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Pattern of Carbohydrate Distribution in Girdled and Intact Squash Petioles

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PATTERN OF CARBOHYDRATE DISTRIBUTION IN GIRDLED AND INTACT SQUASH PETIOLES

(TITLE)

BY

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THESIS

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PATTERN OF CARBOHYDRATE DISTRIBUTION IN GIRDLED AND INTACT SQUASH PETIOLES

The mechanism of transport of photosynthetic products from areas of systhesis to areas of utilization has long been a controversial subject. Many theories have been proposed and nearly as many have been rejected. Presently, it is generally agreed that organic translocation in angiosperms takes place through the sieve elements of living phloem tissue and that the process requires metabolic energy (9,12). How this energy is used and what the actual mechanism of translocation is are still the subject of much research.

The proposed theories of organic translocation can be grouped into three catagories: (1) protoplasmic streaming, (2) electro-osmosis, and (3) mass flow. The first of these, protoplasmic streaming, has been championed by Thaine (10, 11,12). He suggests that translocation takes place via small mitochondria-like particles moving through fine transcellular strands. The driving force involved would be a transcellular form of protoplasmic streaming. This theory provides a very neat explaination of the bidirectional flow that has been demonstrated by Biddulph and Cory (2). According to this theory streaming could easily take place in opposite directions within a single sieve element. There are several

strong arguments in opposition to this theory. Esau et. al. (5) doubt the existence of protoplasmic streaming in mature sieve elements, instead suggesting that what Thaine described as transcellular strands were simply lines caused by the diffraction of light from cells out of focus in his tissue sections. More evidence casting doubt on this theory has been presented by Swanson and Geiger (14). They showed that cooling a petiole slowed or stopped translocation for only a short time. Translocation resumed at near normal rates at temperatures that are thought to be too low for rapid protoplasmic streaming to take place.

The secound theory, that of electro-osmosis, was originally proposed by Spanner (13). He maintains that the energy necessary to move the photosynthates came from bio-electric forces. The proposed driving force is the result of a circulation of potassium or other ions through the sieve plate and back via the companion cells. This theory presently has few adherents. Crafts and Crisp, in their comprehensive book on phloem transport, discount the theory saying that electro-osmotic pump components do not occur in normal, mature seive elements (4).

The third theory, mass flow, is the most widely accepted. It was first presented by Munch in 1927 (10) and has been studied and revised a great deal since. This theory contends that photosynthates move through the phloem in mass as a solution. The driving force is a hydrostatic pressure

gradient created by high carbohydrate concentrations in the phloem near areas of synthesis. One of the main objections to this theory is its difficulty in explaining bidirectional movement of materials as reported by Biddulph and Cory (2). If it is ever shown, unequivocally, that there is bidirectional movement of materials within a single sieve element, mass flow will have to be eliminated from the list of possible transport mechansims. However, this theory requires the least amount of cytoplasmic involvement and seems to agree with most of our present knowledge of mature phloem structure, points which justify its present popularity.

Webb and Gorham (19) using $^{14}CO_2$ have shown that both stachyose and sucrose are major photosynthetic products in squash and that they are both translocated via the phloem. They saw no apparent relationship between the rates of movement of the labelled sucrose and the labelled stachyose. However, if the mass flow hypothesis is correct, the two sugars should flow at approximately the same rate. Ever since the classical work of Mason and Maskell (9) on cotton, it has been known that blocking the phloem causes a buildup of sugars in the phloem above the blockage. According to Aronoff (1), working with soybeans, translocation will not take place out of a leaflet if the petole has been steam girdled. Gage and Aronoff (6) added that steaming a stem of a seedling soybean plant affected transloction in areas above the blockage, but did not stop the translocation to the steamed point. To this time no one has

observed the pattern of carbohydrate buildup in a petiole that has been steam girdled.

It is the purpose of this study to see if the two carbohydrates that are thought to be translocated in squash behave in the same way at a phloem blockage. The pattern of carbohydrate buildup may yield supporting evidence for one of the proposed theories.

METHODS AND MATERIALS

Straightnecked squash plants (Cucurbita melopepo torticollis, Bailey) were used in this experiment because squash is known to translocate both sucrose and stachyose and has been the subject of much research in the past (6,8,11,18,19). The squash seeds were germinated on moist filter paper in petri dishes for four days. The germinating seeds were then transferred to a layer of cheesecloth suspended over an aerated, 10^{-4} molar $CaSO_4$ solution. After four days the seedlings were moved to a Sherer Controlled Environment Chamber and were grown hydroponically in an aerated, modified Hoagland solution (Table 1) for 18 additional days. The plants recieved 16 hours of light at an intensity of about 1,000 foot candles at the level of the plants and 8 hours of darkness daily. The light period temperature was 24 C and the dark period was 19 C. The plants at this stage of growth had six to eight leaves and flower buds were being initiated.

On the eighteenth day in the controlled environment chamber the plants were removed after approximately seven hours of darkness and placed in individual flasks of fresh nutrient solution. The petioles from the third and fourth nodes were studied because these seemed to have large, healthy and mature leaves. One petiole of each plant was girdled with a fine spray of steam until the petiole appeared shrivelled. This required about one minute of steaming. The narrow girdle was

Table 1. Modified Hoagland Solution

$Ca(NO_3)_2$	3×10^{-3} M
KH_2PO_4	2×10^{-3} M
KNO_3	2×10^{-3} M
$MgSO_4$	2×10^{-3} M
H_3BO_3	800×10^{-7} M
$MnCl_2$	150×10^{-7} M
$ZnCl_2$	15×10^{-7} M
$CuCl_2$	8×10^{-7} M
MoO_3	7×10^{-7} M
Fe (as Fe-EDTA)	900×10^{-7} M

made 6 cm below the leaf blade. The other petiole studied on each plant was left untreated as a control. The girdling of the petiole was done under low light intensity (below 10 foot candles) to keep photosynthetic activity to a minimum. The plants were then exposed to incandescent light of 2,500 foot candles for varying lengths of time. The light was filtered through water to minimize any affects of heat given off by the light source. After exposure to the light the petioles were immediately cut into three 2-centimeter sections, meauring from the blade down the petiole. The sections were quickly weighed to the nearest miligram on a Sartorius analytical balance and were frozen at -25 C.

The sugars from each petiole section were extracted separately with 80% (v/v) ethanol for 2 hours using a micro-soxhlet extraction bank. The extracts were diluted to 25 ml with 80% ethanol and stored at -25 C. Separation of the individual sugars was accomplished using paper chromatography techniques. Either 100 or 250 microliters of each petiole extract were streaked across the top of 7 x 46 cm sheets of Whatman #1 chromatography paper. Twenty five micoliters of a 1% solution of sucrose, stachyose and raffinose were spotted on side strips of the paper. The chromatograms were developed with a descending flow of a 1-butanol: acetic acid: water (3:3:2) solvent system for 24 hours. Chromatograms were dried in a forced-draft chromatography oven for 10 minutes at 100 C. The side strips were cut from the chromatograms and sprayed

with benzidine reagent to locate the positions of the known sugars. The benzidine reagent was made up of 0.5 grams benzidine dissolved in 10 ml of 40% (w/v) trichloroacetic acid, and 80 ml of 95% ethanol. The benzidine-sprayed side strips were developed by placing them in the forced-draft oven for 15 minutes at 100 C. The portions of the chromatograms containing sucrose and stachyose were cut from the chromatograms after using the known sugar side strips to locate their positions. The individual sugars were eluted into test tubes with one to two millliliters of distilled water. The eluate was then evaporated to dryness with a Rotary Evapo-Mix under reduced pressure with the water bath at 60 C. One millliliter of distilled water and three milliliters of anthrone reagent (200 mg anthrone + 100 ml concentrated H_2SO_4) were added to each dried eluate. This solution was mixed with a Vortex mixer and placed in a boiling water bath for ten minutes. The samples were transferred to Spectronic 20 tubes and their optical densities were measured at 620 nanometers with a Bausch and Iomb Spectronic 20 Spectrophotometer. Data from standard solutions prepared earlier indicated that in the range of concentrations encountered each 1.2 micrograms stachyose and 0.7 micrograms sucrose caused a 0.01 unit increase in the optical density of the samples.

RESULTS

The results of the sucrose measurements are reported in Table 2 and Figure 1. The sucrose level in the untreated petiole stayed essentially constant with the length of time under illumination. In the steam girdled petiole there was a gradual increase in the sucrose concentration, but it was not large enough to be consistent with what one would expect if sucrose were being actively transported to the steammed point.

The stachyose concentration measurements are reported in Table 3 and Figure 2. The stachyose concentrations in the untreated petiole sections were similar to the sucrose concentrations and varied little with the length of time of illumination. In the steam girdled petiole there was little stachyose build up in the sections having mean distances from the blade of 1 and 3 cm. In contrast, the section 5 cm from the blade and next to the steam girdle, there was a dramatic increase in the stachyose concentration after 10 minutes of light. After the initial increase in concentration the stachyose level dropped gradually for the next 50 minutes until it reached near normal levels.

The concentrations of raffinose were not measured because it was felt that the results of Webb and Gorham (19) who report that raffinose does not assimilate ^{14}C when a leaf is feed $^{14}CO_2$ and data obtained in a preliminary experiment that indicated raffinose does not build up at a steam girdle indicates that raffinose is not a major transport sugar.

Table 2. Comparison of sucrose concentrations in 2-cm petiole sections of plants exposed to light varying lengths of time.

Untreated petiole - mg sucrose/gram fresh wt.

		Length of time exposed to light (minutes)			
		5	10	25	60
Mean distance of 2-cm petiole section from blade (cm)	1	3.6	7.4	8.3	5.7
	3	3.8	3.0	6.0	9.8
	5	3.6	3.5	5.5	10.1

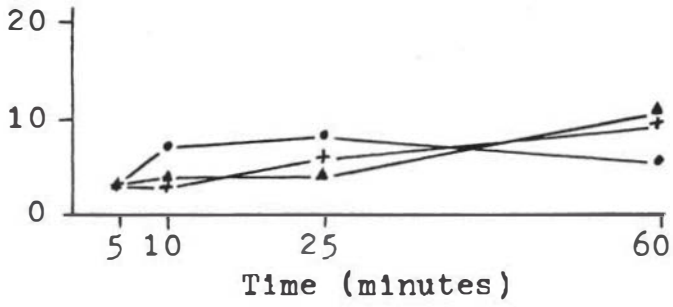
Steam girdled petiole - mg sucrose/gram fresh wt.

		Length of time exposed to light (minutes)			
		5	10	25	60
Mean distance of 2-cm petiole section from blade (cm)	1	4.9	5.6	6.3	16.1
	3	5.1	5.1	7.5	10.7
	5	7.4	7.1	4.0	9.8

Figure 1. Fluctuations in sucrose concentrations in 2-cm petiole sections of plants exposed to light varying lengths time.

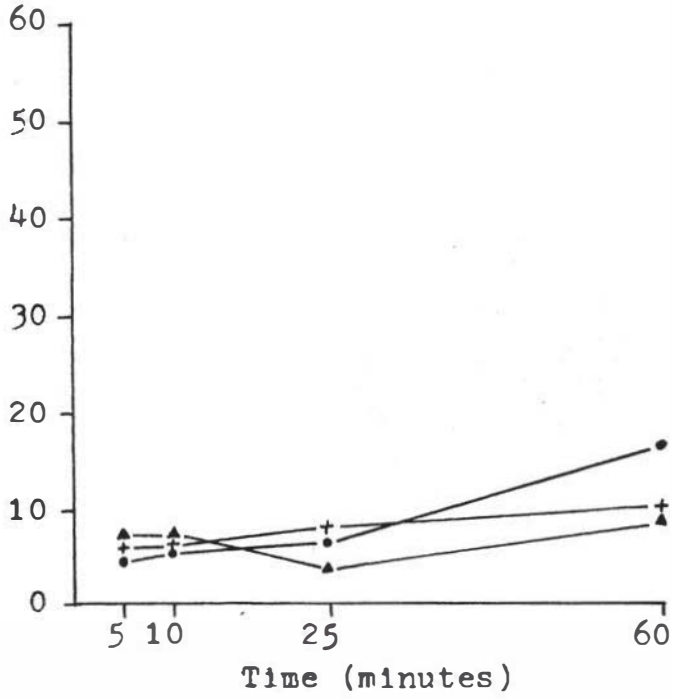
Untreated Petiole

mg. stachyose/ gram fresh wt.



Steam Girdled Petiole

mg. stachyose/ gram fresh wt.



▲ = section from 0 to 2 cm from blade
+ = section from 2 to 4 cm from blade
• = section from 4 to 6 cm from blade

Table 3. Comparison of stachyose concentrations in 2-cm petiole sections of plants exposed to light varying lengths of time.

Untreated petiole - mg stachyose/gm fresh wt.

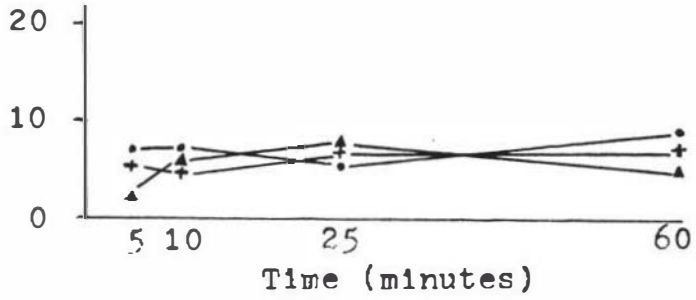
		Length of time exposed to light (minutes)			
		5	10	25	60
Mean distance of 2-cm petiole section from blade (cm)	1	6.8	7.3	5.2	8.1
	3	5.9	5.4	6.3	6.5
	5	2.6	6.1	6.5	4.5

Steam girdled petiole - mg stachyose/gm fresh wt.

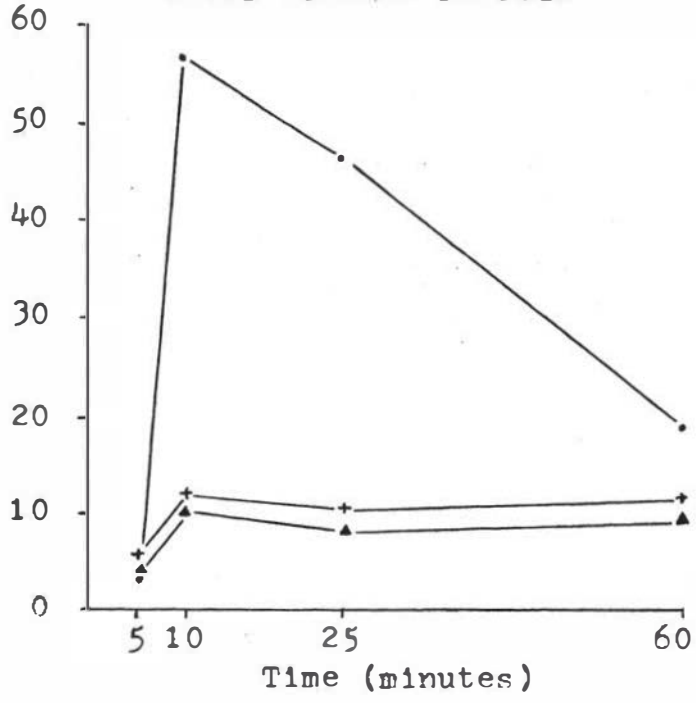
		Length of time exposed to light (minutes)			
		5	10	25	60
Mean distance of 2-cm petiole section from blade (cm)	1	3.0	56.6	46.1	18.3
	3	5.1	12.6	10.2	11.1
	5	4.7	10.4	8.7	9.6

Figure 2. Fluctuations of stachyose concentrations in 2-cm petiole sections of plants exposed to light varying lengths of time.

Untreated Petiole



Steam Girdled Petiole



▲ = section from 0 to 2 cm from blade
\+ = section from 2 to 4 cm from blade
• = section from 4 to 6 cm from blade

DISCUSSION

When a leaf is exposed to light and photosynthesis takes place, carbohydrates are produced. These must be transported to the growing points and storage organs of the plant. It has been found that most angiosperms translocate carbohydrates in the form of sucrose (4). The data presented here clearly shows that during the first hour of photosynthesis sucrose does not build up at a phloem blackage and therefore is probably not being transported in any great quantity in squash. Stachyose does build up and is probably the main transport sugar. The evolution of selectively transporting large molecules such as stachyose seems resonable since by transporting large molecules a greater amount of carbon can be moved without upsetting the osmotic pressure of cells.

This selective transport of one sugar over another, when both are known to be translocatable, is difficult to explain using the mass flow hypothesis. A solution flowing in mass could not have this selective nature. The one possible explaination could be that stachyose is selectively transported out of the cells adjacent to the phloem, so that the selectivity would be a part of the photosynthetic or bundle sheath tissue, not the vascular tissue.

Another aspect of the data presented that is difficult to explain using the mass flow hypothesis is the large build up of stachyose. Transport driven by a hydrostatic pressure

gradient should not allow such a large concentration of a sugar to develop. The concentration of sugars in the petiole must have been higher in the petiole near the steamed point than in the leaf. Transport should have stopped when the 5 cm section and the leaf had approximately the same concentration.

If the mechanism is protoplasmic streaming, as described earlier, it would be interesting to see if there is an increase in the number of mitochondria-like particles in the petiole sections with high stachyose concentrations. It would also be interesting to see if the same pattern of sugar concentrations appears when a petiole is steam girdled after the plant has been exposed to light for several hour. There may be a different pattern, for Webb and Gorham (19) report that little newly synthesized sucrose is exported during the first 30 minutes of photosynthesis in squash. In conclusion, the data presented does not suggest what what transport mechanism is involved, only that it is probably not mass flow.

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