations. The decreased rate persisted for 26 hours, the duration of the longest experiment.

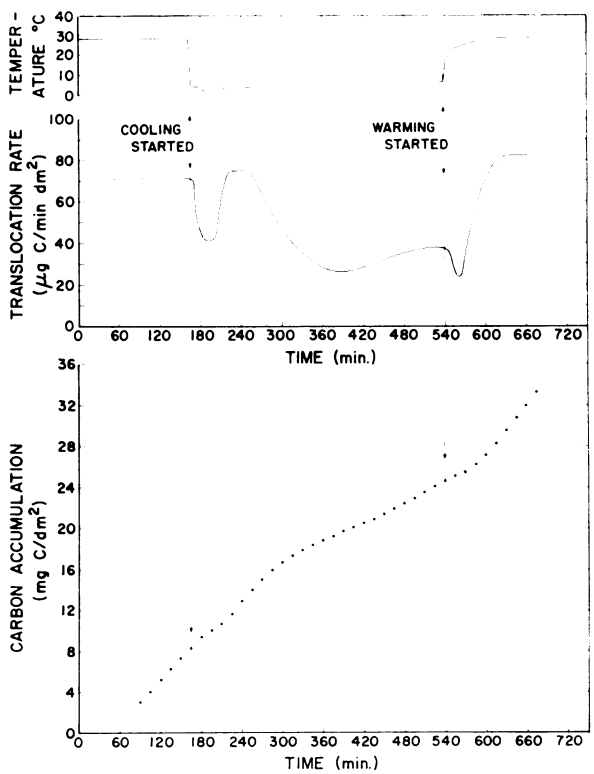
Although there was no sign of wilting, the possibility that the decrease in translocation rate was the result of a water deficit caused by cooling the roots was investigated by keeping the roots at 25° while the beet, crown and monitored sink leaf were cooled to 2 to 5°. The resulting translocation time course was indistinguishable from the curve obtained when roots were also cooled, ruling out decreased water uptake as the cause of reduced translocation into cooled sinks.

In order to study possible diversion of label from a cooled portion of the translocation sink to an uncooled portion, the lamina of 1 side of a developing leaf was cooled to 2 to 5° while the midrib and the lamina of the opposite half of the leaf were kept at 28 to 30°. In 3 experiments the warm half of the leaf was monitored with a G-M detector to determine the time course of any changes in distribution between the cold and the warm half of the sink leaf blade. The sink leaf was assayed for total radioactivity translocated to it and was analyzed chromatographically for labeled ethanol-soluble compounds. The time course of translocation into the warm half of the lamina during cooling of the opposite half is shown in figure 3. Rather than increasing accumulation in the warm portion of the blade, cooling half of the blade caused a decrease in translocation into the warm half (table I, rows 6,7). The translocation time course for the warm portion of the cooled blade shows all of the phases noted for translocation into the cooled sinks described when a major portion of the sink region is cooled, with the possible exception of the first, temporary decrease phase. After 18 hours of cooling, translocation into the warm part of the blade was only 68 % of the rate attained when the opposite, cooled part of the blade was warmed to 28° (table I, row 7). Cooling half the sink leaf caused a gradual reduction in translocation to both the cooled and warm portions of the sink leaf, with a smaller decrease in the warm half than in the cooled half. The average ^{14}C

Table I. *Inhibition of Translocation Rate by Cooling Sinks to 1°*

Experiment No.	Duration of cooling (hrs)	Sink organs cooled	Sink organs in which translocation was measured	Translocation rate* during cooling relative to rate during warm period (%)
1	6.3	Sink leaf, crown, beet, roots base of supply leaf petiole	Sink leaf, crown, beet, roots base of supply leaf petiole	42
2	7.0	Sink leaf, crown, beet, roots base of supply leaf petiole	Sink leaf, crown, beet, roots base of supply leaf petiole	43
3	26	Sink leaf, crown, beet, roots base of supply leaf petiole	Sink leaf, crown, beet, roots base of supply leaf petiole	36
4	5.0	Sink leaf, crown, beet, base of supply leaf petiole	Sink leaf, crown, beet, base of supply leaf petiole	45
5	24	Sink leaf, crown, beet, base of supply leaf petiole	Sink leaf, crown, beet, base of supply leaf petiole	34
			Other half (warm) sink leaf blade	50
6	3.0	One-half sink leaf blade		
7	18	One-half sink leaf blade	Other half (warm) sink leaf blade	68
8	3.0	4-cm of beet	Sink leaf	130

* Translocation rate measured in sink organs listed in column 4 when parts listed in column 3 are cooled.



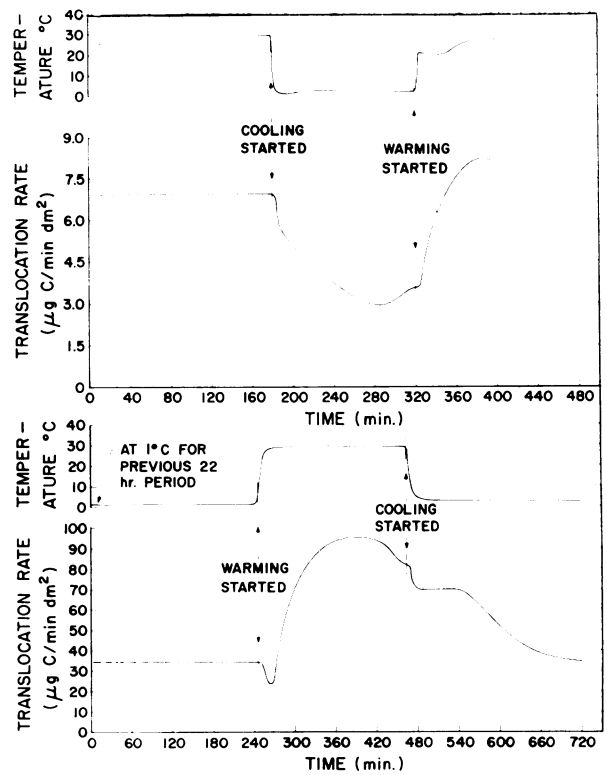


FIG. 2. (left) Rate of translocation of labeled photosynthate during cooling and subsequent warming of the entire sink region (roots, beet, crown, sink leaf). Labeling with $^{14}CO_2$ began at zero min.

FIG. 3. (top, right) Rate of translocation of labeled photosynthate into the warm half of the sink leaf blade during cooling and subsequent warming of the opposite half of the sink leaf blade. The ^{14}C in the monitored warm half of the sink leaf is 14.5 % of the label translocated to the entire sink.

FIG. 4. (bottom, right) Rate of translocation into sink region following prolonged cooling (26 hr total during first cooling period) of entire sink. Labeling began at zero min.

distribution ratio between the halves was 0.6.

Although diversion within a sink organ did not take place as a result of localized cooling, there was an increase in accumulation in uncooled sink organs when another sink organ was cooled. Within 20 minutes after a 4-cm portion of the beet was cooled to 0.5° translocation to the monitored sink leaf increased by 30 % and remained steady during the remaining 130 minutes of cooling (table I, row 8). Within 10 minutes after the beet was warmed, the rate of translocation into the sink leaf decreased to the original, pre-cooling level.

The possibility of a continued decline in rate and significant damage to the cooled region was investigated by using a 20- to 26-hour cooling period which started prior to labeling, and continued through the first 4 hours of labeling (fig 4). In all 3 experiments the translocation rate was found still to be at 35 to 40 % of the usual rate observed for the warm sink, a degree of inhibition similar to that found after 5 to 6 hours of cooling (table I). In experiments with an 18- to 26-hour cooling period (fig 4) as in those with a 5- to 6-hour cooling period (fig 2), warming brought recovery to a rate 2 to 3 times the rate during cooling. This recovery rate was equal to or slightly higher than the rate prior to cooling. Three phases could be distinguished: an initial decline in rate lasting 15 to 30 minutes, a gradual recovery during 30 to 60 minutes and finally, a period of uniform rate. A similar recovery curve was obtained for translocation into the warm side of the sink leaf when the side which had been cooled 18 hours was rapidly warmed.

Chemical Analysis of the Sink Leaf. In those experiments in which only half of the sink leaf was cooled, a comparison was made of differences in distribution of ^{14}C between compounds in the cooled versus the warm half (table II). When the blade half was cooled throughout the labeling period, the proportion of ^{14}C in sucrose was considerably higher in the cooled portion of the leaf. Sucrose was the major labeled compound in the sink leaf blade. If the cooled blade was warmed to 28° for several hours, with continuation of labeling, the pattern of distribution of ^{14}C in sucrose and other fractions was found to be nearly the same in the treated and untreated sink halves. With this cooling schedule sucrose contained a smaller proportion of the label present in the sink leaf.

Discussion

In a review of phloem physiology Esau (6) proposed that sugar may be actively removed from the translocation path in the sink by companion cells and parenchyma cells which lie next to the sieve elements. Zimmermann (19) observed differential removal of sugars from the translocation path, presumably by active transport. In a proposed translocation model, Kursanov (11) regarded active uptake into storage and growing areas as one of the processes which helps to maintain a pressure gradient for mass flow. The inhibition of translocation caused by sink region cooling supports the above models in which a pressure gradient is maintained in part by a temperature-dependent metabolic process such as active transport. In another study (15) it was concluded that the inhibition of translocation brought about by cooling of bean petioles was due to low temperature injury as evidenced by the long, gradual and incomplete recovery when cooling was stopped. In contrast, no permanent inhibition was found when sugar beet petioles were cooled to 0°, indicating neither metabolic inhibition of translocation nor the presence of a serious obstruction. By contrast, the readily reversible inhibition observed for sink cooling in sugar beet suggests that the low temperature is slowing a metabolic process which is responsible in part for motivating translocation.

The gradual decline observed following initiation of sink cooling is interpreted as the result of a gradual decrease in gradient along the translocation path resulting from inhibition of vein unloading. The maintenance of a nearly uniform rate of translocation for up to 26 hours of sink cooling suggests a new rate of vein unloading as a result of metabolic inhibition rather than damage to the translocation system.

Table II. *Distribution of Labeled Compounds in Cooled and Non-cooled Halves of a Sink Leaf*

Treatment schedule during labeling			Warm half		Cool half	
Warm	Cool	Warm	Relative ^{14}C accumulation	Ratio ^{14}C sucrose to ^{14}C present in leaf half (%)	Relative ^{14}C accumulation	Ratio ^{14}C sucrose to ^{14}C present in leaf half (%)
...	3 hrs	...	100	42	97	77
...	18 hrs*	...	100	46	37	60
3 hrs	2.3 hrs	3 hrs	100	21	59	22
...	18 hrs*	2.5 hrs	100	22	30	38

* Labeling was not started until the last 3 hr of the cooling period.

The fact that translocation continues at a 35 to 45 % level even after 26 hours of sink cooling gives support to the existence of active loading at the vein endings in the source, a mechanism suggested by others (1, 3, 4, 6, 8, 11, 13, 15, 17). Recovery curves often gave evidence of an overshoot following warming (fig 4) suggesting an increase in the osmotic potential in the source as a result of the build-up of sugar occasioned by inhibition of translocation.

The 30 % increase in translocation to the sink leaf caused by cooling a portion of the beet agrees with the observation of Linck and Swanson (12) that cooling a developing pea fruit at one node caused a decrease in arrival of ^{32}P at that pod and an increase in translocation of label to a pod at another node. Diversion of label from a cooled region to a warm region conforms to the general pattern of organic translocation toward regions of high metabolic activity. Active vein unloading appears to be a feasible mechanism for controlling distribution to regions upon demand and could explain the reversal of direction of movement under changing conditions noted by others (14, 16).

The inhibition noted in an uncooled portion of a sink organ when part of the sink is cooled is more difficult to explain. If a given file of sieve elements has termini in both the warm and cool portions of a sink organ, an increase in osmotic potential in phloem of the cold region as a result of inhibition of vein unloading would decrease the gradient along the file of sieve elements. The rate of transport to both warm and cool regions would lessen, with the latter being inhibited to a greater extent.

Cooling cells of the sink region should also inhibit metabolic conversion of the translocate and thus increase the proportion of label found in those compounds which are translocated. Earlier studies (9, 10) showed that sucrose was the main molecular species entering the translocation stream. In the present study the proportion of label in sucrose alone increased markedly in the cooled side of the sink leaf, indicating that it is primarily sucrose that arrives in the sink region. No other compound soluble in 80 % (v/v) ethanol contained a significant proportion of ^{14}C. The present findings neither support nor rule out active unloading via temporary phosphorylation as suggested by Kursanov (11).

At present microautoradiographic and histochemical studies are under way to localize the site of inhibition of translocation within sink region tissues.

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Literature Cited

1. Bauer, L. 1953. Zur Frage der Stoffbewegungen in der Pflanze mit besonders Berucksichitgung der Wanderung von Fluorochromen. Planta 42: 367–451.
2. Bieleski, R. L. 1960. The physiology of sugarcane. III. Characteristics of sugar uptake in slices of mature and immature storage tissue. Australian J. Biol. Sci. 13: 203–20.
3. Bieleski, R. L. 1966. Sites of accumulation in excised phloem and vascular tissues. Plant Physiol. 41: 455–66.
4. Crafts, A. S. 1951. Movement of assimilates, viruses, growth regulators and chemical indicators in plants. Botan. Rev. 17: 203–84.
5. Curtis, O. F. and S. D. Herty. 1936. The effect of temperature and light on the translocation of photosynthetic products. Am. J. Botany 23: 528–32.
6. Esau, K. 1961. Plants, Viruses and Insects. Harvard University Press, Cambridge, Massachusetts. p 55.
7. Esau, K. 1965. Parenchyma cells in the conducting system (the "pumps" and "sinks"). Plant Physiol. 40: xxvii.
8. Esau, K., H. B. Currier, and V. I. Cheadle. 1957. Physiology of phloem. Ann. Rev. Plant Physiol. 8: 349–74.
9. Geiger, D. R. and C. A. Swanson. 1965. Sucrose translocation in the sugar beet. Plant Physiol. 40: 685–90.
10. Geiger, D. R. and C. A. Swanson. 1965. Evaluation of selected parameters in a sugar beet translocation system. Plant Physiol. 40: 942–47.
11. Kursanov, A. L. 1963. Metabolism and the transport of organic substances in the phloem. Advances in Botanical Research, Vol. 1. R. D. Preston, ed. Academic Press, New York. p 209–78.
12. Linck, A. J. and C. A. Swanson. 1960. A study of several factors affecting distribution of phosphorus-32 from the leaves of *Pisum sativum*. Plant Soil 12: 57–68.
13. Müller, K. and A. C. Leopold. 1966. The mechanism of kinetin-induced transport in corn leaves. Planta 68: 186–205.
14. Nelson, C. D. 1962. The translocation of organic compounds in plants. Can. J. Botany 40: 757–70.
15. Swanson, C. A. and D. R. Geiger. 1966. Time course of low temperature inhibition of sucrose translocation in sugar beets. Submitted N. B. to Plant Physiol.
16. Thrower, S. L. 1962. Translocation of labelled assimilates in soybean. II. The pattern of translocation in intact and defoliated plants. Australian J. Biol. Sci. 15: 629–49.
17. Wanner, H. 1952. Phosphataseverteilung and Kohlenhydrattransport in der Pflanze. Planta 41: 190–94.
18. Webb, J. A. and P. P. Gorham. 1965. The effect of node temperature on assimilation and translocation of ^{14}C in the squash. Can. J. Botany 43: 1009–20.
19. Zimmermann, M. H. 1958 Translocation of organic substances in trees III. The removal of sugars from the sieve tubes in the white ash (*Fraxinus americana* L.). Plant Physiol. 33: 213–17.