

SUGAR, ACID ACCUMULATION AND METABOLISM

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Most storage components of citrus fruits (such as Brix and acids) originate in the leaves in the form of sucrose, which in itself, is the ultimate product of photosynthesis. Sucrose moves through the phloem strands of the vascular system from mature leaves and into the fruit where it supplies carbons for the energy demands and eventually for storage. In comparison to other evergreen trees, citrus are considered "slow paced organisms" in regards to the rates of photoassimilate translocation from leaves to sink tissues.

Citrus fruits differentially derive photoassimilates depending on their location along the tree branches. Fruit located at the apical ends derive their photoassimilates acropetally from leaves belonging to the same growth cycle. Lateral fruit, however, get their carbon allocation from basipetal root directed carbon from the same leaves [Goldschmidt and Koch, 1996] (Figure 1).

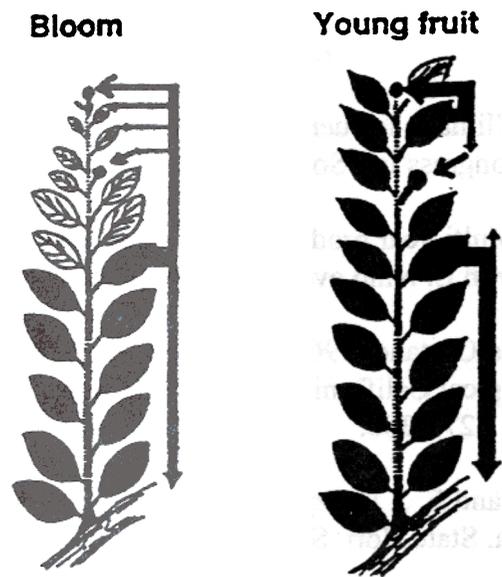


Figure 1. Translocation patterns for assimilates during early flush period (bloom) and after leaves have matured (young fruit). Dark colored leaves indicate mature exporting leaves. (Modified from Golschmidt and Koch, 1996).

Figure 1 also demonstrates the importance of mature leaves during the first month of the new flush. During this time, where young leaves remain as "sinks", photoassimilates must arrive from the photosynthetically active older leaves and possibly from vegetative storage parts. Positive carbon balance for young leaves normally takes 30 to 40 days of development [Goldschmidt and Koch, 1996]. Once the carbon balance becomes positive, photoassimilate availability for fruit growth increases.

Photoassimilates are transported through the phloem from source leaves into the fruit. As the amount of photosynthates increase during development so do the diameter of the phloem tissue. This structural change (in addition to others) facilitates the transport into the growing fruit tissues [Koch and Avigne, 1990]. Assimilate entry into the juice sacs occurs via three vascular bundles located at the segment epidermis of each individual segment [Lowell et al. 1989]. The bundles are located one at the dorsal side and the remaining two along the lateral septa of the segment walls (Figure 2).

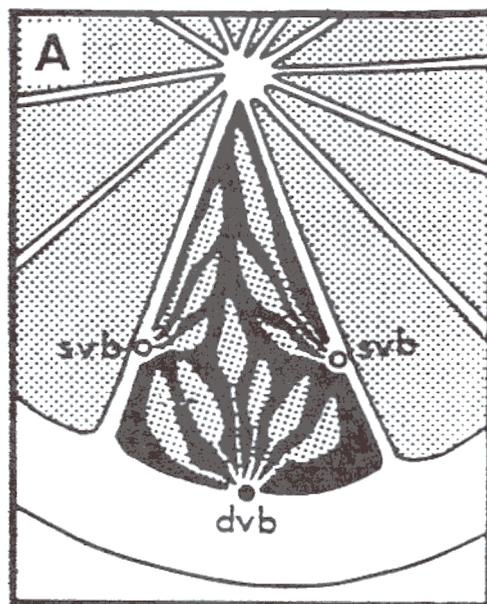


Figure 2. Cross section diagram of a citrus fruit showing the location of the three main vascular bundles supplying photosynthates to the juice cells. (svb, septal vascular bundles; dvb, dorsal vascular bundle). (From Goldschmidt and Koch 1996)

Photoassimilates entering the juice sacs exit the vasculature and enter the segment epidermis transiently. From this area, they move on to the storage cells of the juice sacs in a very slow process (Figure 3).

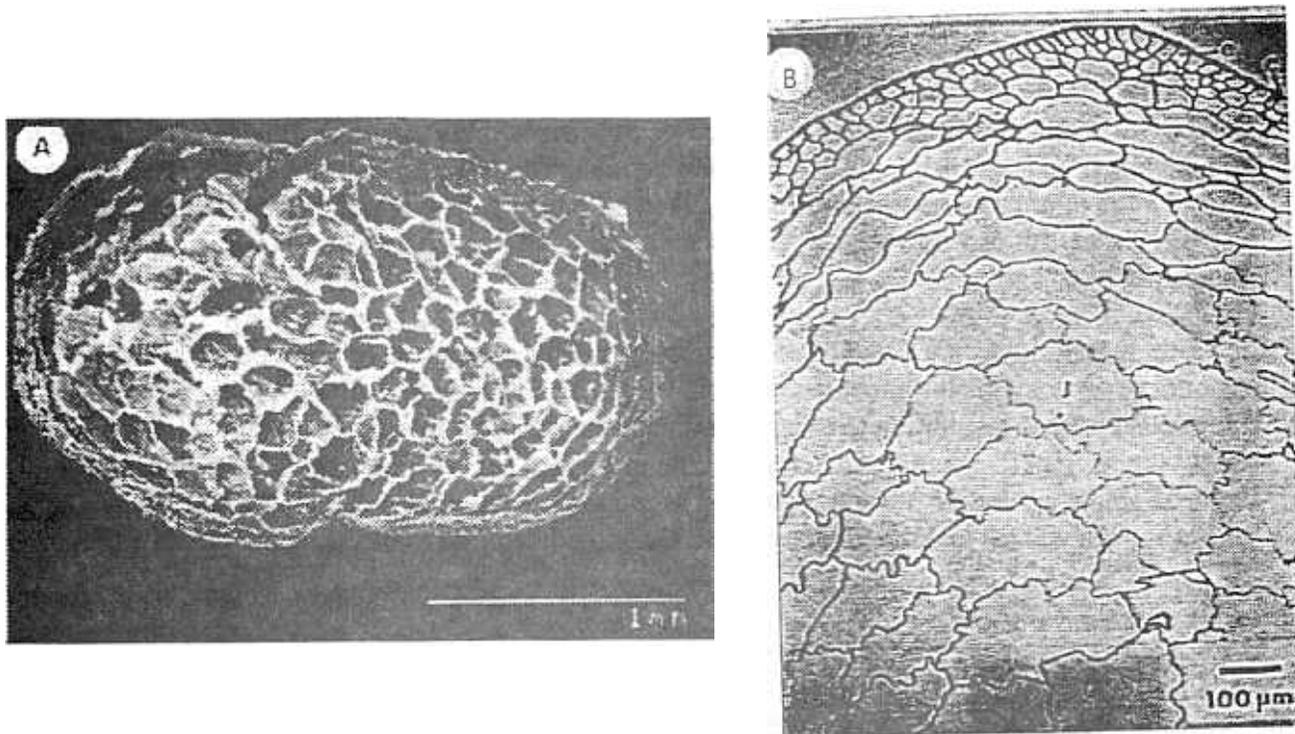


Figure 3. Cross sectional view of maturing citrus juice vesicles. (A.) Scanning electron micrograph. (B.) Light micrograph.

Sucrose is the main form of photoassimilate transport in citrus trees, and moves very slowly within the cells of the juice sacs. Transport of sucrose through the juice vesicle cells appears to occur with minimal sucrose cleavage. In other tissues such as tomato, cleavage of sucrose along the transport pathway is necessary for the creation of a driving force for sucrose movement. [Milner et al, 1996]. Although the transfer rates of photoassimilate is very slow, carbon gains in citrus fruits is a slow process that occurs over a very long time during development (Figure 4). In fact, data suggests that transient compartmentation in vacuoles takes place in route to the juice sac head.

Sugar Accumulation in Juice Cells

The large and highly vacuolated storage cells of the juice sac heads are the final intended location for the transported photoassimilates (Figure 3). These cells are approximately 95% vacuole clearly illustrating their suitability for storing large amounts of reserve materials. Mature juice cells store large amounts of sugars and acids which combined make up to approximately 90% of the total soluble solids. Despite the fact that most photoassimilates arrive into these storage cells in the form of sucrose, large amounts of other sugars such as fructose and glucose are found (Figure 5). More surprisingly, differences in sugar and acid content between different parts of the fruit [Ting, 1969] and on different locations within a given tree [Syvertsen and Albrigo, 1980] have been reported. Figure 4 demonstrates a typical pattern of sugar accumulation in oranges.

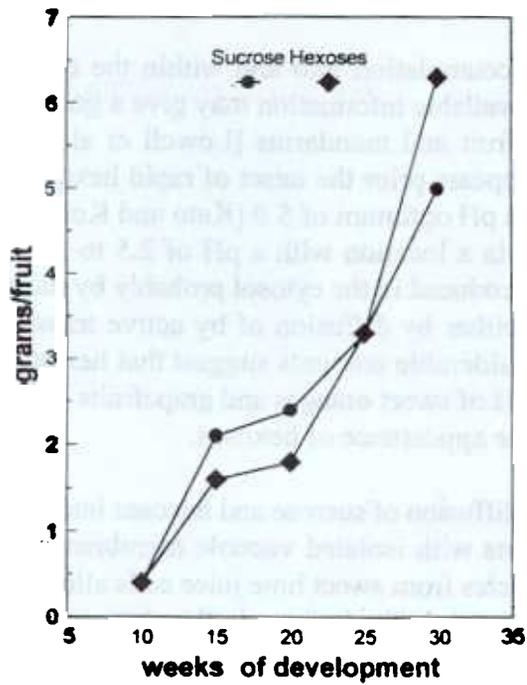


Figure 4. Changes in sucrose and hexoses during Marsh grapefruit development. (Modified from Lowell et al., 1989)

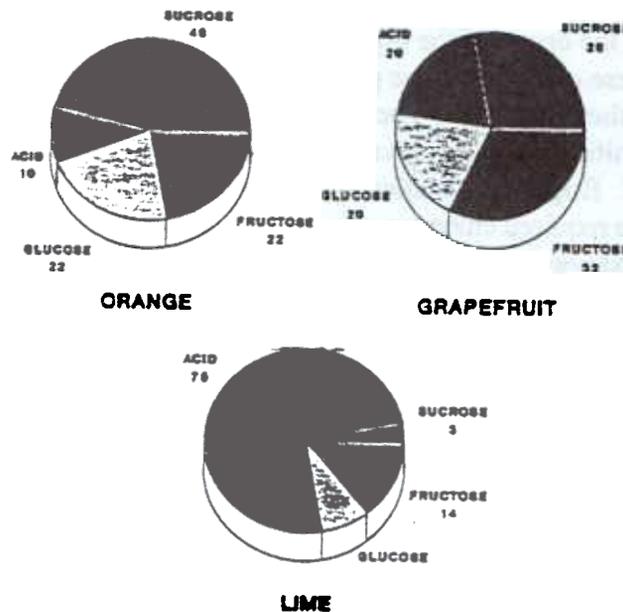


Figure 5. Proportions of sugars and acid as the major components of the total soluble solids in oranges and grapefruits.

The process of sugar accumulation into and within the cells of the juice sacs are not well understood, however, some available information may give a good indication to such mechanisms. For instance, in both grapefruit and mandarins [Lowell et al., 1989; Kato and Kubota, 1978], vacuolar acid invertase disappears prior the onset of rapid hexose accumulation. In addition, the vacuolar acid invertase with a pH optimum of 5.0 [Kato and Kubota, 1978], would not likely be of any metabolic consequence in a location with a pH of 2.5 to 3.0 [Echeverria and Burns, 1989]. Therefore, hexoses must be produced in the cytosol probably by the action of the alkaline invertase and move into the vacuole either by diffusion or by active transport. The fact that hexoses are present in the cytosol in considerable amounts suggest that hexoses are readily diffusible into the vacuole. The low vacuolar pH of sweet oranges and grapefruits (ca. pH 3.0) and can also contribute to a considerable extent to the appearance of hexoses.

Evidence for facilitated diffusion of sucrose and hexoses into the vacuolar compartment of juice cells comes from experiments with isolated vacuole membranes from sweet limes [unpublished data]. Purified tonoplast vesicles from sweet lime juice cells allowed the rapid diffusion of sucrose as well as both principal hexoses into the lumen. In the absence of an active transport mechanism, the vacuolar acid invertase becomes the driving force for assimilate uptake and for the generation of hexoses. In sweet limes, the vacuolar pH of 5.0 coincides with the maximal activity of acid invertase making this enzyme a formidable driving force. It is interesting to note that in acid limes (which do not pose acid invertase as in oranges and grapefruits) the proportion of hexoses to sucrose parallels that of sweet limes which poses the enzyme. However, the internal pH of the vacuole compartment is such that non-enzymatic acid hydrolysis can account for the hexoses produced.

Acid Metabolism

Early studies on the origin of the organic acids in citrus fruits [Varma and Ramakrishnan, 1956] demonstrated that these compounds are produced within the juice cells and are not translocated into the fruit from any other plant part. The observation that in citrus fruits organic acids accumulate rapidly during the initial stages of development is a clear indication that additional CO₂ is fixed within the fruit itself. Bogin and Wallace [1967] and later Roe et al. [1984], were able to measure the presence of all the required enzymes involved in dark CO₂ fixation. According to Yen and Koch [1990], actual CO₂ fixation by the juice tissues increased dramatically after June and remained fairly constant thereafter.

After reaching a peak in concentration midway through development, acids begin to decline and continue doing so after the fruit has been harvested [Hirai and Ueno, 1977] (Figure 6). The decline in organic acids has been attributed to the increase in anaerobic respiration as the fruit matures [Bruemmer and Roe, 1970]. Such utilization of acids for energy production has been demonstrated by Murata [1977] where injected radiolabeled citrate was recovered as CO₂ and in other metabolites. It is very possible that the observed CO₂ fixation in mature fruits leads to the formation of acids as well as some sugars.

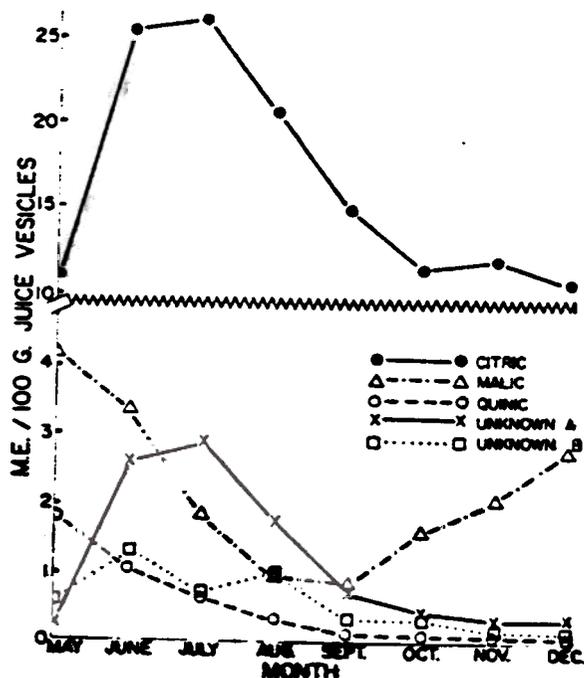


Figure 6. Changes in organic acids concentrations during the development of Hamlin oranges. (From Ting and Vines, 1966).

Transport of citrate into the vacuole of citrus fruits was studied in fruits containing high and low levels of citric acid [Canel et al., 1995]. The results of these studies indicate that the transport of citrate into the vacuoles is a highly energy dependent system not yet described for other tissues. However, how these compounds are moved from the storage compartment in order to be utilized remains unsolved.

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