DOI:10.1021/jf102562n

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Comparison of Leaf and Fruit Metabolism in Two Tomato (*Solanum lycopersicum* L.) Genotypes Varying in Total Soluble Solids

Kietsuda Luengwilai,[†,§] Oliver E. Fiehn,[‡] and Diane M. Beckles*[,†]

[†]Department of Plant Sciences, University of California, One Shields Avenue, Davis California 95616, United States, and [‡]Department of Molecular and Cellular Biology & Genome Center, University of California, 451 Health Sciences Drive, Davis, California 95616, United States. [§]Current Address: Horticulture Department, Kasetsart University, Kampangsaen campus, Kampangsaen, Nakorn Pathom 73140, Thailand.

Sink and source activity in two tomato (*Solanum lycopersicum* L.) genotypes that vary in fruit Brix were investigated to identify differences that potentially underscore this trait. Solara (Brix 9%) accumulated almost twice the glucose, fructose, and sucrose in ripe fruit and had a higher horticultural yield (25% greater) compared to Moneymaker (Brix 5%). ^{14}C-glucose feeding suggested large disparities in sucrose metabolism in ripe fruit between genotypes. Biochemical pathways in the leaf adjacent to a fruiting truss at night were also analyzed since in many species, this is the period when leaf reserves are mobilized to feed the plant. Surprisingly, leaf metabolism, i.e., starch and sugar content, the levels of polar metabolites assayed by GC-TOF MS and $^{14}CO_2$-pulse-chase fluxes in detached leaves, did not change between the day and night in either genotype. Solara has a higher morphological source-to-sink ratio, and this may contribute to higher Brix in that genotype.

KEYWORDS: Tomato; Brix; total soluble solids; metabolite profiling; ^{14}-C feeding

1. INTRODUCTION

The aim of this study was to compare biochemical and eco-physiological traits of two tomato genotypes that are drastically different in fruit Brix. Brix (total soluble solids, TSS) is arguably the most important quality parameter in both fresh-market and processing tomatoes (*1*). Each 1% increase in TSS is estimated to be worth $700,000 a year to the US tomato processing industry (*2*). Although the flavor of table tomatoes is determined by a complex interplay among taste, aromas, and texture, consumers rate fruit sweetness highly in their evaluation of fresh tomatoes (*3*), and there has been a litany of complaints about their poor flavor (*4*).

One of the problems with breeding for high TSS is that there is often an inverse relationship between this trait and fruit size (*5*,*6*). Several wild tomato accessions have poor yield but very high TSS (10−12%). These tomatoes tend to import more sugars to the fruit later in ripening, which is circumvented in modern tomatoes because of abscission of the fruit calyx (*7*). Another feature that differentiates the wild tomatoes is their more efficient sucrose import mechanisms that vary among accessions. For example, in the hexose-accumulators *S. pennellii* and *S. pimpinellifolium*, high acid invertase rapidly converts sucrose to hexose, and in *S. pennellii*, the increased invertase activity in the apoplast may magnify the sucrose gradient between the source and sink causing greater sucrose translocation to the fruit (*8*,*9*). In *S. habrochaites* and to some degree also in *S. pennellii*, more starch conversion from sucrose may accentuate the sucrose gradient early in fruit development, and in the mature fruit, the stored starch is degraded contributing to the sugar pool (*8*, *10*). Finally, experimental data suggests that high accumulation of sucrose in *S. habrochaites*, *S. hirsutum*, and *S. peruvianum* may be due to a steepening of the sucrose gradient later in fruit development via cycles of sucrose degradation and resynthesis involving sucrose phosphate synthase and sucrose synthase (*11*, *12*).

Carbohydrate production in fruit is not just the result of endogenous metabolic processes in this organ; however, it is also partially dependent on the availability of leaf assimilates for import. Evidence for this comes from a range of studies. Changes in the leaf-to-fruit ratio (*13*), modification of leaf photosynthetic activity by shading (*14*), or altering of the activity of some leaf metabolic enzymes (*15*, *16*) have all had repercussions in fruit yield and/or sugar accumulation. In addition, morphological and physiological differences in the features of photosynthetic tissues in *S. pennellii* introgression lines and the self-pruning mutant correlated with differences in fruit TSS; and plants with a high harvest index had a lower TSS and vice versa (*17*). These factors point to the importance of considering both source and sink capacities when trying to elucidate mechanisms determining horticultural yield, as this trait may be both source- and sink-limited.

We had two separate objectives as we undertook this study. Our first objective was to understand key differences in fruit metabolism that may be the basis for increased TSS in Solara. This breeding line has high TSS (9−12%) (*18*) similar to that in *S. pimpinellifolium* (10−12%) from which it is derived, but Solara

*To whom correspondence should be addressed. Phone: ++1 530 754 4779. Fax: ++1 530 752 9659. E-mail: dmbeckles@ucdavis.edu.

 Published on Web 10/29/2010

fruit averages 20−30 g in mass when grown in the greenhouse, which is 20−25-fold greater than the fruit of its *S. pimpinellifolium* parent (*19*). Understanding the biochemical basis for high TSS in Solara fruit may indicate novel mechanisms for developing tomato cultivars with higher horticultural yield. To this end, starch and sugars were measured throughout fruit development, and ^{14}C-glucose fluxes were assayed and compared to those of the research cultivar Moneymaker. Our second objective was to examine source metabolism in these two genotypes. To understand how metabolic pathways and fluxes change in tomato leaves as they transition from light to dark, phenomena not well documented previously, we profiled polar compounds using GC-MS, monitored $^{14}CO_2$ fluxes, and compared the results obtained for the two genotypes. We also measured a range of morphological features of fruits and leaves to put the biochemical data into a physiological context.

2. MATERIALS AND METHODS

2.1. Reagents and Plant Materials. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise stated. Seeds of cv. Moneymaker were obtained from The C.M. Rick Tomato Genetics Resource Center (Davis, CA). Seeds of the breeding line Solara were a kind gift from Dr. Lilliana Stamova (Davis, CA).

2.2. Plant Growth and Tissue Sampling. Tomato plants were grown in a greenhouse from April to September, 2007, at UC Davis, (Davis, CA) as described previously (*18*). Plant developmental stage was recorded weekly for the 48 individual plants, and all side branches were removed as they appeared. A set of 36 plants was used for leaf metabolite profiling and radiolabeling experiments. Another set of 12 plants (6 plants per genotype) were pruned at a level of two leaves above the ninth inflorescence (truss) (Supporting Information, Figure 1A). This set was used to study leaf and fruit physiological characteristics. Flowers were hand-pollinated as they opened, and fruits were harvested when they had reached the appropriate developmental stage. The number of leaves (length ≥5 cm), inflorescences, open flowers, and set fruit, and fruit diameter throughout development were recorded for 6−10 fruits at each developmental stage from each individual plant. Dry weight of individual fruit, leaves, and vegetative tissues was measured after incubation for 14 days in a ventilated oven at 55 °C. Fruits were harvested from among the second to fifth fruits of the first four trusses of each plant. Fruits were harvested at 14, 21, 28, 35, and 42 days postanthesis (DPA), and when fruits were about to change color (breaker) and when fully ripe. If not used immediately, the fruit was quartered, weighed, and flash-frozen in liquid nitrogen. To compare equivalent stages, data was plotted using a developmental index, which is the ratio of the number of days at harvest to the total number of days between anthesis, and the fully ripe fruit for that genotype obtained.

2.3. Fruit and Leaf Starch and Sugar Contents. Fruit starch was determined as previously described (*20*). Fruit and leaf sugars and fruit starch were extracted and analyzed by HPLC as described by Stamova et al. (*21*), while leaf starch was assayed enzymatically exactly as outlined by Luengwilai and Beckles (*22*). Brix was determined as the concentration of total soluble solids (% Brix) measured, using a digital refractometer (RFM-80 BS), on a random sample of 10 fruits per genotype. Mean fruit weight (g) was calculated from a random sample of six representative fruits per plant.

2.4. [U−^{14}C]-Glucose Feeding Experiment. Tomato pericarp disks from 18 fruits at approximately 16 DPA and 18 fruits at red-ripe were sampled with a 10 mm cork borer. Labeling with [U−^{14}C]-glucose (hereafter written as ^{14}C) was as described by Luengwilai et al. (*18*). After labeling for 2 h, the disks were ground to a powder in liquid nitrogen, and the homogenized tissues were then boiled in 80% (v/v) ethanol and separated by centrifugation into the soluble and insoluble fractions. To analyze the starch, the insoluble fraction was digested with amyloglucosidase (10 U per 200 μL of insoluble fraction, Roche Biosciences, Indianapolis, IN) and ^{14}C measured by liquid scintillation counting. The soluble extract was then fractionated into the sugar, organic acid, and amino acid fractions using ion-exchange column chromatography as described by Carrari et al. (*23*), and the incorporation of radioactivity into each fraction was determined by liquid scintillation.

2.5. Leaf Sampling for GC-TOF-MS Profiling. Tissues for carbohydrate measurements and metabolic profiling by GC-TOF MS (Gas Chromatography-Time-of-Flight Mass Spectrometry) were sampled as the first truss was setting fruits. Two leaf disks were harvested from a terminal leaflet immediately above the first truss (Supporting Information, Figure 1C) and used for starch and sugar measurements. Samples were taken 1 h before the onset of darkness (8 p.m.), in the middle of the night (2 a.m.), and then 1 h before dawn (5 a.m.). The rest of the terminal leaflet was immediately frozen in liquid nitrogen and stored at −80 °C for GC-TOF MS analysis. Six replicate plants were used for each of the three timepoints studied, and each plant was sampled only once so that 18 plants were sampled for each genotype.

2.6. Metabolite Extraction and Quantitative Analyses. Polar metabolites were extracted as described previously (*24*) with the following modifications. Frozen leaf tissue (30 mg) was homogenized in liquid nitrogen. One milliliter of prechilled extraction solution (acetonitrile, isopropanol, and water; 3:3:2 ratio) was added. The samples were then vortexed for 10 s and placed on a shaking platform for 5 min at 4 °C. After shaking, the samples were centrifuged for 2 min at 13,000*g*, and the supernatant removed. A 90 μL aliquot of the supernatant was dried completely in a speed vacuum. In order to fractionate complex lipids and waxes, the centrifugation residue was resuspended in 500 μL of 50% (v/v) aqueous acetonitrile and centrifuged at 13,000*g* for 2 min. That supernatant was then transferred to a 1.5 mL Eppendorf tube and concentrated to dryness in a speed vacuum. The extracts were immediately derivatized and GC-TOF mass spectrometry conducted on them (*24*).

2.7. Assessment of Bulk Fluxes in Leaves by $^{14}CO_2$ Pulse−Chase Experiments. The $^{14}CO_2$ feeding experiments were carried out on whole leaves harvested at 8 p.m. (1 h before the start of the dark period). Material was harvested when the plant had 5 trusses. The terminal leaflets of two leaves above and two leaves under the third truss were sampled from six individual plants (Supporting Information, Figure 1B), and each of the 4 leaflets was randomly used for assay as the pulse or chase. Each leaflet was cut with a razor blade under water, and the petiole was placed immediately into a vial containing water where it remained for the entire experiment in order to prevent tissue dehydration. All leaflets in their vials were placed into a chamber kept at 25 °C. $^{14}CO_2$ was generated from (labeled sodium bicarbonate) $NaH^{14}CO_3$ (1.48 MBq) and 500 μL of 1 N perchloric acid ($HClO_4$) in a $^4CO_2$-generating vial. After a 20 min incubation (pulse) under light conditions (400 $W \cdot ms^{-1}$), 500 μL of 10% (w/v) potassium hydroxide (KOH) was added into the $^{14}CO_2$-generating vial to stop the production of CO_2 and in an additional vial to further absorb $^{14}CO_2$ in the chamber air space for 5 more minutes. To end the pulse period, the air exit of each vial was opened, and the radiolabeled air was pumped into a flask containing 10% (w/v) KOH. To flush the system, 500 μL of 1 N $HClO_4$ was injected into 500 μL of 0.5 M of unlabeled $NaHCO_3$ to release CO_2 for 10 min under light conditions. The samples were then transferred into new chambers and kept in the dark for 0, 2, 4, and 8 h (chase). After the chase period, each harvested leaflet sample was flash-frozen in liquid nitrogen before being extracted as described above for the ^{14}C-feeding experiments in fruit tissues.

2.8. Statistical Analysis. Data were analyzed by Student's *t*-test using Microsoft Excel software (Redmond, WA), and *P*-values were determined at the 95% confidence intervals. Statistical analysis of GC-TOF MS results was performed as described in Fiehn et al. (*23*). Box-whisker plots, principal component analysis (PCA) and partial least squares (PLS) were performed using Statistica Data Miner Software (Statsoft Inc., Tulsa, OK). Pearson's correlation coefficients were determined using Microsoft Excel, where $n = 2$ samples × 6 biological replicates = 12 as described by Stamova et al. (*25*)

3. RESULTS

3.1. Fruit Metabolism. *3.1.1. Carbohydrate Accumulation.* Solara fruit accumulated 2-fold more glucose and sucrose, and 1.5-fold more fructose than Moneymaker in the late stages of fruit maturation (**Figure 1A**,**C**). However, there were few differences when the data was expressed on a dry weight basis (Supporting Information, Figure 2). At 14 DPA, the starch content of fruit was similar between the two lines, and starch was degraded at comparable rates (**Figure 1B**,**D** and Supporting Information, Figure 2B,D).

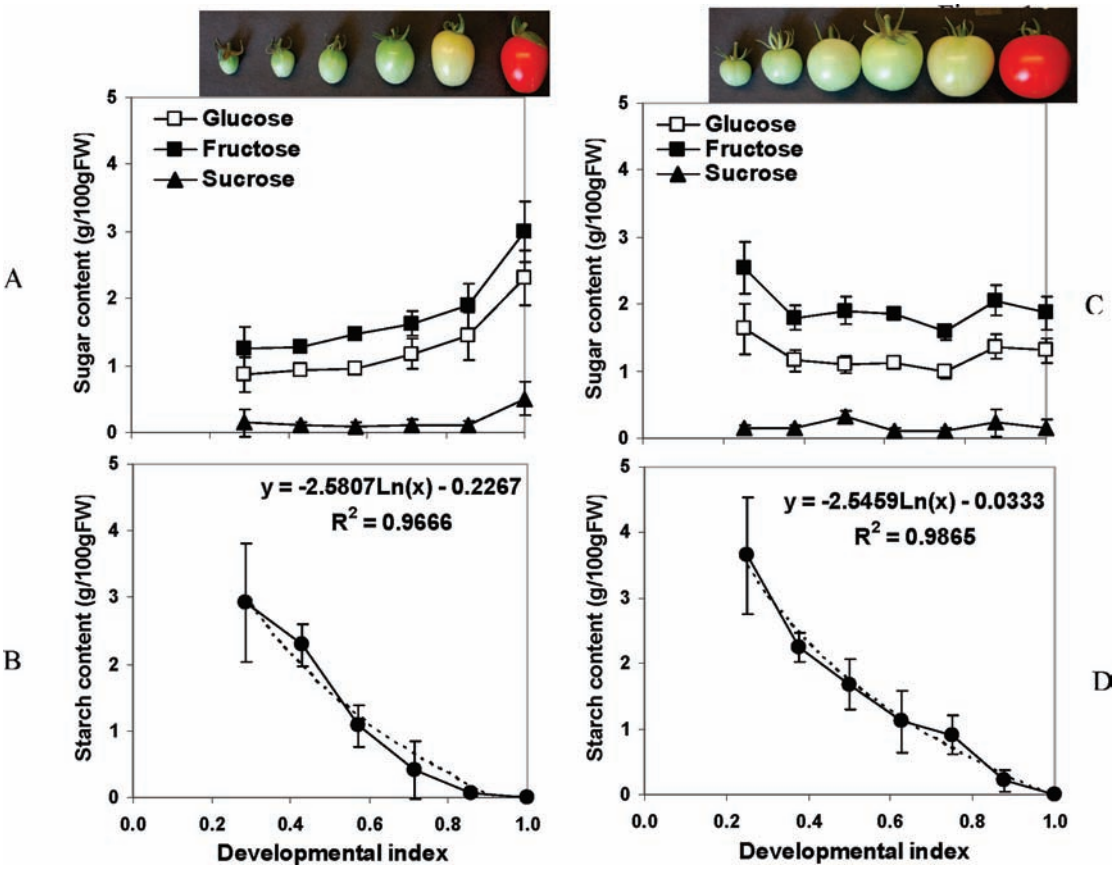

Figure 1. Total soluble sugars (**A**,**C**) and starch (**B**,**D**) content in Solara (**A**,**B**) and Moneymaker (**C**,**D**). The logarithmic regression line for starch accumulation over time is shown as a dashed line (**B**,**D**). Fruit were harvested at 7-day intervals from 14 days postanthesis (DPA) to the fully ripe fruit stage. Definition of the developmental index is given in Materials and Methods. Values represent the mean ± SD of 6 fruits.

3.1.2. ^{14}C-Glucose Uptake and Partitioning. ^{14}C-Feeding experiments showed that there were high levels of dry matter accumulation at 16 DPA (61−64%) in both genotypes, which decreased 30-fold at the red ripe stage (**Table 1**). The proportion of glucose which partitioned to all sugar fractions was similar between genotypes at 16 DPA except for the partitioning to sucrose, which differed, and this difference was magnified in ripe fruit (**Table 1**). While the label in sucrose decreased 4-fold in Moneymaker (from 13 to 3.4%), it did not change significantly in Solara (from 20% to 17%; $P > 0.05$) over the period studied. The partitioning of ^{14}C into glucose and fructose in Moneymaker fruit was approximately the same (∼42−44%) and differed from Solara in that the latter had a smaller amount of label in glucose (26%) (**Table 1**). Solara also had ∼4-fold less ^{14}C in the organic acids (acidic components) fraction (in accordance with low titratable acidity measurements, data not shown, and **Table 1**) and about half the ^{14}C in CO_2, a gauge of respiration, than did Moneymaker (**Table 1**).

3.2. Leaf Metabolism. *3.2.1. Carbohydrate Accumulation.* We measured leaf starch and sugar contents to assess the carbon status of leaves in the dark period. Previous work strongly suggested that the leaf adjacent to a fruiting truss acts as its source, and therefore, these were the leaves sampled (*26*, *27*). Starch content from both Moneymaker and Solara decreased from 20 mg·g FW^{-1} at 8 p.m. to 13−15 mg·g FW^{-1} at 2 a.m. and remained essentially at that level until the end of the dark period (**Figure 2A**). There were no significant differences ($P > 0.05$) in starch accumulation between these time points or between the two tomato varieties (**Figure 2A**,**B**).

We next assayed the diurnal pattern of starch accumulation to see if our results (**Figure 2**) were an anomaly. We measured starch and sucrose every 4 h in another set of plants of similar developmental stage (the leaves were 1 week older). In both Solara and Moneymaker, there was no time-dependent difference in starch accumulation ($P > 0.05$; Supporting Information, Figure 3A). Slightly less starch accumulated in Solara at 2 p.m., 2 a.m., and 6 a.m. when compared to that in Moneymaker; however, both tomato varieties contained nearly identical amounts of sucrose so that the sucrose to starch ratio was slightly higher from 2 to 6 a.m. in Solara (Supporting Information, Figures 3B and 3C). The total sugar (glucose, fructose, and sucrose) to starch ratio was also slightly higher in Solara than in Moneymaker during the night (data not shown).

3.2.2. Leaf Metabolites Assayed by GC-MS-TOF. There is evidence for diurnal shifts in metabolite levels in the leaves of greenhouse-grown plants such as pineapple, Kalanchoe, and potato (*28*, *29*). We wished to see if tomato leaf metabolite levels showed changes in response to the onset of darkness. Metabolite measurements were carried out on the same leaf materials as those that were used for the starch measurements depicted in **Figure 2A**. The complete list of metabolites measured for this study is available at the SetupX public repository (http://fiehnlab.ucdavis.edu:8080/ml/main_public.jsp). Approximately 439 compounds were detected, but only 121 could be accurately identified using retention indices. Moneymaker accumulated higher levels of most metabolites than did Solara, most of which were organic acids and sugar alcohols (data not shown); similar results were obtained on wild tomato species by Schauer et al. (*17*).

To look at the underlying structure of the data, principal component analysis (PCA) was performed. The metabolites did not fall into distinct groups based on time points; rather, they grouped based on genotype (**Figure 3**). Using partial least

Table 1. Metabolism of ^{14}C-Labeled Glucose in Freshly-Excised Pericarp Disks from Moneymaker and Solara Tomato Fruit at 16 Days Post Anthesis (DPA) and in Ripe Fruit[a]

	^{14}C recovered per fraction (as % of total label uptake) from fruit disks incubated in glucose			
	16 DPA		ripe	
fraction	Moneymaker	Solara	Moneymaker	Solara
CO_2	2.1 ± 0.6	2.2 ± 0.6	**2.3 ± 0.2**	**1.2 ± 0.3**
ethanol-insolubles	64.7 ± 4.7	61.0 ± 1.4	1.6 ± 0.4	2.0 ± 0.7
starch	33.9 ± 2.1	35.1 ± 11.8	1.6 ± 0.4	2.2 ± 0.8
ethanol-solubles	30.6 ± 3.4	37.3 ± 1.6	96.1 ± 0.3	96.7 ± 0.4
sucrose	**13.3 ± 2.3**	**20.3 ± 1.9**	**3.4 ± 0.5**	**17.1 ± 6.4**
glucose	0.6 ± 0.1	0.8 ± 0.2	**43.8 ± 5.0**	**26.3 ± 4.6**
fructose	3.4 ± 0.3	4.5 ± 0.8	41.8 ± 2.9	45.5 ± 0.3
acidic components (organic acids)	8.4 ± 1.4	6.0 ± 1.3	**1.6 ± 0.1**	**0.3 ± 0.1**
basic components (amino acids)	4.9 ± 0.4	5.5 ± 0.6	2.5 ± 0.3	2.4 ± 0.7
total ^{14}C recovered				
as kBq	843.9 ± 110	796.0 ± 154	352.7 ± 69	334.3 ± 67
as % supplied	62.8 ± 10	56.9 ± 10	25.2 ± 5	23.9 ± 5

[a] The mean mass of the tissue used was 0.96 g ± 0.1. Disks were incubated in [U−^{14}C] glucose at a specific activity 1.4 kBq·mmol^{-1} together with 10 mM glucose for 2 h. Values are the mean ± SD (n = 3−6 independent measurements). The values in bold type indicate differences between the two genotypes at the 95% confidence level (P < 0.05).

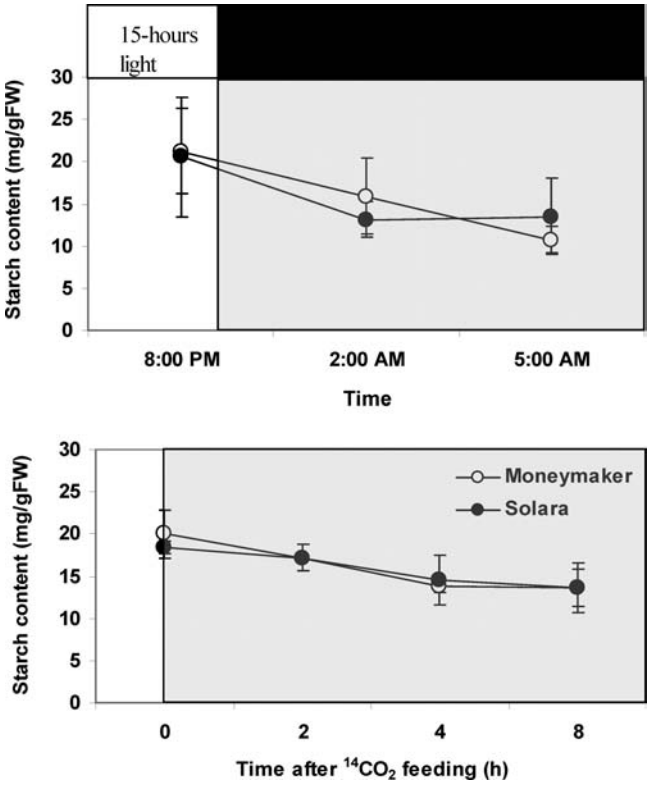

Figure 2. Starch accumulation of Moneymaker (white) and Solara (black) after 9 weeks of growth. Leaves were harvested when the first fruit appeared. (**A**) Starch content from plant-attached leaves. Two leaf disks from the terminate leaflet of the leaf above the first truss were sampled (i) one hour before the onset of darkness (8 p.m.), (ii) in the middle of darkness (2 a.m.), and (iii) an hour before the light (5 a.m.) period. A single plant was used for each sample assayed so that a total of 18 plants were used for each genotype in order to minimize disruption to source−sink relationships. (**B**) Starch content measured from half of the leaflet at each time point that was used for the ^{14}C feeding experiment (**Table 1**). Values represent the mean ± SD from 6 replicate plants.

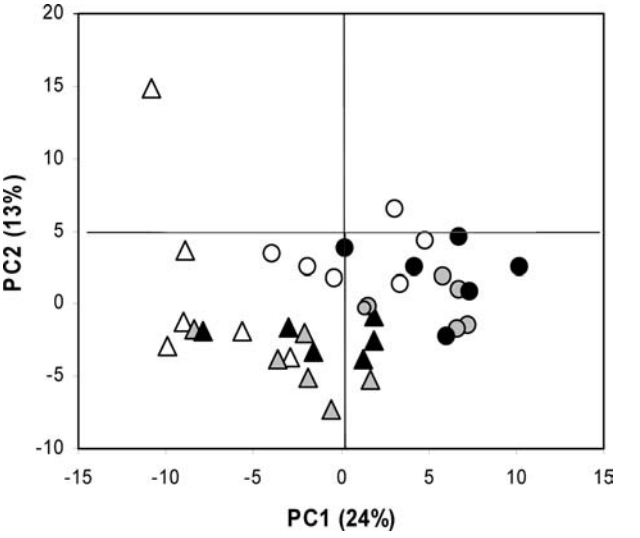

Figure 3. Analysis of metabolites in tomato leaves by GC-TOF MS profiling. Principal component analysis (PCA) of 121 identified metabolites in samples harvested from tomato leaves 1 h before the start (8 p.m.; after 14 h in the light; open/white), the middle (2 a.m.; gray), and 1 h before the end of the night (5 a.m.; black). Moneymaker (circles) and Solara (triangles). Each data point represents an independent sample. Samples that cluster together are more similar to each other, while those that are farther apart are different. The data displayed here are partitioned on the basis of genotype. PC = principal component.

squares-discriminant analysis (PLS-DA), a supervised method that accentuates differences between groups (*25*), similar results were obtained (data not shown). These data indicate that there was no detectable change in metabolite levels between time points in either genotype.

We then calculated the $\log_2$ ratios of the fold-change in metabolite levels relative to that in the 8 p.m. sample. This would magnify differences in compounds accumulated between tomato samples and accentuate changes over the three periods, even though these changes are not statistically significant. Metabolites were grouped into major compound classes (*30*) (see **Figure 4A**). In general, major carbohydrates in tomato leaf (sucrose, fructose, and glucose) either were unchanged or slightly decreased in signal level during darkness. Notably, maltose signal intensity, a strong indicator of starch breakdown did not change at night in either tomato variety (**Figure 4A**). The relative levels of major carbohydrates, i.e., glucose, fructose, and sucrose, identified through GC-MS profiling were identical to those measured using HPLC (data not shown). Metabolites associated with glycolysis, accumulated similarly in both Moneymaker and Solara, increasing in the

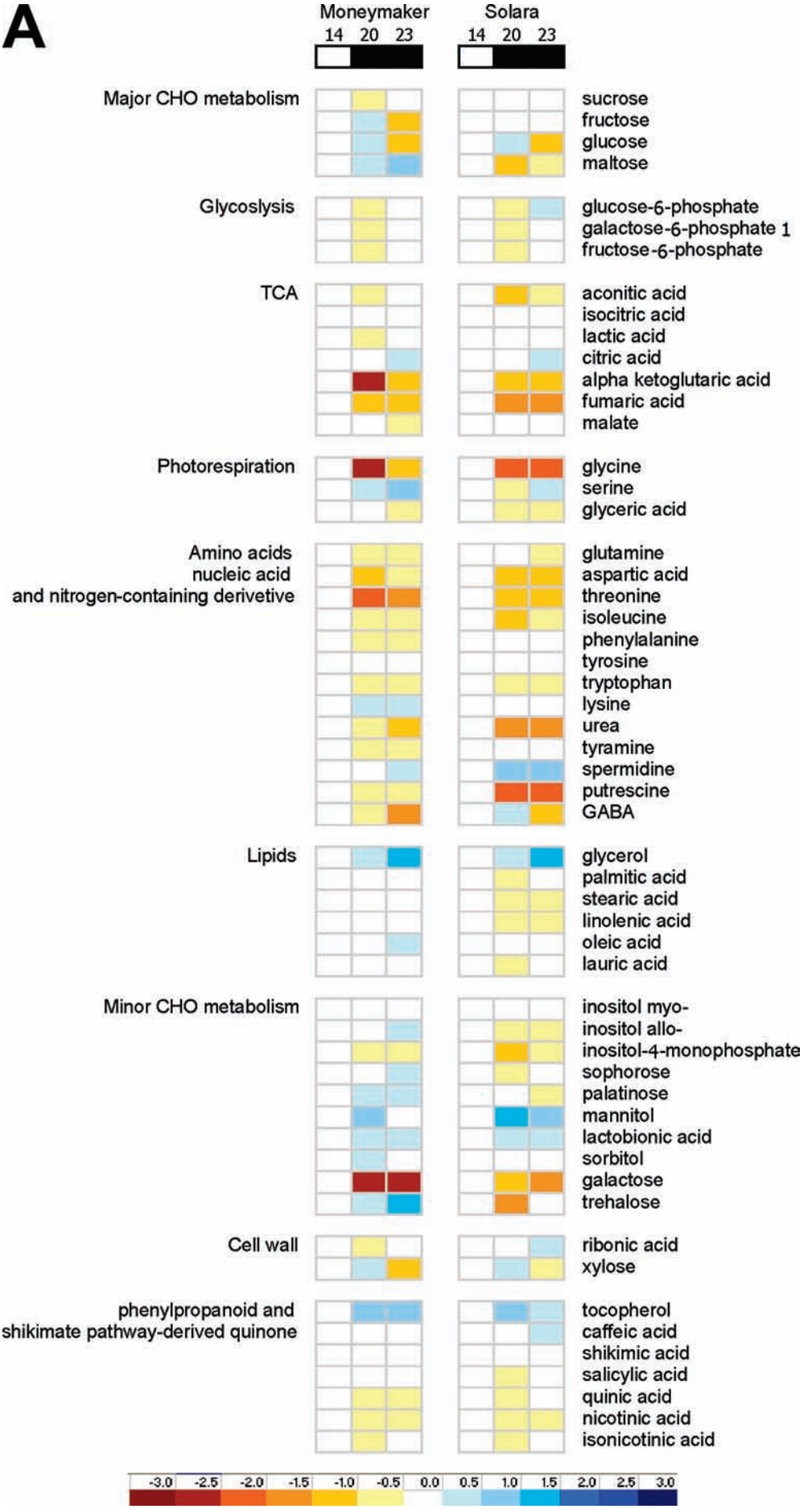

Figure 4. Continued

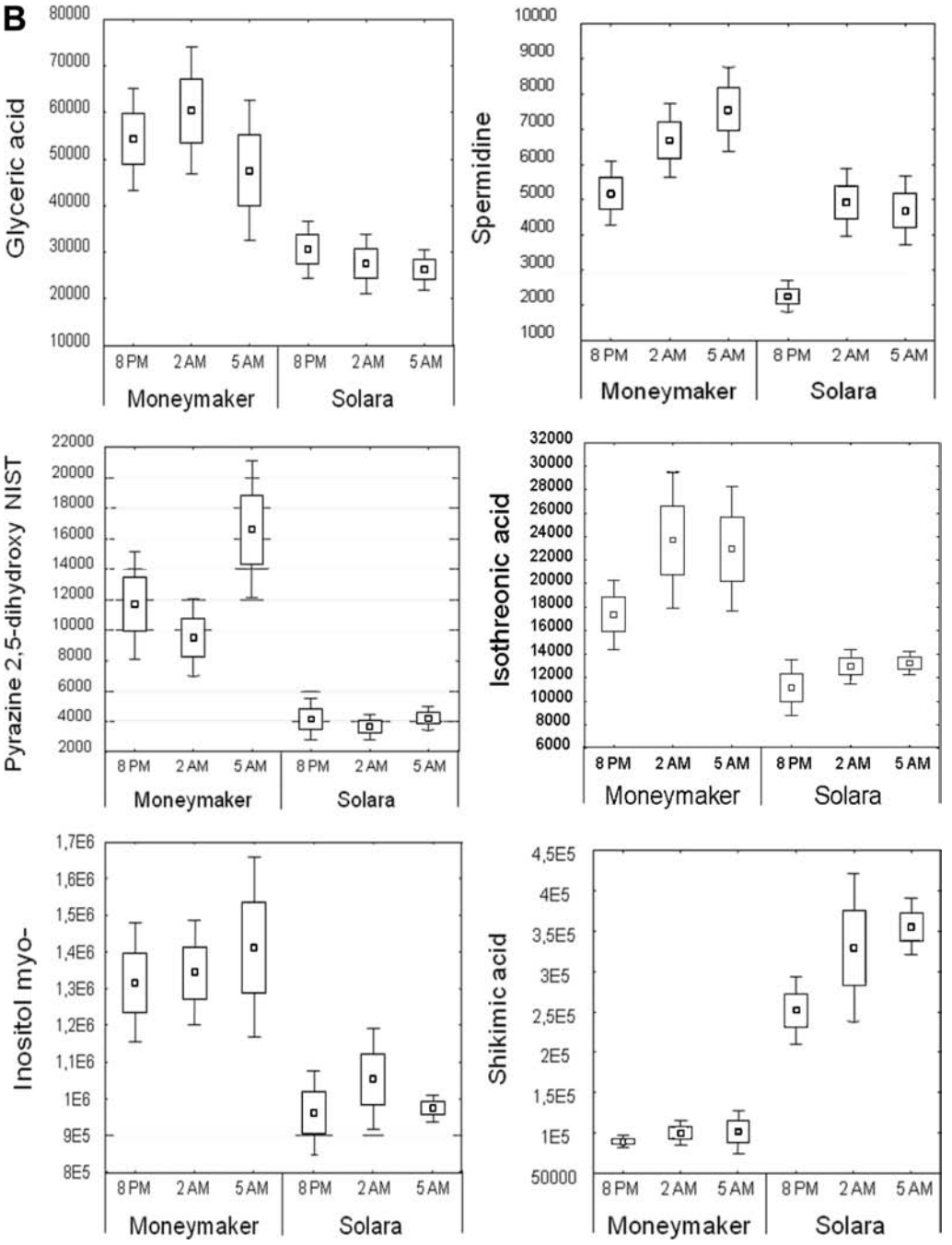

Figure 4. Metabolite levels in leaves of tomato genotypes. (**A**) Heat map showing the changes in metabolite levels between Moneymaker and Solara over the course of a day. Tomato plants were grown under 15 h of light and 9 h of dark cycles. Leaf harvesting and GC-MS-TOF analysis of leaf extracts were performed as described in Materials and Methods. Log_2 ratios were calculated for each value, and the fold-changes in metabolite levels relative to the value measured in samples harvested at 8 p.m. were calculated. Colors indicate the magnitudes of the fold-changes as indicated. The number of hours elapsed in the diurnal cycle at the time of leaf harvest is indicated at the top of the columns. As previously noted, the differences between time points were not statistically significant (B) Box-whisker plots levels of which differed significantly between Solara and Moneymaker. Boxes show the arithmetic means of the levels of each metabolite measured at the same points in the diurnal cycle as those in **A** and the standard error for 1.96 times standard deviation to indicate the 95% confidence intervals.

middle of the night and then at 5 a.m. levels were back to those present in light (**Figure 4A**). Metabolites associated with the TCA cycle pathway and most amino acids decreased during the night. Lipid-associated metabolites generally decreased in Solara leaves but did not change in Moneymaker.

When the levels of the 141 known metabolites were examined, only 6 differed significantly between Moneymaker and Solara ($P < 0.05$) (**Figure 4A**). They included glyceric acid, shikimic acid, spermidine, isothreonic acid, myo-inositol, and pyrazine 2.5-dihydroxy NIST (**Figure 4B**). Only 8 metabolites showed diurnal variation: two, threonine, and serine (1.7% of total), in Moneymaker, and six, spermidine, montanic acid, melibiose, mannitol, lysine, and glycine (5.0% of total; data not shown), in Solara.

3.2.3. ^{14}C-Uptake and Partitioning in Detached Tomato Leaves. Harvested tomato leaves were exposed to $^{14}CO_2$ for 20 min (pulse) in the light, then to air (chase) in the dark. The label incorporated during the pulse provides an estimate of partitioning of carbon into tissues, while the label in tissues during the chase is indicative of degradation. Leaves were harvested for labeling at 8 p.m. when photosynthesis was occurring, and labeled

Table 2. Pulse−Chase Experiment to Assess Primary Metabolism in Tomato Leaves[a]

	^{14}C- expressed as a % of total uptake recovered at the end of the pulse											
	starch		sucrose		glucose		fructose		organic acids		amino acids	
	MM	Sol	MM	Sol	MM	Sol	MM	Sol	MM	Sol	MM	Sol
pulse (20 min)	13 ± 5	15 ± 4	32 ± 2	40 ± 3	16 ± 2	13 ± 1	8 ± 1	3 ± 2	13 ± 1	13 ± 1	7 ± 0	7 ± 1
chase 1 (2 h)	7 ± 2^{ns}	13 ± 10^{ns}	34 ± 2^{ns}	33 ± 3^{ns}	18 ± 0^{ns}	12 ± 2^{ns}	7 ± 3^{ns}	**$10 \pm 2^{**}$**	11 ± 1^{ns}	14 ± 1^{ns}	6 ± 0^{ns}	6 ± 0^{ns}
chase 2 (4 h)	8 ± 0^{ns}	12 ± 2^{ns}	30 ± 3^{ns}	28 ± 3^{ns}	18 ± 2^{ns}	17 ± 3^{ns}	8 ± 1^{ns}	**$10 \pm 1^{**}$**	13 ± 2^{ns}	13 ± 1^{ns}	5 ± 0^{ns}	6 ± 1^{ns}
chase 3 (8 h)	12 ± 2^{ns}	11 ± 5^{ns}	**$21 \pm 1^{*}$**	**$20 \pm 2^{*}$**	14 ± 1^{ns}	16 ± 3^{ns}	12 ± 5^{ns}	4 ± 1^{ns}	**$24 \pm 3^{*}$**	13 ± 2^{ns}	9 ± 1^{ns}	6 ± 0^{ns}

[a] Terminal leaflets were incubated with $NaH^{14}CO_3$ at a specific activity 1.48 MBq · mmol^{-1} for 20 min under light conditions (400 W · ms^{-1}). The $^{14}CO_2$ was removed and the leaflets were flushed with $NaHCO_3$ for 10 min under light conditions (pulse). Then the leaflets were transferred to a new chamber and incubated for 2, 4, and 8 h in the dark. The ^{14}C measured in the pulse is expressed as a percentage of the total radioactivity measured in leaf disks (Bq · gFWT^{-1}). Values are the mean ± SE (n = 3 independent experiments). MM, Moneymaker; Sol, Solara. Statistical significance of the differences in the label compared between the pulse and chases are indicated by bold-faced values with one asterisk ($P < 0.05$) and bold-faced values with two asterisks ($P < 0.01$). Differences with $P > 0.05$ are indicated with ns (not significant).

samples were harvested 2, 4, and 8 h after the pulse in darkened conditions to stimulate degradation. Our results indicated that starch content at each sampling point in the detached leaves used for the feeding experiment was identical to that in leaves attached to the plant at equivalent times (**Figure 2A** and **B**). This suggests that the detached leaves serve as good representatives of in situ starch metabolism.

During the pulse, ^{14}C accumulated in sucrose (~32−40%), organic acid (~13%), starch (~13−15%), amino acids (~7%), and the hexose phosphate pool (~17−27%) (**Table 2** and data not shown). There were no differences in $^{14}CO_2$ uptake or in the percentage of ^{14}C partitioning into the amino acid, starch, sucrose, or glucose bulk pools between genotypes during either the pulse or chase. At the end of the chase there was more label in the organic acid fraction in Moneymaker, but not in Solara, while there was more turnover of the fructose pool in Solara not evident in Moneymaker (**Table 2**). Since the specific activity of the hexose phosphate pool was similar, there are thus no differences in fluxes between samples (*31*).

3.3. Ecophysiological Assessment of Growth. *3.3.1. Fruit.* A number of physiological parameters were examined in the two tomato lines (**Figure 5**). First we reconfirmed the higher TSS in Solara, which was 9% and nearly twice as high as that measured in Moneymaker, which was 5% (**Figure 5F**). Solara developed fewer trusses, but its fruits were smaller (**Figure 5D**,**E**) and greater in number (**Figure 5B**). Moneymaker and Solara both exhibited some indeterminate growth, but in this study, they were pruned or cut at the ninth inflorescence to produce a determinate growth habit. This made it possible to make comparable assessments of horticultural yield (TSS × yield), which was 25% higher for Solara than for Moneymaker (**Figure 5C**). Therefore, higher TSS in Solara was not accompanied by an equally dramatic decrease in fruit mass.

3.3.2. Vegetative Growth. We also measured various aspects of plant and leaf physiology, including the number of leaves, flowers, and trusses. Moneymaker flowered one week earlier than Solara so that at the time of measurement this genotype had 2 inflorescences with 10 flowers each, whereas Solara had one inflorescence with 6 flowers opened (**Figure 6**). However, truss production slowed in Moneymaker so that both genotypes had the same number of leaves and trusses by 9 weeks after germination (data not shown). Leaf size and plant height were also measured in the two lines and correlation networks drawn for each genotype (Supporting Information, Figure 4). Plant height correlated strongly with flower number (r = 0.915) in Moneymaker but with leaf number (r = 0.951) in Solara, which also had a larger leaflet size. The number of flowers is indicative of sink potential, while the size of the leaf is indicative of source capacity. Therefore, these results illustrate that sink development more strongly tracks plant growth in Moneymaker than in Solara, while in the latter, leaf size does. The overall picture is that Solara has a higher proportion of vegetative tissue than Moneymaker. This is borne out when all the individual ecophysiology measurements are examined but is succinctly described by the network analysis.

4. DISCUSSION

We performed a comprehensive comparative analysis of leaf and fruit metabolism to understand the basis for high TSS in the tomato variety Solara. Our data showed that Solara fruit accumulated approximately 2-fold more glucose, fructose, and sucrose in ripe fruit than Moneymaker (**Figure 1A**,**C**), which in turn led to higher soluble solids and higher horticultural yield at ripening in the former (**Figure 5C**,**F**). Ecophysiology measurements show that Solara has a higher proportion of vegetative tissue than Moneymaker, which may also be an important contributory factor (**Figure 6** and Supporting Information, Figure 4). In contrast to fruit, leaf metabolism showed no difference between the two genotypes at the stage of development we examined. Somewhat surprisingly, leaf starch, sucrose, and polar metabolite levels were essentially the same after 14 h of light and when followed by 8 h of darkness (**Figures 2**, **3**, **4A**, and Supporting Information, Figure 3).

4.1. Fruit Metabolism. Solara showed some similarities to other tomato species with high TSS. In *S. chmiewlewskii*, *S. cheesmanii*, *S. pennellii*, *S. habrochaites*, *S. peruvianum*, and *S. pimpinellifolium* a peak in sugar content late in fruit development is attributed to the continued import of sugars. In Solara, a sharp increase in glucose, fructose, and sucrose content in the pericarp was seen between 35 and 49 DPA. We saw no differences between the genotypes in pericarp ^{14}C-uptake, which implies similar sugar import capacity in Solara compared to Moneymaker. However, this difference could occur in the columella, which we did not analyze. Our suggestion is plausible because Baxter et al. (*8*) found differences in ^{14}C-uptake in the columella but not the pericarp in an *S. pennellii*-introgressed high TSS line (IL9−2−5). In spite of this, Solara may still have a different mechanism for achieving high TSS as compared to both IL9-2-5 and the *S. pimpinellifolium* accession studied by Husain et al. (*19*). While we observed altered ^{14}C-metabolism of sucrose, acids, and respiration, and altered steady-state levels of sugars in Solara pericarp, metabolism in this tissue was essentially unchanged in IL9-2-5 as compared to that in the control (*8*). Solara accumulates significant amounts of sucrose, while the *S. pimpinellifolium* studied by Husain et al. accumulated in levels similar to that in *S. lycopersicum* (*19*).

As noted earlier, the percentage of label that partitioned into sucrose remained essentially constant in Solara fruit from 16 DPA to the red ripe stage. In ripe fruit, there was 5-fold more label in sucrose but only 2-fold more steady-state accumulation of sucrose in Solara than in Moneymaker (**Table 1**; **Figure 1A**,**C**).

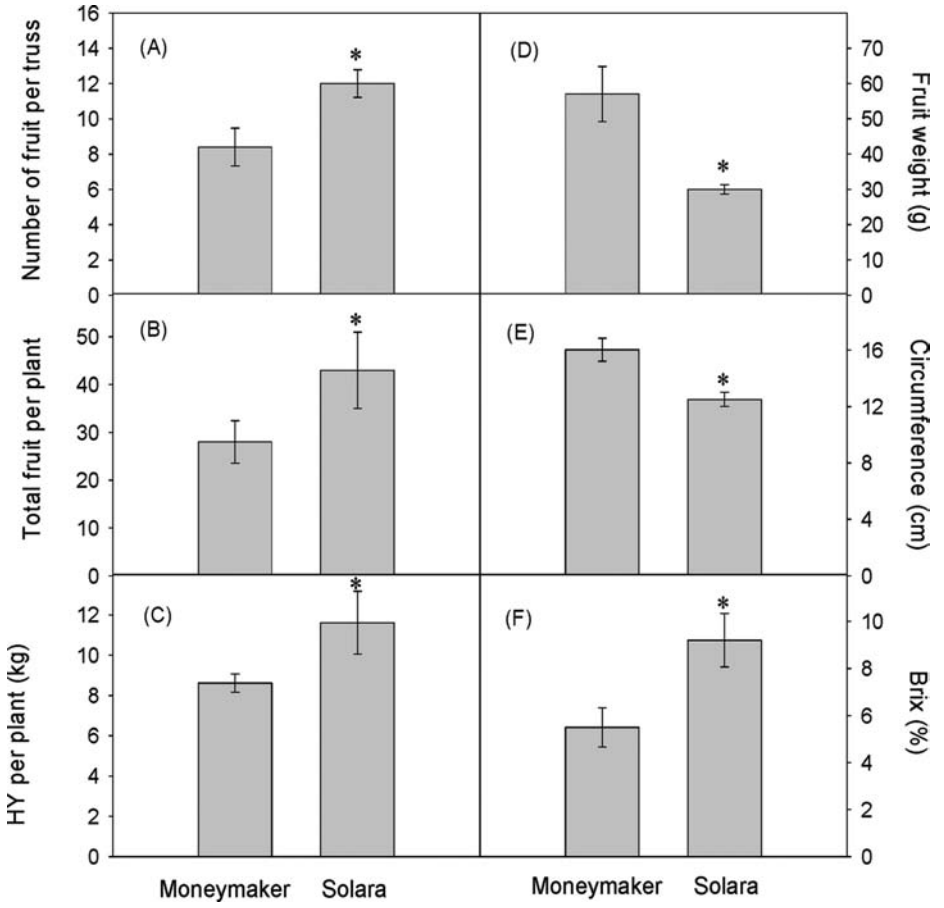

Figure 5. Comparison of plant and fruit parameters in Moneymaker and Solara. (**A**) number of fruits per truss; (**B**) total fruit; (**C**) horticultural yield (HY) per plant (number of fruit × mass of fruit × TSS); (**D**) average ripe fruit weight; (**E**) circumference of the ripe fruit, and (**F**) Brix (total soluble solid content) were measured in ripe fruits. Fruits were assayed from four equivalent trusses in each line. The data represent the mean ± SD from 5 to 6 plants per line for **A**−**E** and from 12 fruits from a different plant for Brix measurements (**F**). * indicates values in Solara that are significantly different from those in Moneymaker ($P \leq 0.05$) as determined by Student's *t*-test. Plants were grown in a greenhouse during the summer of 2007.

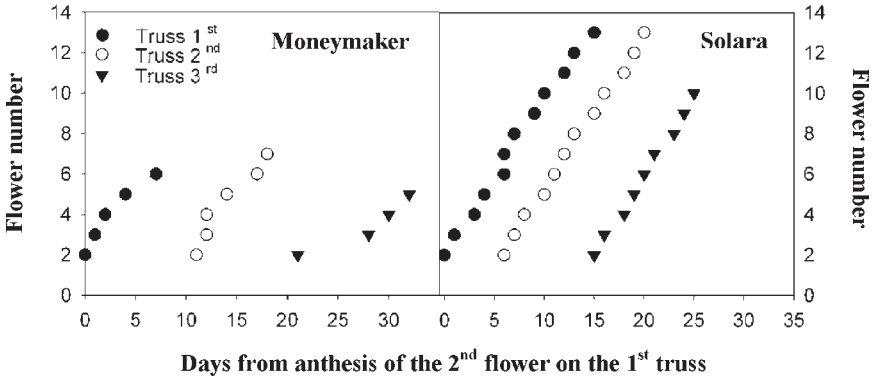

Figure 6. Comparison of the numbers of flowers between Moneymaker and Solara. Timing, in days, of the appearance of flowers on the first, second, and third trusses after anthesis of the second flower on the first truss. Each data point represents the mean from 6 plants per line. See Supporting Information, Figure 1C for full descriptions of the tissues sampled.

This indicates the possibility that the sucrose pools in Solara are turned over more often, a mechanism thought to influence tomato fruit sugars (*9*, *12*), and we speculate that sucrose cycling may also be an important basis for high TSS in Solara. Unfortunately, we were unable to make reliable measurements of the hexose phosphates in our fruit samples and therefore could not calculate the specific activity of the hexose phosphate pool, which would have permitted the translation of partitioning data (%) into fluxes (nmol hexose·gFWT^{-1}·h^{-1}) (*31*).

Similar levels of starch accumulated in Solara and Moneymaker, and therefore, it is doubtful that starch metabolism made appreciable contributions to fruit TSS in Solara as has been reported for *S. pennellii* and *S. habrochaites* (*10*, *32*). Starch accumulation in tomato is dependent on sucrose supply (*33*) and is subject to turnover (*34*). In Solara, more ^{14}C-glucose was metabolized to sucrose at the period of maximal starch synthesis (**Table 1**), a finding consistent with higher starch synthesis as proposed for IL9-2-5 (*8*, *9*). However, starch content was not

significantly different between Solara and Moneymaker (**Table 1**, **Figure 1B**,**D**) suggesting that starch synthesis or degradation may be regulated differently in Solara.

S. pimpinellifolium is uncharacteristic of most wild tomato species in that it accumulates low levels of organic acids in the fruit (*35*). In agreement with this we observed less ^{14}C-glucose partitioning into organic acids in Solara (**Table 1**). Respiration in ripe fruit was also lower in Solara (**Table 1**). It is therefore reasonable to assume that the conversion of glucose to organic acids, an energy-requiring process, occurs to a greater extent in Moneymaker. It is possible that in Moneymaker sugars are respired to a greater extent resulting in further loss of sugars that would normally be stored, while in Solara this flux is reduced with the result that the endogenous sugar content is preserved.

Overall, an important difference between these genotypes may lie in the relative influence of water and carbon fluxes on fruit sugar accumulation. Since fruit dry weights are similar (Supporting Information, Figure 2), but there is a disparity in fresh weight (**Figure 5**), then it can be concluded that there are differences in water flux between genotypes. This phenomenon may disproportionately influence fruit sugar concentration and eating quality (*36*).

4.2. Leaf Metabolism. Our examination of leaf metabolism was carried out first, to understand the biochemical processes ongoing in tomato leaves in the dark, and second, to determine whether genotypic differences in those biochemical processes could possibly contribute to the observed differences in horticultural yield (HY). Our experimental design did not allow us to establish a causal link between leaf biochemistry and sink capacity, however. We found that leaf starch (**Figure 2**) and sucrose contents (net accumulation; **Figure 3** and Supporting Information, Figure 3) were constant throughout the dark period. Fluctuations in environmental conditions, plant-to-plant variability in the greenhouse, and the presence of small fruit and flowers which are only weak sinks may all contribute to the lack of change in leaf carbohydrate content over the day in these lines. The feeding experiment showed that there was a loss of ^{14}C from the sucrose pools at the end of the dark period (8 h of chase) in both Moneymaker and Solara (**Table 2**). This sucrose could have been used as a source of energy through respiration, and as a consequence, the ^{14}C-organic acid fraction increased as sucrose decreased in Moneymaker (**Table 2**). The remaining sucrose may not have fallen below the critical threshold needed to trigger the degradation of starch as a source of carbon. In addition, the net amount of leaf sucrose in the intact leaves did not change in the dark (**Figure 2A**), while the ^{14}C flux of sucrose in detached leaves decreased (**Table 2**). This suggests that translocation of sucrose from other source leaves likely occurred on the plant. This phenomenon may occur in planta to ensure that the fruit truss is continually fed from the entire source-leaf network as well as from the adjacent source leaf.

Leaf metabolite pathways were investigated using GC-MS-TOF. To our knowledge, data on metabolite levels in tomato leaves during the night have not been previously published. Schauer et al. (*17*) looked broadly at metabolite levels in tomato leaves of five wild species, but this study was limited to one time-point during the day. The levels of metabolites involved in metabolic pathways that produce the leaf assimilate did not vary between our two genotypes. A similar result was found by Baxter et al. (*8*). They examined $^{14}CO_2$ uptake in leaf disks and concluded that the higher TSS in the IL9-2-5 introgression line was not due to increased photosynthetic activity (*8*). In spite of the overall similarity in leaf metabolism between our genotypes, Solara had a higher morphological source-to-sink ratio (Supporting Information, Figure 4), larger leaf size, and flowered one week later than Moneymaker so that at the time of leaf harvesting it had fewer sinks and reduced demand for photoassimilate (**Figure 6**) than Moneymaker. This would mean that Solara potentially had more sources to "feed" the developing fruit supporting high sugar accumulation. Such an assumption is predicated on both genotypes having similar leaf photosynthetic capacity per unit area and similar phloem uptake rates and would require further investigation.

The onset of darkness did not coincide with detectable changes in levels of leaf metabolites in this study (**Figures 3**, **4**, and **5**). Diurnal shifts in metabolite levels coordinating with changes in starch and sugar levels have been characterized in many species and are related to circadian rhythms; growth and development are dependent on these cycles (*37*). The plants we used had only flowers and one small fruit and may have been sink-limited (*38*). We did not examine leaves on trusses with a heavier fruit load because they were less healthy, and many were senescing. Still, strong diurnal changes in leaf starch have been documented in multiple plants which did not have a reproductive sink at the time of sampling that would have created strong demand for the photoassimilate including sweet potato (*39*), spinach (*40*), sweet pepper (*41*), tobacco (*42*), and potato (*29*). Thus, we find our results surprising. Additional studies have indicated that starch does not change significantly in tomato leaves throughout the diurnal period (*43*, *44*) even when sink load was significant (*45*).

Our results lead us to believe that in tomato, sugar levels can override light and circadian regulation of leaf metabolism. Leaf metabolism is powerfully regulated both by light and sugars (*30*, *46*). At the onset of darkness, photosynthesis ceases, leaf starch breakdown is initiated, and there are changes in leaf sugars, redox, and pH (*46*). These changes collectively lead to massive diurnal shifts in global metabolites through changes in the regulation of metabolic enzymes (*37*). We sampled leaves that were subjected to a light−dark transition, but the leaves maintained the same sugar content due, we assume, to translocation from other leaves. The unvarying sugar levels correlated perfectly with the lack of change of global metabolites in spite of a drastic shift from light to dark. Therefore, the regulation of metabolite levels by light-dependent changes in the leaf such as pH, redox, and Mg^{2+}, which normally change in the dark, may be overridden by sugars which remained unchanged (*46*).

We have made comprehensive biochemical and physiological measurements of source and sink tissues in two tomato genotypes, Solara and Moneymaker. The key differences we observed in fruit metabolism that may contribute to increased TSS in Solara were higher hexose accumulation throughout development and altered sucrose metabolism during ripening. In contrast, during early flowering, these two lines did not differ in their ability to produce carbon for export. Irrespective of similarities in source metabolism, however, higher source-to-sink ratio (low harvest index) at all of the stages of development examined, along with differences in fruit metabolism, may contribute to higher TSS in Solara fruit as compared to the fruit of Moneymaker.

5. ABBREVIATIONS USED

TSS, total soluble solids; DPA, days post anthesis; HY, horticultural yield.

ACKNOWLEDGMENT

We thank Nadia Bertin and Marion Prudent for advice on the eco-physiological measurements and Liliana Stamova for the gift of Solara plants. We are also appreciative of the comments made by Nadia Bertin, Belinda Martineau, and the anonymous reviewers.

Supporting Information Available: (1) Description of the physiological characteristics used in the experiment; (2) Total soluble sugars and starch content in Solara and Moneymaker expressed as g/100g of fruit dry weight; (3) Leaf starch and sugar contents, and sucrose-to-starch ratio of Moneymaker and Solara; and (4) Correlation networks representing the association among the physiological characteristics of Moneymaker and Solara. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Schaffer, A. A.; Petreikov, M.; Miron, D.; Fogelman, M.; Spiegelman, M.; Bnei-Moshe, Z.; Shen, S.; Granot, D.; Hadas, R.; Dai, N.; Levin, I.; Bar, M.; Friedman, M.; Pilowsky, M.; Gilboa, N.; Chen, L. Modification of carbohydrate content in developing tomato fruit. *Hortscience* **1999**, *34*, 1024−1027.

(2) Purdue-University Genetically Engineered High Solid Tomato. http://www.hort.purdue.edu/newcrop/Default.html

(3) Gough, C.; Hobson, G. E. A comparison of the productivity, quality, shelf-life characteristics and consumer reaction to the crop from cherry tomato plants grown at different levels of salinity. *J. Hortic. Sci.* **1990**, *65*, 431−439.

(4) Causse, M. Genetic and Genomic Approach for the Improvement of Sensory Quality in Tomato. In *Seed Biotechnology Center 10th Anniversary Symposium*, Seed Biotechnology Center: Davis, CA, 2009.

(5) Eshed, Y.; Zamir, D. An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated Qtl. *Genetics* **1995**, *141*, 1147−1162.

(6) Yousef, G. G.; Juvik, J. A. Evaluation of breeding utility of a chromosomal segment from *Lycopersicon chmielewskii* that enhances cultivated tomato soluble solids. *Theor. Appl. Genet.* **2001**, *103*, 1022−1027.

(7) Balibrea, M. E.; Martinez-Andujar, C.; Cuartero, J.; Bolarin, M. C.; Perez-Alfocea, F. The high fruit soluble sugar content in wild *Lycopersicon* species and their hybrids with cultivars depends on sucrose import during ripening rather than on sucrose metabolism. *Funct. Plant Biol.* **2006**, *33*, 279−288.

(8) Baxter, C. J.; Carrari, F.; Bauke, A.; Overy, S.; Hill, S. A.; Quick, P. W.; Fernie, A. R.; Sweetlove, L. J. Fruit carbohydrate metabolism in an introgression line of tomato with increased fruit soluble solids. *Plant Cell Physiol.* **2005**, *46*, 425−437.

(9) Husain, S.; N'Tchobo, H.; Nguyen-Quoc, B.; Kingston-Smith, A. H.; Thomas, B. J.; Foyer, C. H. Changes in activities of acid invertase isoforms affect sugar accumulation and composition during ripening of tomato fruit. *J. Hortic. Sci. Biotechnol.* **2003**, *78*, 182−192.

(10) Petreikov, M.; Yeselson, L.; Shen, S.; Levin, I.; Schaffer, A. A.; Efrati, A.; Bar, M. Carbohydrate balance and accumulation during development of near-isogenic tomato lines differing in the AGPase-L1 allele. *J. Am. Soc. Hortic. Sci.* **2009**, *134*, 134−140.

(11) Stommel, J. R. Enzymatic components of sucrose accumulation in the wild tomato species *Lycopersicon-peruvianum*. *Plant Physiol.* **1992**, *99*, 324−328.

(12) Miron, D.; Schaffer, A. A. 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.* **1991**, *95*, 623−627.

(13) Prudent, M.; Bertin, N.; Genard, M.; Munos, S.; Rolland, S.; Garcia, V.; Petit, J.; Baldet, P.; Rothan, C.; Causse, M. Genotype-dependent response to carbon availability in growing tomato fruit. *Plant Cell Environ.* **2010**, *33*, 1186−1204.

(14) Gautier, H.; Massot, C.; Stevens, R.; Serino, S.; Genard, M. Regulation of tomato fruit ascorbate content is more highly dependent on fruit irradiance than leaf irradiance. *Ann. Bot.* (*Oxford, U.K.*) **2009**, *103*, 495−504.

(15) Carrari, F.; Nunes-Nesi, A.; Gibon, Y.; Lytovchenko, A.; Loureiro, M. E.; Fernie, A. R. Reduced expression of aconitase results in an enhanced rate of photosynthesis and marked shifts in carbon partitioning in illuminated leaves of wild species tomato. *Plant Physiol.* **2003**, *133*, 1322−1335.

(16) Worrell, A. C.; Bruneau, J.-M.; Summerfelt, K.; Boersig, M.; Voelker, T. A. Expression of a maize sucrose phosphate synthase in tomato alters leaf carbohydrate partitioning. *Plant Cell* **1991**, *3*, 1121−1130.

(17) Schauer, N.; Semel, Y.; Roessner, U.; Gur, A.; Balbo, I.; Carrari, F.; Pleban, T.; Perez-Melis, A.; Bruedigam, C.; Kopka, J.; Willmitzer, L.; Zamir, D.; Fernie, A. R. Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. *Nat. Biotechnol.* **2006**, *24*, 447−454.

(18) Luengwilai, K.; Tananuwong, K.; Shoemaker, C. F.; Beckles, D. M. Starch molecular structure shows little association with fruit physiology and starch metabolism in tomato. *J. Agric. Food Chem.* **2010**, *58*, 1275−1282.

(19) Husain, S. E.; James, C.; Shields, R.; Foyer, C. H. Manipulation of fruit sugar composition but not content in *Lycopersicon esculentum* fruit by introgression of an acid invertase gene from *Lycopersicon pimpinellifolium*. *New Phytol.* **2001**, *150*, 65−72.

(20) Beckles, D. M.; Craig, J.; Smith, A. M. ADP-glucose pyrophosphorylase is located in the plastid in developing tomato fruit. *Plant Physiol.* **2001**, *126*, 261−266.

(21) Stamova, B. S.; Laudencia-Chingcuanco, D.; Beckles, D. M. Transcriptomic analysis of starch biosynthesis in the developing grain of hexaploid wheat. *Int. J. Plant Genomics* [Online early access]. DOI: 10.1155/2009/407426. Published Online: Mar 8, **2009**.

(22) Luengwilai, K.; Beckles, D. M. Climacteric ethylene is not essential for initiating chilling injury in tomato (*Solanum lycopersicum* L.) cv. Ailsa Craig. *J. Stored Prod. Postharvest Res.* **2010**, *1*, 1.

(23) Carrari, F.; Baxter, C.; Usadel, B.; Urbanczyk-Wochniak, E.; Zanor, M. I.; Nunes-Nesi, A.; Nikiforova, V.; Centero, D.; Ratzka, A.; Pauly, M.; Sweetlove, L. J.; Fernie, A. R. Integrated analysis of metabolite and transcript levels reveals the metabolic shifts that underlie tomato fruit development and highlight regulatory aspects of metabolic network behavior. *Plant Physiol.* **2006**, *142*, 1380−1396.

(24) Fiehn, O.; Wohlgemuth, G.; Scholz, M.; Kind, T.; Lee, D. Y.; Lu, Y.; Moon, S.; Nikolau, B. Quality control for plant metabolomics: reporting MSI-compliant studies. *Plant J.* **2008**, *53*, 691−704.

(25) Stamova, B. S.; Roessner, U.; Suren, S.; Laudencia-Chingcuanco, D.; Bacic, A.; Beckles, D. M. Metabolic profiling of transgenic wheat over-expressing the high-molecular-weight Dx5 glutenin subunit. *Metabolomics* **2009**, *5*, 239−252.

(26) Kunio, O.; Yuka, S.; Shin'Ichi, W. Source-sink relationship of ^{13}C-photosynthates in single-truss tomato. *Bull. Natl. Res. Inst. Veg., Ornamental Plants Tea* **2001**, *16*, 351−361.

(27) Bertin, N.; Tchamitchian, M.; Baldet, P.; Devaux, C.; Brunel, B.; Gary, C. Contribution of carbohydrate pools to the variations in leaf mass per area within a tomato plant. *New Phytol.* **1999**, *143*, 53−61.

(28) Chen, L. S.; Lin, Q.; Nose, A. A comparative study on diurnal changes in metabolite levels in the leaves of three crassulacean acid metabolism (CAM) species, *Ananas comosus*, *Kalanchoe daigremontiana* and *K-pinnata*. *J. Exp. Bot.* **2002**, *53*, 341−350.

(29) Urbanczyk-Wochniak, E.; Baxter, C.; Kolbe, A.; Kopka, J.; Sweetlove, L. J.; Fernie, A. R. Profiling of diurnal patterns of metabolite and transcript abundance in potato (*Solanum tuberosum*) leaves. *Planta* **2005**, *221*, 891−903.

(30) Gibon, Y.; Usadel, B.; Blaesing, O. E.; Kamlage, B.; Hoehne, M.; Trethewey, R.; Stitt, M. Integration of metabolite with transcript and enzyme activity profiling during diurnal cycles in *Arabidopsis* rosettes. *Genome Biol.* **2006**, *7*: R76.

(31) Sweetlove, L. J.; Tomlinson, K. L.; Hill, S. A. The effect of exogenous sugars on the control of flux by adenosine 5′-diphosphoglucose pyrophosphorylase in potato tuber discs. *Planta* **2002**, *214*, 741−750.

(32) Baxter, C. J.; Sabar, M.; Quick, W. P.; Sweetlove, L. J. Comparison of changes in fruit gene expression in tomato introgression lines provides evidence of genome-wide transcriptional changes and reveals links to mapped QTLs and described traits. *J. Exp. Bot.* **2005**, *56*, 1591−1604.

(33) Nguyen-Quoc, B.; Foyer, C. H. A role for 'futile cycles' involving invertase and sucrose synthase in sucrose metabolism of tomato fruit. *J. Exp. Bot.* **2001**, *52*, 881−889.

(34) Luengwilai, K.; Beckles, D. M. Starch granules in tomato fruit show a complex pattern of degradation. *J. Agric. Food Chem.* **2009**, *57*, 8480−8487.
(35) Schauer, N.; Zamir, D.; Fernie, A. R. Metabolic profiling of leaves and fruit of wild species tomato: a survey of the *Solanum lycopersicum* complex. *J. Exp. Bot.* **2005**, *56*, 297−307.
(36) Liu, H. F.; Genard, M.; Guichard, S.; Bertin, N. Model-assisted analysis of tomato fruit growth in relation to carbon and water fluxes. *J. Exp. Bot.* **2007**, *58*, 3567−3580.
(37) Zeeman, S. C.; Smith, S. M.; Smith, A. M. The diurnal metabolism of leaf starch. *Biochem. J.* **2007**, *401*, 13−28.
(38) Ho, L. C. Partitioning of assimilates in fruiting tomato plants. *Plant Growth Regul.* **1984**, *2*, 277−285.
(39) Kitahara, K.; Uchino, T.; Okizono, I.; Suganuma, T. Diurnal-Nocturnal Changes of Assimilated Starch Structures in Sweetpotato Leaves. *J. Applied Glycosciences* **2004**, *51*, 81−85.
(40) Stitt, M.; Gerhardt, R.; Kurzel, B.; Heldt, H. W. A Role for fructose 2,6-bisphosphate in the regulation of sucrose synthesis in spinach leaves. *Plant Physiol.* **1983**, *72*, 1139−1141.
(41) Hammond, J. B. W.; Burton, K. S. Leaf starch metabolism during the growth of pepper (*Capsicum-Annuum*) plants. *Plant Physiol.* **1983**, *73*, 61−65.
(42) Matheson, N. K.; Wheatley, J. M. Diurnal-nocturnal changes in starch of tobacco leaves. *Aust. J. Biol. Sci.* **1963**, *16* (1), 70−76.
(43) Nunes-Nesi, A.; Carrari, F.; Lytovchenko, A.; Smith, A. M. O.; Loureiro, M. E.; Ratcliffe, R. G.; Sweetlove, L. J.; Fernie, A. R. Enhanced photosynthetic performance and growth as a consequence of decreasing mitochondrial malate dehydrogenase activity in transgenic tomato plants. *Plant Physiol.* **2005**, *137*, 611−622.
(44) Hammond, J. B. W.; Burton, K. S.; Shaw, A. F.; Ho, L. C. Source-sink relationships and carbon metabolism in tomato leaves 0.2. Carbohydrate pools and catabolic enzymes. *Ann. Bot.* (*Oxford, U.K.*) **1984**, *53*, 307−314.
(45) Gary, C.; Baldet, P.; Bertin, N.; Devaux, C.; Tchamitchian, M.; Raymond, P. Time-course of tomato whole-plant respiration and fruit and stem growth during prolonged darkness in relation to carbohydrate reserves. *Ann. Bot.* (*Oxford, U.K.*) **2003**, *91*, 429−438.
(46) Geigenberger, P.; Kolbe, A.; Tiessen, A. Redox regulation of carbon storage and partitioning in response to light and sugars. *J. Exp. Bot.* **2005**, *56*, 1469−1479.

Received for review July 1, 2010. Revised manuscript received October 6, 2010. Accepted October 11, 2010. The Anandamahidol Foundation supported K.L. This work was funded by the National Science Foundation grant MCB-0820823 to OEF and in part by the France Berkeley Fund, National Science Foundation MCB-0620001, and University of California Hatch projects CA-D*-PLS-7198-H and CA-D*-PLS-7821-H to DMB.