Sugar Content and Uptake in the Strawberry Fruit

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Abstract. Sucrose was not detected in developing fruit of 'Brighton' strawberry (Fragaria ×ananassa Duch.) until 10 days after anthesis. Thereafter, its concentration increased rapidly but then declined as fruit became red ripe. The concentration of glucose and fructose were similar and higher than that of sucrose during early fruit growth and in ripe fruit. Uptake of 14C-sugars was followed in excised disks of cortical tissue from fruit 15−17 days old. The addition of CaCl2 was necessary to maintain tissue respiration. Sucrose uptake into tissue disks was nearly constant over 4 hr and had a pH optimum of 5.0. Kinetic analysis of sucrose uptake revealed both linear and saturable components. The kinetic characteristics of fructose uptake was similar to those for sucrose. Glucose, however, was taken up much more rapidly than either sucrose or fructose and only demonstrated saturation kinetics. The metabolic inhibitors NaCN (5 mM), dinitrophenol (DNP, 3 mM) and carbonyl cyanide m-chlorophenyl hydrazone (CCCP, 100 μM) stimulated sucrose uptake 34%, 94%, and 54%, respectively. DNP eliminated the saturable component. Uptake of sucrose was inhibited by 36% with 10 μM DNP, 16% with 5 mM glucose, and 16% in a 100% N2 atmosphere. After incubation in 14C-sugars for 2 hr, about 90% of the label recovered from disks was in a neutral fraction. Half or more of this was in either glucose or fructose, depending upon the sugar fed. The distribution of 14C between glucose and fructose moieties of sucrose isolated from tissue fed (14C-fructosyl) sucrose indicated that a portion of the sucrose recovered underwent hydrolysis and randomization. Similar results were found with sucrose isolated from attached, whole fruit 8 hr after abraded leaves were fed labeled sugars. Results suggest that sucrose may be hydrolyzed prior to uptake into fruit tissue.

The process of phloem unloading and the subsequent movement of sugars into cells of a sink organ seems important in controlling the availability of metabolites needed for organ growth. Identification of pathways and mechanisms by which translocated sugars are accumulated by receiving cells may point to ways of regulating partitioning of dry matter to economically important organs.

Transport of sugars from the phloem to other plant tissues may be symplastic, through plasmodesmata, or apoplastic. Both pathways have been shown to occur in different plants and plant organs (8, 10, 23, 26). In plant tissues having apoplastic phloem unloading, sugar uptake is, at least in part, an active, carrier-mediated process (10, 16, 26).

Little is known about the processes involved in the accumulation of sugars in fleshy fruit. Sucrose has been shown to be the major carbohydrate translocated to strawberry fruit (5), and rates of sucrose uptake in strawberry fruit cortical tissue correlate with fruit growth rates (6). In ripe fruit, however, sucrose is accumulated to lower levels than that of its component hexoses, glucose, and fructose (25). The absolute and relative amounts of these sugars apparently vary with the degree of fruit ripeness (21). In this study, the concentrations of sugars in strawberry fruit were determined throughout fruit development. Using 14C-sugars, the rate of uptake and metabolism of sugars by excised fruit tissue were examined and metabolite distribution compared to that of intact fruit.

Material and Methods

Fruit were obtained from plants of the day-neutral strawberry 'Brighton', which were grown in a greenhouse under a 16-hr photoperiod.

Sugar concentrations. Primary flowers were tagged at anthesis and the fruit were harvested at 2−5 day intervals over the following 25 days. Upon harvest, calyces were removed, and the fruit were weighed and then stored at −20°C. Frozen fruit were heated to boiling in a microwave oven, then held in a boiling water bath for 30 sec, cooled by immersion in an ice bath, and weighed (21). To correct weight loss, distilled water was added. Fruit were homogenized with a Polytron, the mixture diluted to 100 ml with distilled water, and filtered through Whatman No. 1 paper. Concentrations of sugars were determined enzymatically with glucose/fructose and sucrose/glucose kits from Boehringer-Mannheim Biochemicals (Indianapolis, Ind.).

Tissue disk incubation. Unripe primary fruit at 15−17 days postanthesis were used. A 7-mm diameter plug of tissue was removed from the fruit cortex with a cork borer and cut into 1.3 mm thick disks with a razor blade and Plexiglas jig. Disks were pooled in a buffer solution and allowed to equilibrate for 30 min at 25°C under constant agitation.

Ten disks (0.52 g fresh weight) were placed in 5 ml of solution in a 50 ml Erlenmeyer flask and incubated at 25°C and 80 rotations/min in a gyratory water bath shaker. The solution, unless otherwise noted, contained 10 mM sucrose, 50 mM CaCl2, and 20 mM citrate buffer (pH 5.0).

To measure tissue respiration, flasks were sealed with serum caps and the internal atmosphere sampled immediately and again after 60 min. Air samples (2 ml) were analyzed for CO2 using a flow-through infrared gas analyzer system (3). Between measurements, flasks were flushed with air and resealed.

Sugar uptake into tissue disks was determined by incubating disks in 14C-labeled sugars (20 μCi·mmol−1; 1 Ci = 37 GBq) for 2 hr, followed by a 30 min rinse in 4 changes of ice-cold unlabeled solution to remove labeled sugar from the free space. Rinsed disks were extracted overnight in 80% ethanol at 80°C. The ethanol soluble fraction was dried at 50°C, taken up into one ml of H2O, and radioactivity was determined by liquid scintill-
ulation spectroscopy. The insoluble fraction was combusted in a Packard Oxidizer and assayed. Kinetic analysis was performed using Lineweaver-Burk plots. Where 2 components were present, the first order rate coefficient $k$ was calculated as the slope of the linear regression equation for points on the total uptake curve at or above 25 mM substrate concentrations. The contribution of the linear component to total uptake was estimated as $k$ multiplied by the substrate concentration at each point. The linear component was subtracted from total uptake to give rates due to the saturable component.

**Distribution of $^{14}$C-labeled metabolites.** Strawberry fruit tissue disks were incubated 2 hr in solutions containing 10 mM (U-$^{14}$C) sucrose, ($^{14}$C-fructosyl) sucrose, $^{14}$C-fructose, or $^{14}$C-glucose. Disks were separated into soluble and insoluble fractions as described previously. Soluble fractions were passed through coupled Dowex 50-X8 (H$^+$) and Dowex 1-X8 (formate) cation and anion exchange columns, resulting in a neutral fraction (1). Basic and acidic fractions were eluted from the cation and anion resin, respectively, with 2 $\text{N}$ NH$_4$OH or 2 $\text{N}$ HCl. Fractions were dried under reduced pressure, the residue dissolved in a known volume of 50% ethanol and radioactivity determined. The neutral fraction was analyzed for individual sugars by descending paper chromatography. Known sugars were cochromatographed with samples on Whatman No. 1 filter paper and developed 72 hr in 9:2:1 butanone:1 acetic acid:1 boric acid saturated water (by volume) (20). Radioactivity of individual sugars was measured by cutting 18 x 18 mm sugars from the chromatograms and counting activity according to Wang and Jones (24). Sucrose was eluted from chromatograms and hydrolyzed with 10-20 units of invertase (Sigma Chemical Company, St. Louis, Mo.) per $\mu$mol sucrose for 2 hr at 50°C. The hydrolysate was rechromatographed to determine the ratio of $^{14}$C-glucose to $^{14}$C-fructose.

To compare sugar metabolism in fruit tissue disks with intact fruit, strawberry plants were reduced to one leaflet and one primary fruit 15 days postanthesis. Leaflets were lightly abraded with 400 carborundum paper to facilitate sugar uptake. The following day, 2 $\mu$Ci of (U-$^{14}$C) sucrose, ($^{14}$C-fructosyl) sucrose, or $^{14}$C-fructose were fed to abraded leaflets. Fruit were harvested 8 hr after feeding, frozen, and freeze-dried. Fruit tissue was ground and analyzed in the manner previously described for tissue disks.

**Results**

**Sugar concentrations.** Concentrations of fructose and glucose in the strawberry fruit remained constant or declined for the first 10 days following anthesis (Fig. 1B). Between days 10 and 20, fruit fresh weight increased rapidly, and the concentration of these sugars rose (Fig. 1A and B). Sucrose was not detected in fruit until 10 days after anthesis. Thereafter, its concentration rapidly increased. During fruit ripening, from 18 to 25 days after anthesis, sucrose levels declined. When fruit were red ripe, 25 days after anthesis, they contained 1.2% glucose, 1.5% fructose, and 0.6% sucrose.

**Respiration and sugar uptake.** The respiration rate of fruit tissue disks in 10 mM sucrose initially was similar to that of intact ‘Brighton’ fruit (data not shown), but dropped rapidly during the first 4 hr of incubation (Fig. 2A, control). Addition of sorbitol as an osmoticum decreased but did not eliminate the decline in respiration. CaCl$_2$ had a greater stabilizing effect than sorbitol at equivalent osmotic potentials, and 50 mM CaCl$_2$ maintained tissue respiration at high rates. Addition of CaCl$_2$ also decreased the rate of sucrose uptake. Fruit disks incubated 2 hr in 10 mM $^{14}$C-sucrose in the absence of CaCl$_2$ accumulated twice as much radioactivity as those incubated with 50 mM CaCl$_2$ (data not shown).

The pH of the bathing solution has been shown to affect sugar uptake (10, 15). Measurement of the uptake of $^{14}$C-sucrose into strawberry fruit disks over the pH range of 4–8 showed that uptake was greatest at pH 5.0 (Fig. 2B). Sucrose uptake at pH 8 was 40% lower. Some browning of the tissue was observed at pH 7.0 and above.

Sucrose uptake by tissue disks was relatively linear over 4 hr (Fig. 2C). The rate of incorporation of $^{14}$C-sucrose into insoluble material was low for the first 90 min but then increased, with 16% to 22% of the radioactivity recovered in this fraction after 2–4 hr.

Temperature strongly influenced sucrose uptake, showing an optimum at about 40°C (Fig. 2D). Incorporation of radioactivity into insoluble components peaked at about 30° declining to near zero at 40°.

Kinetic analysis of sucrose uptake revealed 2 components.
Fig. 2. Respiration or sucrose uptake by tissue disks of strawberry fruit cortex. (A) Respiration over time in various concentrations of CaCl₂ and sorbitol. Control contained 10 mM sucrose and 20 mM MOPS buffer (pH 7.0). (B) Uptake of ¹⁴C-sucrose in 20 mM citrate-phosphate (pH 4 and 5), 2(N-morpholino) ethanesulfonic acid (MES, pH 6), 3(N-morpholino) propanesulfonic acid (MOPS, pH 7), and N-2-hydroxyethylpiperazine propane sulfonic acid (EPPS, pH 8) buffers. (C) Uptake and incorporation of ¹⁴C-sucrose by strawberry fruit tissue in 10 mM sucrose over 4 hr. (D) Effect of temperature on uptake and incorporation of ¹⁴C-sucrose. Points represent the mean ± se of 2 (c), 3 (A,B), or 4 (D) replicates.

(Fig. 3). At low sucrose concentrations, a saturable component following Michaelis–Menten kinetics was predominant, whereas above 25 mM a linear component following first order kinetics dominated. Sucrose uptake can be described by the equation $v = V_{\text{max}} S/(S + K_M) + kS$ (17), where $v$ is the rate of sucrose uptake, $S$ is the sucrose concentration in the medium, $V_{\text{max}}$ and $K_M$ are Michaelis–Menten constants, and $k$ is the first order rate coefficient. The values for sucrose uptake were: $V_{\text{max}}$, 0.65 μmol·g⁻¹·fresh weight·hr⁻¹; $K_M$, 12.5 mM; $k$, 0.018 μmol·g⁻¹·fresh weight·hr⁻¹·mmol⁻¹.

The metabolic inhibitors NaCN (5 mM), dinitrophenol (DNP, 3 mM), and carbonyl cyanide m-chlorophenyl hydrazone (CCCP, 100 μM) all increased sucrose uptake from 10 mM sucrose while inhibiting respiration and incorporation of ¹⁴C-label into the


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insoluble fraction (Table 1). Sucrose uptake in the presence of 3 mM DNP showed first order kinetics with a rate coefficient of 0.103 μmol·g⁻¹·hr⁻¹·mmol⁻¹, which was 6 times greater than in control tissue. The saturable component of sucrose uptake was not apparent in tissue bathed in 3 mM DNP (data not shown). When tissue disks were incubated for 2 hr in ¹⁴C-sucrose prior to placement in 3 mM DNP, efflux of radioactivity after one hour in DNP was 11% higher than the loss from control disks. Metabolism of labeled sucrose and sequestering of sugars in the vacuole may have reduced the release of ¹⁴C-activity. The greater uptake of sucrose in the presence of DNP was similar to that of disks in solutions without Ca. When all disks were incubated without Ca, 3 mM DNP only stimulated sucrose uptake 30%. At lower concentrations of DNP, sucrose uptake was inhibited (Table 2). Uptake from 10 mM sucrose was reduced 36% by 10⁻³ M DNP. At this concentration, incorporation of radioactivity into the insoluble fraction was strongly inhibited (78%), whereas respiration was slightly stimulated (7%). At 10⁻⁴ M DNP, respiration was nearly eliminated, but sucrose uptake was only moderately depressed. Both a N₂ atmosphere and 5 mM glucose inhibited sucrose uptake 16% while incorporation of ¹⁴C into insoluble substances decreased by over 50% (Table 1).

Because of the reported presence of cell wall bound acid invertase in strawberry fruit (19), the kinetics of glucose and fructose uptake into strawberry fruit disks were measured and compared with that of sucrose (Fig. 4). Fructose uptake was very similar to that of sucrose, having both a linear and a saturable component. For fructose, the values of \( V_{max} \), \( K_M \) and \( k \) were 0.55 μmol·g⁻¹·hr⁻¹, 13.2 mM and 0.017...
depending on the labeled sugar supplied to the tissue. Together, at all concentrations tested. Unlike sucrose and fructose, glucose metabolized by the fruit tissue. After 2 hr incubation in labeled sugars, over 80% of all radioactivity was recovered in the soluble fraction, 5% to 8% in the acid fraction and 2% to 3% in the basic fraction, regardless of the labeled sugar fed (Table 3). The distribution of sugars within the neutral fraction, however, depended on the labeled sugar supplied to the tissue. Together, glucose and fructose accounted for 68% to 79% of the radioactivity, 5% to 8% in the acid fraction and 2% to 3% in the basic fraction of radioactivity in the neutral fraction. In disks fed (U-14)C-sucrose or 14C-glucose, half of the radioactivity in the neutral fraction was in glucose, whereas fructose accounted for over half of the radioactivity when disks were fed (14C-fructosyl) sucrose or 14C-fructose.

If sucrose is hydrolyzed and subsequently resynthesized, the distribution or radioactivity between its glucose and fructose moieties would be, because of isomerase activity, different than that of the 14C-sucrose offered the tissue. Glucose/fructose (G/F) ratios of sucrose isolated from tissue disks were all below 1.0, regardless of the sugar fed. The G/F ratio of sucrose from tissues fed (14C-fructo) sucrose was significantly higher than the ratio of the original sugar (i.e., 0.01), but lower than that from disks incubated in 14C-fructose (0.10 vs. 0.35), suggesting that some, but not all, of the exogenously supplied sucrose had undergone hydrolysis and randomization. To compare the metabolite distribution in tissue disks with that in intact fruit, labeled sugars were fed to source leaves, and metabolites in fruit were analyzed 8 hr later. The distribution of radioactivity among metabolites was similar regardless of the sugar fed (Table 4). Intact fruit had a 2–3 times higher proportion of activity in the insoluble fraction than was found in tissue disks. Also, 50% of the radioactivity in the neutral fraction was in sucrose, with the remaining equally distributed between glucose and fructose. Sucrose isolated from plants fed (14C-fructose)sucrose or (U-14)C-sucrose had a G/F ratio of about 0.5 indicating that sucrose underwent considerable hydrolysis and randomization.

Discussion

The failure to detect sucrose in very young strawberry fruit and the subsequent rise in the concentration of sucrose from 5 to 18 days after anthesis may have resulted from changes in invertase activity. Poovaiah and Veluthambi (19) reported that soluble acid invertase activity in strawberry fruit is highest at anthesis but declines rapidly over the next 20 days. The opposite patterns of sucrose accumulation and soluble invertase activity in strawberry fruit are similar to those in young sugar beet roots (7). The drop in sucrose concentration as 'Brighton' fruit ripened may be associated with the observed rise in invertase activity as fruit reach maximum fresh weight (19).

The strawberry fruit at 15 days after anthesis is undergoing many rapid changes. At about this time, the rate of fruit expansion is at its peak (6), sugars are rapidly accumulating and the middle lamella of cortex parenchyma is beginning to break down (13). The ability of Ca to maintain respiration in disks cut from fruit of this age was not due solely to an osmotic effect. Calcium may have prevented cell separation and maintained fruit tissue integrity. Neal (18) demonstrated the importance of divalent cations, such as Ca2+, in preserving cell adhesion in ripening strawberry fruit.

While maintaining respiration, Ca appeared to inhibit sucrose uptake. Ehwald et al. (4) found Ca reduced sugar efflux from sugar beet root disks. They proposed that Ca decreased membrane permeability or that it mechanically stabilized the structure of the cell wall-membrane complex, thus enabling cells to tolerate the high turgor pressures that result from incubation in dilute solutions. ATPase, which appears to be involved in active sugar uptake (15), was inhibited by Ca in strawberry tissue extracts (2). Since respiration, which would be necessary for ATP production, required the addition of Ca, it appears that decreased sucrose uptake in the absence of CaCl2 was a result of greater membrane permeability and leakage of sucrose into the tissue.

Uptake of 14C-sucrose by strawberry tissue disks was not dependent upon its conversion into insoluble substances. There was no lag in the initiation of sucrose uptake, yet the incorporation of 14C into the insoluble fractions was initially slow. Temperature from 30° to 40°C reduced the recovery 14C in insoluble material, whereas total sucrose uptake and partitioning of 14C into the soluble fraction greatly increased. Presumably, the soluble fraction was derived mostly from vacuolar material from the large strawberry cortex cells (11).

The 2 kinetic components of sucrose uptake observed in strawberry fruit tissue have been reported in other plant tissues (16, 17, 22). The biphasic nature of sucrose uptake could in-

<table>
<thead>
<tr>
<th>Fraction</th>
<th>(14C-fructosyl)sucrose</th>
<th>(U-14C)sucrose</th>
<th>14C-fructose</th>
<th>14C-glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insoluble</td>
<td>12 ± 2</td>
<td>18 ± 1</td>
<td>19 ± 1</td>
<td>16 ± 1</td>
</tr>
<tr>
<td>H2O Soluble</td>
<td>88 ± 2</td>
<td>82 ± 1</td>
<td>82 ± 1</td>
<td>84 ± 1</td>
</tr>
<tr>
<td>Acidic</td>
<td>5 ± 1</td>
<td>8 ± 1</td>
<td>7 ± 1</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Basic</td>
<td>3 ± 1</td>
<td>2 ± 1</td>
<td>3 ± 1</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Neutral</td>
<td>93 ± 1</td>
<td>90 ± 1</td>
<td>90 ± 1</td>
<td>91 ± 1</td>
</tr>
<tr>
<td>Glucose</td>
<td>14 ± 2</td>
<td>48 ± 2</td>
<td>16 ± 2</td>
<td>69 ± 3</td>
</tr>
<tr>
<td>Fructose</td>
<td>54 ± 2</td>
<td>25 ± 1</td>
<td>59 ± 1</td>
<td>10 ± 5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>17 ± 1</td>
<td>19 ± 1</td>
<td>11 ± 1</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>G/F ratio</td>
<td>0.10 ± 0.02(\ast)</td>
<td>0.49 ± 0.05</td>
<td>0.35 ± 0.05</td>
<td>0.59 ± 0.03</td>
</tr>
<tr>
<td>Total uptake ((\mu)mol sugar · 2 hr(^{-1}))</td>
<td>0.417</td>
<td>0.671</td>
<td>0.671</td>
<td>1.563</td>
</tr>
</tbody>
</table>

\(\ast\)Values represent the means of 3 replicates ± se.

\(\ast\)Ratio of radioactivity ± se in glucose (G) and fructose (F) moieties of recovered sucrose.
The strawberry cortex tissue used in these experiments is not homogeneous, but contains several cell types, including parenchyma cells as well as vascular tissue. In cotyledons of Ricinus communis, Komor (14) suggested the 2 components of sucrose uptake were due to a linear diffusional component superimposed upon a saturable, carrier mediated transport system. In sugar beet roots, however, the linear component was inhibited by reduced temperatures and metabolic inhibitors (22), indicating an energy dependent mechanism that has not been adequately explained.

The effect of metabolic inhibitors and temperature on sucrose uptake were measured to determine if uptake is dependent on metabolic energy. The increased rates of sucrose uptake observed in 3 mM DNP, 100 μM CCCP, and 5 mM NaCN were opposite of effects observed on sucrose uptake in tissue disks from the taproot of sugar beet (26). We also measured sucrose uptake by sugar beet tap root tissue under the same conditions used for strawberry tissue and found that 3 mM DNP and 5 mM NaCN reduced sucrose uptake similar to the reduction reported by Wyse (26). In strawberry fruit tissue, these relatively high inhibitor concentrations may eliminate any active uptake component and greatly increase the permeability of the sucrose limiting membrane. Jackson (12) demonstrated with barley roots that at acid pH, DNP increased membrane permeability to ions. The increase in the linear component of sucrose uptake as well as larger efflux caused by 3 mM DNP in strawberry tissue is consistent with an increased permeability of the tissue to sucrose. At reduced DNP concentrations, membrane permeability may be affected to a lesser extent, and reduced sucrose accumulation may indicate the involvement of an active uptake system.

In some plant tissues sucrose is believed to be hydrolyzed by an invertase in the free space before uptake (10). A cell wall bound invertase with a pH optimum of 4.6 is reportedly present in strawberry fruit (19). The similar pH optimum observed for sucrose uptake may indicate the involvement of invertase. If sucrose is hydrolyzed before uptake, strawberry fruit tissue might be expected to accumulate the resulting hexoses more rapidly than sucrose. This accumulation was found to be the case, with the rate of glucose uptake much higher than that of sucrose. The saturable nature of glucose uptake indicates a different mechanism than that involved in sucrose accumulation. In sugar cane internodes, in which sucrose is hydrolyzed in the free space, glucose is also taken up more rapidly than sucrose (9). In the present study, the inhibition of sucrose uptake by glucose may also indicate inversion of sucrose before it is accumulated in the tissue.

The 14C-metabolite distribution in fruit disks fed (U-14C) sucrose was very similar to the expected distribution if fruit had been fed equal amounts of 14C-fructose and 14C-glucose. Because of the increased rate of glucose uptake, higher accumulation of glucose than of fructose would be expected. This accumulation probably contributed to the greater proportion of radioactivity in glucose than fructose in tissues fed (U-14C)-sucrose. In contrast, metabolite distribution in tissues fed (14C-fructosyl) sucrose was similar to those fed 14C-fructose, both having high levels of labeled fructose. The alteration of the G/F ratio of (14C-fructosyl)sucrose fed to tissue discs indicates that some sucrose underwent hydrolysis, but that a portion entered the fruit tissue intact. The fact that the G/F ratio of sucrose from (U-14C) sucrose fed tissue is not 1.0 also suggests hydrolysis. 14C-glucose may be diluted by a pool of unlabeled glucose resulting in a G/F ratio of less than 1.0 when sucrose is resynthesized. From these observations, it appears that sucrose is hydrolyzed and taken up as hexoses, with some sucrose entering the tissue intact, possibly by a passive leakage.

Asymmetrically labeled sucrose was randomized similarly to uniformly labeled sucrose in intact fruit, which would be consistent with apoplastic unloading and hydrolysis in the free space. Because sucrose fed to leaves may have taken 3-4 hr to arrive at the fruit (5), labeled sucrose may have had a longer period of time to be metabolized than 14C-sugars fed directly to fruit disks. This time difference explains why a large portion of the activity occurred in the insoluble fraction and why the levels of radioactivity recovered in sucrose were higher than in tissue disks. Accumulations of sucrose in 'Brighton' strawberry fruit at this stage of development is more rapid than that of glucose and fructose (Fig. 1). Sucrose could also have been accumulated in intact fruit without hydrolysis, with hydrolysis and randomization occurring in the leaves prior to loading or in fruit cells after uptake.

### Literature Cited