Changes in Carbohydrates and Carbohydrate Metabolizing Enzymes during the Development, Maturation, and Ripening of Buttercup Squash (*Cucurbita maxima* D. 'Delica')

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Abstract. During this study, we divided the developmental growth pattern of buttercup squash into three phases: 1) early growth, from flowering up to 30 days after flowering; 2) maturation, from 30 days until 60 days after flowering (or harvest); and 3) ripening, from 60 days (or harvest) until ≈ 100 days after flowering. Harvest occurred at 48 days after flowering. Fruit growth (expansion), starch, and dry matter accumulation were largely completed during early growth, and there was a progressive decline in the respiration rate. Extractable activities of acid and alkaline invertases, sucrose synthase, alkaline α -galactosidase, and sucrose phosphate synthase (assayed with saturating substrates) were high initially but declined markedly during this phase. Glucose, fructose, and low concentrations of raffinose saccharides were present, but no sucrose was detected. During maturation, starch and dry matter remained nearly constant and sucrose began to accumulate. During ripening, starch was degraded, sucrose synthase activity was significant but relatively constant, sucrose phosphate synthase activity increased, and sucrose continued to accumulate.

The Japanese market is a target for buttercup squash growers in Mexico and New Zealand. This market demands sweet squash. We are seeking to understand carbohydrate partitioning in the starch-accumulating fruit of buttercup squash with a view to increasing the sweetness of New Zealand squash.

There has been little published work describing the enzymology of carbohydrate partitioning in buttercup squash fruit. Nagao and Indou (1991) measured α -amylase activity together with total sugar and starch concentrations during growth in 'Ebisu'. They found total sugars and α -amylase activity were low during development, but higher from 40 to 45 d after flowering as starch content began to decline. In contrast, considerable literature on carbohydrate partitioning now exists for crops such as banana (*Musa cavendishii* Lamb ex Paxton) (Cardenunsi and Lajolo, 1995; Hubbard et al., 1990), tomato (*Lycopersicon esculentum* Mill. and *L. hirsutum* Humb. and Bonpl.) (Miron and Schaffer, 1991; Wang et al., 1993), potato (*Solanum tuberosum* L.) (Ross et al., 1994; Sung et al., 1989), carrot (*Daucus carota* L.) (Sturm et al., 1995), muskmelon (*Cucumis melo* L.) (Hubbard et al., 1989), and sweet pepper (*Capsicum annuum* L.) (Hubbard and Pharr, 1992; Nielsen et al., 1991). Many of these carbohydrate sinks degrade translocated sugars and accumulate hexoses (e.g., muskmelon, sweet pepper, and carrot), while in others, the sugars are degraded and converted into starch. In these latter sink organs, sugar accumulation usually begins during ripening, or following harvest (e.g., banana, potato).

Together with the partitioning patterns exhibited by these sink organs, there are often distinct transitions in the activities of enzymes responsible for carbohydrate metabolism during development. For example, there may be a transition from acid invertase to sucrose synthase as the prime enzyme responsible for sucrolysis during early sink initiation and expansion (Dali et al., 1992;

Hubbard and Pharr, 1992; Hubbard et al., 1991; Miron and Schaffer, 1991; Ross et al., 1994; Sung et al., 1994). In many sink organs accumulating starch, sucrose synthase often dominates sucrolysis earlier in development (Sung et al., 1989). Subsequently, sucrose synthase activity declines and sucrose phosphate synthase activity increases later in development (Cordenunsi and Lajolo, 1995; Geigenberger and Stitt, 1993). During ripening, the appearance of sucrose phosphate synthase often coincides with sucrose accumulation (Hubbard et al., 1989, 1991). These examples illustrate how the expression of different enzymes during development impinges on the composition of carbohydrates in sink organs.

In most sink organs studied, the main transport form of carbon from the leaves is sucrose. Some plants transport the raffinose series of oligosaccharides (Dey, 1990), and both muskmelon (Hubbard et al., 1989) and summer squash (*Cucurbita pepo* L.) (Richardson et al., 1982) transport stachyose and raffinose. To our knowledge, there is no information available on the forms of carbohydrate entering the fruit of buttercup squash, but if raffinose saccharides are translocated from leaves, then it is possible some will enter fruit. One enzyme thought to be involved in degrading raffinose saccharides is α -galactosidase, which has acid and alkaline forms (Gaudreault and Webb, 1982). The presence of α -galactosidase in fruit should provide evidence that raffinose saccharides are present (Dey, 1990).

In this paper, we provide some basic data on carbohydrate concentrations in developing fruit, and the extractable activities of a range of enzymes involved in carbohydrate metabolism to aid our understanding of carbon partitioning, particularly into sweet sugars, in buttercup squash. We harvested and stored some fruit and left other fruit in the field unharvested to determine whether harvest produced different patterns of carbohydrate partitioning.

Materials and Methods

PLANT MATERIAL. Seed of *Cucurbita maxima* D. 'Delica' were purchased from Yates Seeds (Auckland, New Zealand) and sown

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at the Levin Research Centre (0.5 m within rows with 3 m between rows) on 16 Nov. 1993 and 1 Dec. 1994. Normal crop husbandry was followed (irrigation and spraying with powdered sulphur to control powdery mildew (*Sphaerotheca fuliginea* Schl.) as required, except that vines were trimmed to prevent them entering adjacent rows. At flowering (the day female flowers opened), the expanded ovaries of 200 fruit were tagged.

At flowering, and frequently thereafter, six fruit were harvested for measurement of respiration rate, ethylene production, carbohydrate composition, and the determination of enzymic activities. The growth (increase in fruit circumference) of 18 marked fruit (two from each of nine rows) was monitored twice each week.

At 48 d after flowering, fruit were adjudged to be commercially mature by skin, ground spot, and stalk color (Hawthorne 1990). Half of the fruit that remained in the field were hand-harvested, washed, weighed, placed into produce crates, and stored at 12 °C (Hurst et al., 1995) for up to 50 d. The other half of the crop remained intact in the field.

RESPIRATION AND ETHYLENE PRODUCTION. Fruit were either taken from the storeroom (12 °C) and left at 20 °C for 6 h to warm before measurements or were taken from the field and left for 16 h at 20 °C to warm before measurements were made. Individual fruit were placed into 2-, 4-, or 10-L plastic containers for up to 60 min at 20 °C. Two 5-mL gas samples from the head space were injected into a (series 204; Pye Unicam, Cambridge, England) gas chromatograph with thermal conductivity detector for determining CO₂ concentration or a (model GC-9A; Shimadzu, Kyoto, Japan) gas chromatograph, with flame ionisation detector for determining ethylene concentration. Baseline concentrations of CO₂ and ethylene were determined from identical plastic containers that contained no fruit.

CARBOHYDRATE COMPOSITION. Samples of mesocarp (≈10 g) were obtained from opposite sides of each fruit, taking care to

avoid the ground spot. The samples were free of seeds, placenta, and skin and were frozen, freeze-dried, powdered, and stored at -80 °C for determining carbohydrate composition. Other samples were frozen in liquid N and stored at -80 °C for determining enzyme activities.

Dry matter content, sugar (sucrose, glucose, and fructose), and starch concentrations were determined according to Hurst et al. (1995). Oligosaccharides, as raffinose saccharides, were extracted in 62.5% methanol at 55 °C for 15 min, and quantified using (Boehringer Mannheim, Mannheim, Germany) raffinose test kits (catalogue no. 428 167).

ENZYME EXTRACTION AND ASSAYS. Frozen mesocarp samples (2 g) were homogenized with an Ultraturrax (Janke and Kunkel, Staufen, Germany) in 8 mL extraction buffer composed of 50 mM Hepes-KOH (pH 7.5), 2 mM Na₂EDTA, 10% (v/v) glycerol, 10 mM MgCl₂, and 2 mM dithiothreitol. The extract was squeezed through laboratory cloth, spun at 10,000× g for 10 min at 4 °C, desalted using mini columns of Sephadex G-25 (Pharmacia, Uppsala, Sweden) (Neal and Florini, 1973) and stored at -80 °C. Activity in all assays reported was proportional to the amount of desalted extract added and linear for the duration of the incubation. All reported data are means ±SE of the means of three to four replicate fruit.

Acid invertase in 50 µL extract was assayed in 50 mM sodium acetate buffer (pH 5.0) and 33 mM sucrose in a final volume of 0.55 mL. The blank contained boiled enzyme, and hexose production was measured after 30 min at 30 °C using Nelson's reagent (Nelson, 1944) with equal amounts of glucose and fructose in the standard. Alkaline invertase and sucrose synthase in 50 µL extract were assayed in 50 mM Hepes-KOH buffer (pH 7.5), 33 mM sucrose, and 3 mM uridine diphosphate (for sucrose synthase) in a final volume of 0.55 mL. Blanks contained boiled enzyme. Hexose production was measured after 30 min at 30 °C using Nelson's reagent (Nelson, 1944).

α-Galactosidases in 50 µL extract were assayed in 50 mM sodium acetate buffer (pH 5.0) (acid form) or in 50 mM Hepes-KOH buffer (pH 7.5) (alkaline form) and 1 mM *p*-nitrophenyl-α-D-galactoside in a final volume of 1 mL. The assay was run for 30 min at 30 °C and was stopped by adding 3 mL 0.1 M NaOH. For the blanks, the extract was added after the NaOH. Rates of reactions were calculated from the absorbance at 410 nm and taking the extinction coefficient of *p*-nitrophenol as 18.75 L·mmol⁻¹·cm⁻¹ (Farley et al., 1981).

Sucrose phosphate synthase in 50 µL extract was assayed in 50 mM Hepes-KOH buffer pH 7.5, 15 mM MgCl₂, 25 mM glucose-6-phosphate, 12 mM fructose-6-phosphate, and 12 mM uridine diphosphoglucose in a final volume of 1 mL. The assay was run for 30 min at 30 °C. After boiling 3 min, 0.1 mL was taken and added to 0.1 mL 30% (v/v) KOH and the mixture boiled for 11 min. After cooling, 3 mL anthrone reagent was added, the solutions heated for 15 min at 40 °C and sucrose production was determined (van Handel 1968). The blanks were incubated without fructose-6-phosphate or glucose-6-phosphate, and the reaction was started with uridine diphosphoglucose.

Soluble protein concentrations in the extracts were determined using a dye-binding kit (Bio-Rad, Hercules, Calif.) based on Bradford (1976) with bovine serum albumin as the standard.

Results

Buttercup squash fruit grew very rapidly after flowering, but growth in circumference stopped ≈20 d after flowering (Fig. 1a). Fruit dry matter content increased after flowering until ≈30 d (Fig.

Fig. 1. (a) Growth in circumference and dry matter accumulation and (b) respiration as CO₂ evolution on a fresh mass basis of buttercup squash fruit during growth in the field (solid symbols). From 48 d after flowering, harvested fruit, which were stored at 12 °C (open symbols), are compared with attached fruit.

1a). There was a small loss in dry matter after harvest, and the loss was delayed slightly when fruit remained in the field.

Respiration rates of young fruit were very high (Fig. 1b) and declined gradually during development. Respiration rate was higher in those fruit that were harvested and stored than in those left in the field. We did not detect any ethylene production (<0.01 $\text{nmol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$), on a fresh mass basis, from buttercup squash fruit at any time during the experiment.

Starch accumulation (Fig. 2a) followed the dry matter accumulation curve. A week after harvest (48 d after flowering) or ≈ 2 weeks later for fruit left in the field, starch concentration began to decline. Concentrations of hexose (Fig. 2b and c) increased until 14 d after flowering and then declined gradually until around day 35. After harvest, hexose concentrations increased slowly in intact and harvested fruit, but in harvested fruit, the increase occurred sooner. We found little sucrose in fruit until ≈ 35 d after flowering (Fig. 2d). Sucrose concentration increased from this point in both harvested and intact fruit. Raffinose saccharides were quite low during the first 14 d after flowering (Fig. 2d). There was a sudden, comparatively large, increase between days 20 to 30, and thereafter, concentrations were lower but steady.

Extractable activity of soluble acid invertase was high at flowering (Fig. 3a) and slowly declined until 30 d. After this time, there was little activity in fruit that were harvested and stored or in those left in the field.

Extractable activities of sucrose synthase and alkaline invertase also declined until ≈ 25 to 30 d after flowering (Fig. 3b). Following harvest, activity of sucrose synthase increased slightly during the remainder of the experiment. Activities of alkaline invertase were low after harvest but increased slightly between days 77 and 84 in stored fruit.

Extractable activities of alkaline and acid forms of α -galactosidase (Fig. 3c) gradually declined after flowering until ≈ 21 d with little further change, irrespective of whether the fruit were stored or remained in the field. Initially, activities of the alkaline form were far higher than those of the acid form. Extractable activity of sucrose phosphate synthase, assayed with saturating substrate, declined after flowering (Fig. 3d), and then from 21 d after flowering, increased gradually when fruit remained in the field. Following harvest, the activity of sucrose phosphate synthase remained relatively steady in stored fruit.

The pattern of change in soluble protein concentration was the same as that shown by sucrose phosphate synthase enzyme activity. Soluble protein concentrations were around $8\text{ mg}\cdot\text{g}^{-1}$ on a fresh mass basis at flowering and $2.5\text{ mg}\cdot\text{g}^{-1}$ at 14 d, and they gradually increased to $\approx 5\text{ mg}\cdot\text{g}^{-1}$ from day 48 to 98. In stored fruit, soluble protein concentrations remained around $4\text{ mg}\cdot\text{g}^{-1}$.

Discussion

The developmental growth pattern of buttercup squash fruit can be conveniently divided into three phases: 1) early growth, during the first 30 d after flowering; 2) maturation, from 30 until 60 d after flowering (or harvest); and 3) ripening, from 60 d (or harvest) until ≈ 100 d after flowering. In common with other developing carbohydrate sinks, there were distinct transitions in carbohydrate metabolism in buttercup squash that delineated these developmental phases. The first transition was characterized by the rapid loss in glucose and fructose between days 25 to 35; the modest accumulation of raffinose saccharides between days 20 to 30; and the decline in activities of acid and alkaline invertases, sucrose synthase, and the α -galactosidases during the first 30 d after flowering. These events coincided with a decline in the respiration

Fig. 2. Starch concentrations, (a) as hexose units, (b) glucose concentrations, (c) fructose concentrations, and (d) sucrose and raffinose saccharide concentrations, all on a fresh mass basis, in buttercup squash fruit during growth in the field (solid symbols). From 48 d after flowering, harvested fruit, which were stored at 12 °C (open symbols), are compared with attached fruit.

rate and cessation of fruit expansion and dry matter accumulation. Following this phase, starch and dry matter remained relatively constant until the third phase, which was characterized by starch degradation, accumulation of sucrose, and comparatively high activity of sucrose phosphate synthase.

The act of harvest had little influence on the ripening pattern and carbon partitioning. The only differences were higher respiration rates and slightly higher hexose levels in harvested fruit compared with intact fruit. Perhaps, the cooler evenings soon after the normal harvest time and the dew that dampened the leaves provided conditions suitable for mildew establishment. Collapse of leaves would have effectively reduced photosynthesis and the translocation of carbon to the fruit. Starch breakdown may have been initiated just as it had been in harvested fruit only slightly later.

Photosynthate is translocated from the leaves of cucurbits as stachyose, although proportions of raffinose, sucrose, and hexoses increase closer to the sink organs (Richardson et al., 1982; Webb and Gorham, 1964). In this study, raffinose saccharides entered the mesocarp of buttercup squash fruit (Fig. 2d), but they were in very low concentrations compared with those for hexoses. This was the same pattern found by Hubbard et al. (1989) in muskmelon fruit. We believe alkaline α -galactosidase is responsible for the degradation of raffinose saccharides in rapidly growing buttercup squash

fruit; the extractable activity was high initially, and concentrations of raffinose saccharides increased once the enzyme disappeared. The alkaline α -galactosidase from *Cucurbita pepo* leaves has a pH optimum of 7.5 (Gaudreault and Webb, 1982) and declines in activity as the leaves develop from carbohydrate sinks to autonomous carbohydrate sources. The activity of acid α -galactosidase in buttercup squash remained relatively constant throughout and may not have a role in degrading incoming raffinose saccharides. Gaudreault and Webb (1982) also reported no change in activity of this enzyme during the development of *Cucurbita pepo* leaves from carbohydrate sinks to sources, and suggested the acid forms of the enzyme are extracellular. Richardson et al. (1982) reported that α -galactosidase is not active in the alkaline phloem of *Cucurbita maxima*, although it is not clear whether the alkaline or acid form was assayed.

The initially high activity of acid invertase that we found in buttercup squash is typical of that found in a range of organs undergoing rapid expansion [e.g., muskmelon (Hubbard et al., 1989), potato (Ross et al., 1994), and sweet pepper (Nielsen et al., 1991)]. By cleaving sucrose into two hexose moieties, the osmotic potential would be doubled, thus assisting in cell expansion. Since fruit expansion is fast and of considerable magnitude in buttercup squash, acid invertase may assist in this expansion. Overall, acid invertase may have a minor role in sucrolysis in buttercup squash fruit during early growth because the extractable activity was low compared with those of the cytoplasmic enzymes alkaline invertase and sucrose synthase.

The high activity of sucrose synthase observed during initial growth suggests that it may play a major role in sucrolysis. High sucrose synthase activity is common in other organs that are accumulating starch [e.g., banana (Cordenunsi and Lajolo, 1995) and potato (Ross et al., 1994)] and is probably responsible for providing the carbon for starch synthesis. Another role for sucrose synthase could be in the supply of nucleotide sugars for biosynthesis of cellulose in the rapidly expanding fruit. The high level of cellular biosynthetic activity was reflected in the high rates of respiration measured during early fruit growth. The simultaneous presence of sucrose synthase and alkaline invertase during early growth is interesting as they are both cytosolic but the enzymes may be supporting different processes. Alkaline invertase may be responsible for providing carbon for respiratory activity and energy production in the cells.

The maturation phase was characterized by cessation of starch and dry matter accumulation and the beginnings of sucrose accumulation. Sucrose phosphate synthase and sucrose synthase were the only enzymes we assayed which had appreciable activity. Sucrose phosphate synthase may have been responsible for the synthesis of sucrose, but the symplastic import of sucrose from the parent plant cannot be ruled out, at least in those fruit that remained in the field. There also may have been some contribution to sucrose accumulation during maturation and ripening from sucrose synthase, which can operate in the synthesis direction (Geigenberger and Stitt, 1993). The reason for the high activity of sucrose phosphate synthase during early growth is not known, but sucrolysis by sucrose synthase and alkaline invertase would have contributed to the virtual absence of sucrose during that time.

Ripening was characterized by starch breakdown and substantial accumulation of glucose, fructose, and sucrose. It is clear from the patterns presented in data from harvested and field-ripened fruit, that harvest had only a minor effect on the accumulation of soluble carbohydrates. To some extent this might suggest that by 48 d after flowering, translocation of assimilates may have largely ceased due to competition for assimilates by other fruit on the vine,

Fig. 3. Extractable activities of (a) acid invertase, (b) sucrose synthase (SS) and alkaline invertase, (c) all as production of hexose, alkaline- and acid- α -galactosidase, as production of *p*-nitrophenol, and (d) sucrose phosphate synthase, as production of sucrose, all on a fresh mass basis, in buttercup squash fruit during growth in the field (solid symbols). From 48 d after flowering, harvested fruit, which were stored at 12 °C (open symbols) are compared with attached fruit.

or reduction in photoassimilate production due to shading of leaves or infection of leaves with the powdery mildew fungus. From 55 to 98 d after flowering, starch breakdown, expressed as hexose units on a fresh mass basis in harvested fruit ($13.3 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$) was nearly balanced by sucrose synthesis ($2.7 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$), hexose production ($2.2 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$) and whole-fruit respiration rate ($9.6 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$), a total of $14.5 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$. Since the activity of sucrose synthase was $1440 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ and that of sucrose phosphate synthase was only $85 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$, the combined presence of sucrose synthase and sucrose phosphate synthase during maturation and ripening suggests the sucrose that accumulated must have been stored in a separate compartment from the cytosol, such as the vacuole.

Literature Cited

- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254.
- Cordenunsi, B.R. and F.M. Lajolo. 1995. Starch breakdown during banana ripening: sucrose synthase and sucrose phosphate synthase. *J. Agr. Food Chem.* 43:347–351.
- Dali, N., D. Michaud, and S. Yelle. 1992. Evidence for the involvement of sucrose phosphate synthase in the pathway of sugar accumulation in sucrose-accumulating tomato fruits. *Plant Physiol.* 99:434–438.
- Dey, P.M. 1990. Oligosaccharides, p. 195–218. In: P.M. Dey, and J.B. Harborne (eds.). *Methods in plant biochemistry*. vol. 2 Carbohydrates. Academic, London.
- Farley, J.R., C.H. Chestnut, and D.J. Baylink. 1981. Improved method for quantitative determination in serum of alkaline phosphatase of skeletal origin. *Clinical. Chem.* 27:2002–2007.
- Gaudreault, P.R., and J.A. Webb. 1982. Alkaline α -galactosidase in leaves of *Cucurbita pepo*. *Plant Sci. Lett.* 24: 281–288.
- Geigenberger, P., and M. Stitt. 1993. Sucrose synthase catalyses a readily reversible reaction in vivo in developing potato tubers and other plant tissues. *Planta* 189: 329–339.
- Hawthorne, B.T. 1990. Age of fruit at harvest influences incidence of fungal storage rots on fruit of *Cucurbita maxima* D. hybrid 'Delica'. *N.Z. J. Crop Hort. Sci.* 18:141–145.
- Hubbard, N.L., S.C. Huber, and D.M. Pharr. 1989. Sucrose phosphate synthase and acid invertase as determinants of sucrose concentration in developing muskmelon (*Cucumis melo* L.) fruits. *Plant Physiol.* 91:1527–1534.
- Hubbard, N.L., D.M. Pharr, and S.C. Huber. 1990. Role of sucrose phosphate synthase in sucrose biosynthesis in ripening bananas and its relationship to the respiratory climacteric. *Plant Physiol.* 94:201–208.
- Hubbard, N.L., D.M. Pharr, and S.C. Huber. 1991. Sucrose phosphate synthase and other sucrose metabolizing enzymes in fruits of various species. *Physiol. Plant.* 82:191–196.
- Hubbard, N.L. and D.M. Pharr. 1992. Developmental changes in carbohydrate concentration and activities of sucrose metabolizing enzymes in fruits of two *Capsicum annuum* L. genotypes. *Plant Sci.* 86:33–39.
- Hurst, P.L., V.K. Corrigan, P.J. Hannan, and R.E. Lill. 1995. Storage rots, compositional analysis and sensory quality of three cultivars of buttercup squash. *N.Z. J. Crop Hort. Sci.* 23:89–95.
- Miron, D., and A.A. Schaffer. 1991. Sucrose phosphate synthase, sucrose synthase and invertase activities in developing fruit of *Lycopersicon esculentum* Mill. and the sucrose accumulating *Lycopersicon hirsutum* Humb. and Bonpl. *Plant Physiol.* 95:623–627.
- Nagao, A. and T. Indou. 1991. Studies on changes in components of squash fruit during maturation to determine harvesting time. *Hokkaido Prefecture Agr. Expt. Sta. Bul.* 62:63–68.
- Neal, M.W., and J.R. Florini. 1973. A rapid method for desalting small volumes of solution. *Anal. Biochem.* 55:328–330.
- Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* 153:375–380.
- Nielsen, T.H., H.C. Skjaerbaek, and P. Karlsten. 1991. Carbohydrate metabolism during fruit development in sweet pepper (*Capsicum annuum*) plants. *Physiol. Plant.* 82:311–319.
- Richardson, P.T., D.A. Baker, and L.C. Ho. 1982. The chemical composition of cucurbit vascular exudates. *J. Expt. Bot.* 33:1239–1247.
- Ross, H.A., H.V. Davies, L.R. Burch, R. Viola, and D. McRae. 1994. Developmental changes in carbohydrate content and sucrose degrading enzymes in tuberising stolons of potato (*Solanum tuberosum*). *Physiol. Plant.* 90:748–756.
- Sturm, A., V. Sebková, K. Lorenz, M. Hardegger, S. Lienhard, and C. Unger. 1995. Development- and organ-specific expression of genes for sucrose synthase and three isoenzymes of acid β -fructofuranosidase in carrot. *Planta* 195:601–610.
- Sung, S.S., W.J. Sheih, D.R. Geiger, and C.C. Black. 1994. Growth, sucrose synthase, and invertase activities of developing *Phaseolus vulgaris* L. fruits. *Plant Cell Environ.* 17:419–426.
- Sung, S.S., D.P. Xu, and C.C. Black. 1989. Identification of actively filling sucrose sinks. *Plant Physiol.* 89:1117–1121.
- van Handel, E. 1968. Direct microdetermination of sucrose. *Anal. Biochem.* 22:280–283.
- Wang, F., A. Sanz, M.L. Brenner, and A. Smith. 1993. Sucrose synthase, starch accumulation, and tomato fruit sink strength. *Plant Physiol.* 101:321–327.
- Webb, J.A. and P.R. Gorham. 1964. Translocation of photosynthetically assimilated C^{14} in straight-necked squash. *Plant Physiol.* 39:663–672.