Parameters Controlling Sucrose Content and Yield of Sugarbeet Roots*

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Agronomists and plant breeders working with sugarbeets have long been frustrated by the inverse relationship that exists between sucrose content and root yield. Genetic selection and agronomic practices that tend to increase yield decrease sucrose content, and those that increase sucrose content decrease yield.

In the past, plant breeders made significant gradual improvements in the potential sucrose content of commercial cultivars. In recent years, progress has slowed and we seem to have reached an impasse. Significant progress in the production of new genotypes possessing both high yield and high sucrose will require new, innovative selection criteria. Simple selection criteria based on limited physiological factors may be the answer. Such criteria could also be used to evaluate chemical growth regulators for their ability to regulate partitioning of photosynthate to maximize yields.

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This paper will discuss partitioning of photosynthate in sugarbeet and report the results of recent investigations on the inverse relationship between sucrose content and root yield. The objective of this work was to delineate areas for needed research and to propose several physiological principles for use as a basis in efficient genetic selection.

Evidence for balanced partitioning of photosynthate between root growth and sucrose accumulation will be presented. This partitioning is regulated in the root and it operates independently of photosynthate supply.

Sucrose is translocated to the root via the phloem, and evidence will be presented to show that it then passes into the free space between the root cells and then diffuses into the interring area. Our hypothesis is that the final sucrose content of storage parenchyma cells is regulated by length of the diffusion path and by the proportion of cells located near the vascular tissue where free-space sucrose concentrations are highest. Large cells and wide rings are related to high yield, whereas small cells and narrow rings are related to high sucrose content.

Allocation of Photosynthate

Photosynthate is allocated to the sugarbeet root continuously throughout the growing season. The priorities for allocation proposed by Fick et al. (3) are respiration, top growth, fibrous root growth, and storage root growth including sucrose accumulation. However, the proportion of available photosynthate allocated to each sink varies continuously throughout the season depending on the relative "sink strength" of the individual plant part. This type of continuous season-long partitioning is termed "balanced" as opposed to the "phasic" partitioning that occurs in potatoes, corn, wheat, etc. (5).

The photosynthate allocated to the root is partitioned between growth and sucrose storage. Root growth here includes both fibrous and tap roots. Snyder et al. (9) have good evidence that genetically controlled partitioning occurs between the fibrous roots and tap root and that this partitioning may be an important component of yield.

Some controversy exists concerning the pattern of partitioning between root growth and sucrose storage throughout the growing season. Based on results of greenhouse and growth chamber studies, Ulrich (10) proposed that a major portion of the photosynthate translocated to the root was allocated for root growth until late in the growing season when growth was retarded by low temperature and nitrogen deficiency. This confirmed the previous work of Bouillene et al. (2) and van de Sande Bakhuyzen (12) who were able to distinguish three growth phases in the development of the sugarbeet plant; i.e., leaf formation, root formation, and a ripening phase. This work suggested a phasic partitioning of photosynthate for sucrose storage and assumed that the sucrose stored in the root was sucrose in excess of that utilized for growth and maintenance. In recent work by our group at Logan, we have been unable to confirm a phasic pattern for sucrose storage. Our results confirmed those of Bergen (3), van Ginnekin (11), Milford (6), Storer et al. (8), and Follett et al. (4), and indicated that partitioning of photosynthate between root and shoot and partitioning between growth and sucrose accumulation within the root (Figure 1) occur continuously throughout the growing season. The results of our work and of others are summarized in Figure 2. The only difference between these results and those of Ulrich is the linear increase in sucrose concentration throughout the season.

The theory that only sucrose not required for growth is available for storage (10) suggests that increasing photosynthetic rates should enhance sucrose concentrations. However, if sucrose partitioning is balanced between growth

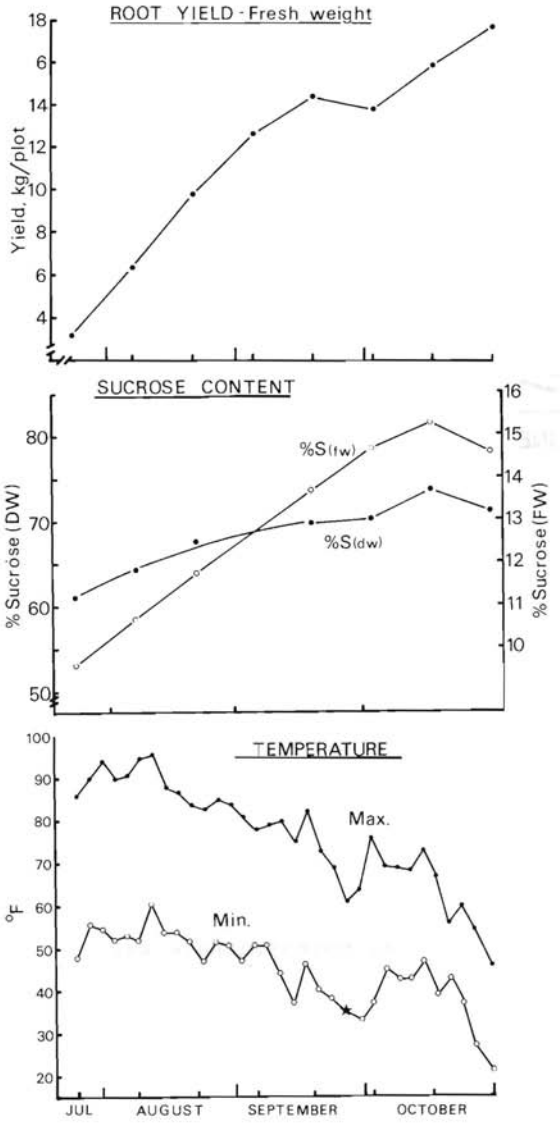


Figure 1. Seasonal growth patterns for root yield and sucrose content at Logan, Utah, in 1972. Data represents the mean of six cultivars grown in a replicated field trial and harvested at two-week intervals. Temperature data are three-day averages.

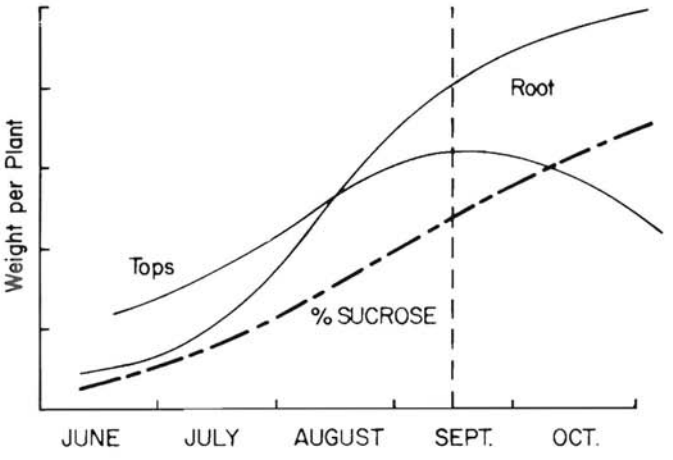


Figure 2. Seasonal pattern of root, tops, and percent sucrose. Note linear pattern of sucrose accumulation. See Bergen (1); van Ginnekin (11); Milford (6); Storer et al. (8); Follett et al. (4).

and storage, then carbohydrate supply should increase yield but have no effect on sucrose content. Field photosynthetic rates and thus photosynthate supply can be increased by CO_2 enrichment of the air within the leaf canopy. Results of such an experiment are given in Table 1. Increasing the CO_2 level to 700 ppm increased root yield by 21%, but reduced the sucrose content from 15.4 to 15.1. Therefore, the additional photosynthate was not utilized for sucrose storage. This conclusion is confirmed by the work of Watson et al. (13) who used shading to reduce photosynthate supply. Shading reduced root dry weight yield but did not alter the sucrose to dry weight ratio. Thus translocated photosynthate was partitioned within the root between growth and sucrose storage and was independent of photosynthate supply.

These data further substantiated the hypothesis of a balanced partitioning of sucrose between storage and growth

in the sugarbeet root. This balanced partitioning concept is important to an understanding of the sucrose-yield relationship.

The discrepancy between the works of Ulrich and of Bergen (1), van Ginekin (11), Milford (6), Storer et al. (8), and Follett et al. (4) may be explained in several ways. In Ulrich's work the plants were grown in containers in nutrient solutions. Such conditions present a physical restraint to root growth and provide much more abrupt environmental changes (temperature, nitrogen supply, etc.) than field conditions do. The more complex field environment would tend to dilute the effects of a change in any one environmental factor. Thus, the results of experiments conducted in relatively simple environments may not be transferable to the field.

Table 1. Effect of CO_2 enrichment on the sucrose content and root yield of sugarbeets.

Treatment	Yield	Sucrose	Sucrose Yield
	lbs/plot	percent	lbs/plot
Control	36.2	15.4	5.6
700 ppm CO_2	43.7	15.1	6.6
LSD (.05)	2.2	.34	.37

Carbon dioxide was supplied to the canopy via perforated tubes located between the rows throughout the growing season. All plots were surrounded (top open) by an 80 cm high clear plastic shield to help maintain the CO_2 level. CO_2 levels were determined within the canopy by gas chromatography.

The site for control of partitioning between sucrose storage and root growth is of obvious importance. The site of control should be apparent if reciprocal grafts of roots and shoots are made between sugar and yield type plants. Such a study was conducted at Logan, Utah in 1977.

The results indicated that control of sucrose storage is located in the root (Table 2). For example, the L19 root increased the sucrose storage capabilities of Fodder tops by 35%, but the L19 top only increased the sucrose concentration in Fodder roots by 13%. Conversely, Fodder tops reduced the sucrose content of the L19 root by only 5%. However, root weight was about equally controlled by the shoot and root. Therefore, the photosynthetic capacity of the leaves and the growth potential of the root are both important for maximizing root growth, but the partitioning between growth and sucrose storage is controlled primarily in the root.

Table 2. Relative effects of root and shoot on sucrose content and root size. Data are from grafts of L19 and fodder.

		Effect on	
		Sucrose	Yield
		percent change	
L19[1]	Scion	+13	-21
	Stock	+35	-25
Fodder	Scion	- 5	CT*
	Stock	-30	CT

*Curly top infected

[1]L19 has a high sucrose content but low root yield. Fodder (Blanca) has a low sucrose content but a high root yield.

Lateral Movement of Sucrose

Determination of the pathway of sucrose movement within the storage root and of the biochemical mechanism of its uptake into root storage cells may help explain the balance between growth and sucrose storage.

Before biochemical studies can be initiated, we must know the morphological pathway of sucrose movement from the

phloem cells to the storage parenchyma. Two possible pathways exist (Figure 3). It is possible for sucrose to move

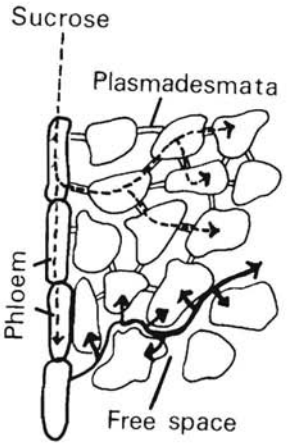


Figure 3. Diagramatic representation of possible pathways of lateral sucrose movement in the sugarbeet root. Solid line, apoplastic; dashed line, symplastic.

from the phloem directly into adjacent cells via plasmadesmata. In this case, sucrose would be actively held and actively transported at all times throughout its movement from the phloem to the interior of the vascular ring. This is an example of movement through the symplast. The second possible mechanism is movement through the apoplast or free space. In this case the phloem cells would unload sucrose directly into the free space between the parenchyma cells where it would move by diffusion away from the vascular ring. Sucrose moving via this pathway would not be actively held while in the free space and, therefore, could easily be washed out of the tissue.

Two experiments were conducted to determine which of these pathways is operable in the sugarbeet root. In Experiment 1, sugarbeet plants growing in the field were

exposed to $^{14}CO_2$ for 30 minutes. Roots were then harvested at regular intervals over a 24-hour period after exposure to the $^{14}CO_2$. At each harvest, a piece of tissue was removed from the root and cut into 1-mm slices. Small disks (4 mm diameter) were punched out of the slices and water solubles were extracted for either 30 seconds or 60 minutes in running tap water. Then the radioactivity remaining in the tissue was determined and the percentage of activity washed out was calculated.

The results indicated that a major portion of the sucrose could be washed out immediately after translocation to the root, but, after 24 hours of uptake, the sucrose was actively held by the tissue (Table 3). This is consistent with movement in the apoplast.

In a second experiment, the inhibitory properties of glucose on sucrose uptake were utilized to substantiate the apoplastic movement theory. Glucose strongly inhibits sucrose uptake. Previous studies in our laboratory with glucose and glucose analogs have shown the site of inhibition to be at the plasmalemma (Wyse, unpublished data). Therefore, if glucose is introduced into the free space of a root, it should prevent sucrose uptake into the cytoplasm. This lack of uptake would leave a greater proportion of the sucrose in the free space, thus allowing a greater proportion to be washed out of the tissue.

Glucose (0.1M), sucrose (0.1M) or water were introduced through a small hole punched into the root with an 18 gauge needle. Uptake of the solutions was started 18 hours prior to $^{14}CO_2$ exposure and continued throughout a 24-hour chase period. The water soluble compounds in the area around the cavity were then extracted as described previously. Glucose significantly increased the percentage of translocated photosynthate washed out of the tissue, which is consistent with the theory of apoplastic movement of sucrose in sugarbeet root (Table 4).

Table 3. Proportion of translocated photosynthate in the free space of sugarbeet root tissue during a 24-hour chase period

Time after $^{14}CO_2$ Exposure	Percent Wash out
30 min	88
60 min	63
90 min	53
2 hr	29
4 hr	24
6 hr	15
24 hr	5

Leaves of field grown sugarbeet plants were exposed to $^{14}CO_2$ for 30 min. At regular intervals the plants were harvested and sections of root excised. Disks 1 X 4 mm were prepared. Samples of disks were washed for either 30 sec or 60 min and the amount of radioactivity remaining in the tissue was determined by 80% ethanol extraction. A 30 sec wash removed soluble materials from the cut cells on the surface, a 60 min wash removed soluble materials from the free space, and 80% hot ethanol extraction removed the remaining soluble sugar, presumably that stored in the vacuole. The percent of total counts in the free space was calculated as:

$$\frac{\text{30 sec wash tissue - 60 min wash tissue}}{\text{30 sec wash tissue}} \times 100$$

Table 4. Effect of free space inhibitors on wash out of translocated photosynthate.

Competing Sugar	Percent Wash out
Control	27
Sucrose	34
Glucose	52

Sucrose (0.1M), glucose (0.1M), or water were introduced into the root free space via a cavity punched into the root with an 18 ga needle. The cavity was filled and connected to a reservoir via a glass capillary tube. The solutions were administered continuously 18 hours prior to $^{14}CO_2$ exposure of the leaves and during a 24-hour chase period. Extraction was as previously described in Table 3.

Since sucrose moves through the free space, a potential factor limiting sucrose accumulation may be the ability of root cells to actively move sucrose from the free space into the vacuole of the storage cells. This process is against a concentration gradient and thus requires considerable energy.

To determine if the uptake mechanism may be the limiting factor, a comparison of the mechanism in several cultivars differing greatly in yield and sucrose storage potential was made (Table 5). The cultivars selected were Blanca (Fodder type, KWS), GWD2 (commercial hybrid, GWS), and L53XL19 (high sugar experimental hybrid). The sucrose content of the cultivars was 63, 70, 71 percent (dry weight), respectively, at the time of the experiment.

Samples of root tissue (1 X 4 mm disks) were exposed to radioactive sucrose, glucose, and fructose for 3 hours, and the rate of uptake into the vacuole of each variety determined. Labeled sugar held by the tissue after a 30-minute wash with cold tap water was assumed to be located in the vacuole. No significant differences in the rate of sucrose uptake existed in the three cultivars (Table 6.) The disks represented a constant volume of tissue; therefore, on a dry-weight basis, the fodder beet was capable of taking up considerably more sucrose than the sugar types. These data showed no cause and effect relationship between the uptake capacity of the tissue and the sucrose concentration in that tissue. The rates of glucose and fructose uptake were much lower than that of sucrose in all varieties.

Table 5. Comparison of percent dry matter, percent sucrose (fresh weight basis), and percent sucrose (dry weight basis) of Blanca, L53XL19, and GWD2 at harvest.

	Dry Matter	Sucrose	Sucrose
	Percent	Percent of fresh wt.	Percent of dry wt.
Blanca	15.0	9.5	63
L53XL19	24.5	17.5	71
GWD2	23.0	16.0	70

Table 6. Interaction between sucrose, glucose, and fructose during uptake into the storage cells of Blanca, L53XL19, and GWD2.

Variety	Uptake Sugar	Uptake Rate	Inhibition		
			Sucrose	Glucose	Fructose
		µmol./3 hrs/20 disks		Percent	
GWD2	Sucrose	5.49 ± 0.33	--	81.3	59.4
	Glucose	0.56 ± 0.05	-11.4	--	- 6.8
	Fructose	0.29 ± 0.03	2.6	74.1	--
L53XL19	Sucrose	5.17 ± 0.36	--	86.3	68.6
	Glucose	0.99 ± 0.01	7.7	--	2.7
	Fructose	0.88 ± 0.07	5.1	31.8	--
Blanca	Sucrose	5.72 ± 0.71	--	82.5	61.4
	Glucose	0.69 ± 0.02	-15.3	--	-14.0
	Fructose	0.48 ± 0.07	12.8	63.1	--

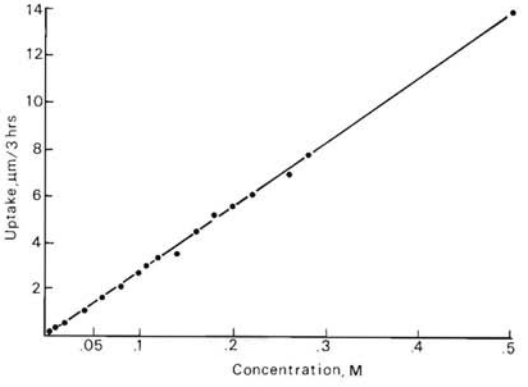
Disks were washed 30 min in running tap water before incubation. The incubation media contained the uptake sugar [0.1M, sp. act (dpm/µmol.): sucrose, 1.25×10^4; glucose, 1.15×10^4; fructose, 2.2×10^4] and the inhibiting sugar (0.05M) in 5 mM PO_4 buffer (pH 6.5). After a 3 hr incubation the disks were washed for 30 min in tap water before extraction in hot 80% EtOH. Radioactivity in the EtOH fraction was determined by liquid scintillation counting.

The fodder beet was intermediate to both the sugar types and again showed a much higher rate of uptake on a dry-weight basis.

If two sugars are transported across a membrane by the same carrier, each sugar should competitively inhibit the uptake of the other. This principle was used to determine if sucrose, glucose, and fructose were accumulated via the same mechanism in each variety. Glucose and fructose strongly inhibited the uptake of sucrose in all three varieties (Table 6). The similarity in the degree of inhibition would indicate that the same mechanism was operating in each case. Sucrose had little effect on the uptake of glucose and fructose. The very similar pattern of inhibition indicated a similar biochemical mechanism in each case. Therefore, the greater sucrose storing capacity of the sugar types cannot be explained on the basis of biochemical differences in the uptake mechanism.

Since sucrose moves from the phloem to the center of the vascular ring by diffusion, it is entirely possible that the rate-limiting step is the rate of diffusion. Factors such as distance and diffusive resistance could determine the relative number of cells exposed to high concentrations of sucrose in the free space. The greater the proportion of total cells exposed to adequate concentrations, the higher the sucrose content.

Figure 4. Effect of concentration on the uptake of sucrose.



Disks of sugarbeet root tissue were incubated for 3 hours in solutions containing various concentrations of sucrose. After incubation, the tissue was washed for 30 minutes in running tap water to remove free-space sugars. The tissue was then extracted with hot 80% ethanol and the concentration of labeled soluble sugars in the ethanol extract was determined by liquid scintillation counting.

The rate of sucrose uptake into root storage cells is directly proportional to the sucrose concentration in the free space (Figure 4). Therefore, cells nearest the sites of phloem unloading should be exposed to the highest concentrations of sucrose for longer periods of time and thus should contain the highest concentrations of sucrose.

A morphological comparison of the roots of the same cultivars used in the uptake study indicated considerable differences in ring number, ring diameter, and cell size distribution of sugar types and fodder beets. A comparison of the inter-ring area between vascular rings 3 and 4 is given in Table 7. The width of the ring was approximately 1 cm in the fodder beet and about 0.6 cm for GWD2 and about 0.4 cm for L53XL19. However, the number of cells across the ring were the same for all three cultivars. The total volume of the largest cells of the fodder type was five-fold greater than that of the high sugar hybrid, but the mean cell volume was ten-fold greater.

Table 7. Cell number, cell volume, and ring width in fodder and sugar types. Measurements are from ring 3 at final harvest.

	Ring Width	Cell Number[1]	Cell Volume Max.[2]	Cell Volume Ave.[3]
			$cm^3 \times 10^{-8}$	
Blanca	10.1	98	189.0	58.8
GWD2	5.7	87	50.5	14.6
L53XL19	4.2	87	32.8	5.9

[1]Number of cells across ring from cambium of ring 3 to cambium of ring 4.

[2]Maximum cell size at center of ring.

[3]Average cell volume for entire ring.

Therefore, the high sugar hybrid had more small cells near the vascular bundles and the low-sugar fodder beet had large cells and very wide rings. The sugar types, L53XL19 in particular, produced narrow rings and many small cells near the vascular bundles. The path of diffusion is, therefore, much shorter in the sugar types, and the sucrose passes many small cells as it diffuses into the ring. Milford (6) found that large cells contained proportionately less sucrose and more nonsucrose soluble solids than small cells. The additional cell volume is essentially made up of water.

Diffusion Controlled Partitioning

Since sucrose moves in the free spaces by diffusion, the concentration would be highest near the unloading site of the vascular bundles. Also, since the rate of sucrose uptake by parenchyma cells is directly proportional to the sucrose concentration in the adjacent free spaces, these parenchyma cells would contain the highest concentrations of sucrose. The proposed relationship between cell size and free space concentration is illustrated in Figure 5. Cells furthest from the vascular area are exposed to low concentrations of sucrose in the free space and, therefore, accumulate less in

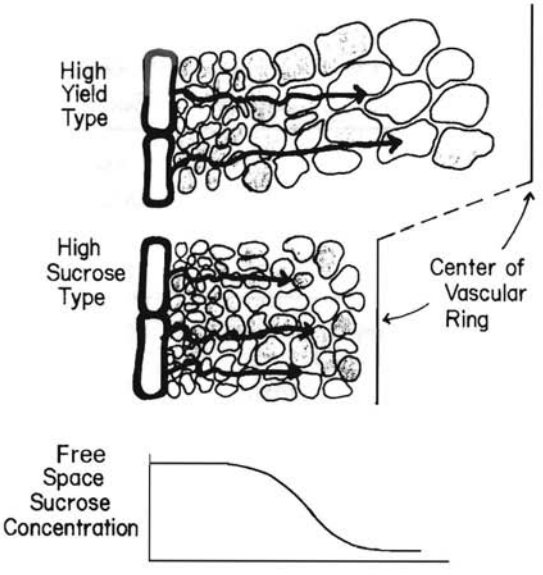


Figure 5. Diagram of diffusion-controlled partitioning of sucrose within the sugarbeet root. Note that the high sucrose root has a high proportion of its cells located in the area where free space sucrose concentrations should be highest.

their vacuoles. Roots containing a high proportion of their cells near the vascular system are roots with a high sucrose concentration. Since the capacity for sucrose uptake was the same in fodder beet and L53XL19 (Table 6), the factor controlling sucrose uptake apparently is not cell size per se but rather the distance of the cell from the vascular system. Cell size and/or cell number determines this distance.

Thus agronomic practices that promote narrow rings should promote high sucrose content. For example, excessive nitrogen fertilization increases root size by cell enlargement. Narrow row spacings or high stand density decrease root size by limiting cell expansion (7). Therefore, the effects of genotype, environment, and nitrogen fertilization on sucrose concentration can be explained by a diffusion-limited or a diffusion-controlled partitioning of photosynthate within the sugarbeet root.

If this hypothesis is confirmed by further study, it is apparent that high yield and high sucrose are possible only if increased yield results from an increase in ring numbers not from an enlargement of cells. Therefore, growth regulators and breeding lines should be screened for their ability to promote proliferation of secondary cambia and to control cell enlargement.

There are still many other factors that may affect sucrose partitioning within the sugarbeet root. For example, we know very little about how the phloem unloads sucrose into the free space. A sophisticated control mechanism allows part of the translocated sucrose to move in the phloem through the tap root into the fibrous root system. Given the dominant sink strength and the large surface area of the vascular system within the tap root, this control system is indeed impressive.

The hormone relationships regulating secondary cambial development, cell division and cell expansion are not well known but appear to be crucial in the control of the sucrose-yield relationship.

Summary

1. Root yield of sugarbeet is determined by photosynthate supply and balanced partitioning of photosynthate between shoot and root. There does not appear to be conflict between the sink strength of the root and its ability to store sucrose (19) Root size is controlled both by the ability of the shoot to provide photosynthate and the growth potential of the root.
2. Photosynthate translocated to the sugarbeet root is partitioned between growth and sucrose storage. This partitioning is balanced and appears to be independent of photosynthate supply, but is influenced by environmental and genetic factors.
3. Because sucrose diffuses from the vascular tissue through the free space of the root, diffusive resistance and length of the diffusion path may be factors controlling sucrose accumulation within the root. Narrow rings allow a large proportion of the total number of cells to be exposed to the high concentration of sucrose in close proximity to the phloem.
4. Research efforts should be directed toward production of large roots with an increased number of rings. This criterion should be useful in selecting superior genotypes.

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