Time-dependent Changes in the Longitudinal Sugar and Respiratory Profiles of *Asparagus* Spears During Storage at 0 °C

Sven Verlinden

Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506

Silvanda M. Silva

Departamento de Tecnologia Rural, Universidade Federal da Paraíba, Campus IV-CFT 58220-000, Bananeiras, Paraíba, Brazil

Robert C. Herner¹

Department of Horticulture, Michigan State University, East Lansing, MI 48824

Randolph M. Beaudry²

Department of Horticulture, Michigan State University, 1066 Bogue Street, East Lansing, MI 48824

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ABSTRACT. The rate of respiration and the concentrations of sucrose, glucose, and fructose were measured along the length of intact asparagus (*Asparagus officinalis* cv. Jersey Giant) spears during storage at 0 °C. Carbon dioxide production by each of five sections along the spear was initially high but underwent a rapid and extensive decline within the first 24 hours after harvest with the rate of decline slowing thereafter. The respiration rate was highest at the tip (Section 1), decreasing as the distance from the tip increased (Sections 2 through 5 with Section 5 being more basal). Initially, the respiration rate of the tip was approximately four times that of the base, but after 23 days at 0 °C, the respiration rate of the tip was only twice that of the base. Sugar levels were measured in Sections 1 through 4. Sugar levels declined with time, but increased, unlike respiration, with distance from the tip. Sucrose underwent a rapid decline within the first 24 hours of storage in the tip and Sections 3 and 4. Sucrose depletion was most extensive in the tip, reaching more than 95% by Day 23. Glucose underwent the most rapid decline in Section 2. The relatively higher rate of glucose depletion in Section 2, the zone of rapid cell elongation, may have been to support a relatively higher rate of cell wall biosynthesis in this section. For the first day after harvest, sugar depletion far outstripped hexose equivalents respired as CO₂. Afterward, however, the rate of respiration (as hexose equivalents) was similar to the rate of sugar depletion for all sections except the most basipetal, which lost carbohydrate faster than could be accounted for by respired CO₂. The data suggest that hexoses were exported from more basipetal tissues to support the metabolic activity of more acropetal sections.

In asparagus, there is a tight link between carbohydrate supplies and the metabolic activity of the spear (Irving and Hurst, 1993; King et al., 1990; Lill et al., 1990; Papadopolou et al., 2001; Saltveit and Kasmire, 1985). In intact plants, the storage carbohydrates are primarily fructans, which are located in the roots (Shiomi, 1993). Mobilization in support of spear growth is initiated by hydrolysis of fructans to sucrose and subsequent transport of the sucrose to the above-ground portion of the plant (Bhowmik et al., 2001; Robb, 1984; Wilson et al., 2008). As the harvest season progresses, fructan depletion in the roots leads to reduced spear production (Morse, 1916; Shelton and Lacy, 1980). Spear shelf life, soluble solids and carbohydrates, carbon partitioning, and sucrose metabolism change throughout the harvesting season (Bhowmik et al., 2002; Hurst et al., 1993b; Wilson et al., 2008; Zurawicz et al., 2008). Harvest, which is accomplished by cutting or snapping the spear at its base, prevents access of the metabolically active spear to the stored carbohydrate of the root and ends the rapid preharvest growth

(Kays and Paull, 2004). After harvest, the spear is forced to depend solely on energy resources it contains at harvest, which primarily take the form of sucrose, glucose, and fructose (Robb, 1984; Wilson et al., 2008).

Loss of carbohydrate supply in harvested asparagus spears causes soluble carbohydrate levels, especially sucrose, to be rapidly depleted (Hurst et al., 1993b; Irving and Hurst, 1993; Saltveit and Kasmire, 1985). This reduction in carbohydrate may limit respiration (Kays and Paull, 2004; King et al., 1990), which declines rapidly in the hours after harvest (Platenius, 1942). Loss of carbohydrate stores may, in turn, decrease the ability of the tissue to produce sufficient energy for some metabolic processes. Inability to maintain metabolic homeostasis is suggested to promote the spear's rapid senescence (Irving and Hurst, 1993). As the level of carbohydrates in the spear declines, the transcription of genes related to senescence increases (Davies et al., 1996; Irving et al., 2001; King et al., 1995).

The tip section of asparagus spears is comprised of highly active meristematic tissues and is considered to be a strong sink that is particularly susceptible to deteriorative changes (Lill et al., 1996). The spear tip is usually the first portion to show symptoms of postharvest deterioration and physiological

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¹Professor Emeritus.

²Corresponding author. E-mail: beaudry@msu.edu.

decline (Eason et al., 2002; King et al., 1990). Tissue deterioration then proceeds to the area just basipetal to the tip, which is the zone of cellular elongation (Robb, 1984). The spear base is comprised of more mature tissues. In the base, cell elongation has ceased and the vascular tissue has begun to lignify and phenolics accumulate (Rodriguez-Arcos, et al., 2002). The spear base is the tissue most resistant to deterioration (King et al., 1990; Lill et al., 1996) and may act as a source of metabolites for the tissues of the tip (Saltveit and Kasmire, 1985).

Although the general pattern of postharvest changes in the concentration of soluble sugars in asparagus spears has been documented (Hurst et al., 1993a; Irving and Hurst, 1993; Saltveit and Kasmire, 1985), the relationship between respiratory carbon use and the loss in carbohydrates along the longitudinal profile has not been investigated. The base of the spear has elevated levels of sugars relative to the more apical sections of the spear and may act as a carbohydrate source for the more apical sections of the spear. The objective of this research was to establish the carbon balance between respiration and hexose catabolism as a function of the longitudinal position of harvested asparagus spears during storage at 0 °C to determine if the spear base acts as a source of carbohydrate for the more apical portion of the spear.

Material and Methods

PLANT MATERIAL AND TREATMENTS. Asparagus cv. Giant Jersey spears were grown using standard commercial cultural practices at the Michigan State University Horticultural Teaching and Research Center, East Lansing (lat. 42.673976°, long. -84.484456°). Spears were harvested by cutting at the soil level using a sharp knife. The harvests took place between 0600 and 0900 HR during the height of the asparagus season (from mid-May to early June). Harvested spears were placed on ice in insulated chests in the field and immediately transported to the laboratory, sorted for obvious defects, and stored at 0 °C. All spears were at least 12 mm in diameter at the base and were at least 180 mm in length.

EXPT. 1. This experiment was designed to determine the respiratory profile along the length of harvested spears during storage at 0 °C. Use of intact spears was desirable to eliminate the elevation in respiration caused by wounding when sections were cut apart. To measure respiration of the different portions of the spear, we used a custom-built, segmented glass respirometry chamber (Fig. 1). Each chamber segment was 4.5 cm in length and 2.2 cm in diameter and could be adhered to additional segments to enclose the entire spear. Each segment was sealed off from adjacent segments with a handmade, thin, flexible silicone rubber diaphragm constructed from silicone sealant (3140 RTV Silicon Adhesive; Dow Corning Corp., Midland, MI). The diaphragm had a hole in its center to accommodate the spear. The spear was inserted through the diaphragm, which was sealed using a bead of silicone sealant. A bead of silicone sealant was also applied to the joints of the chamber segments to create an airtight seal. The silicone was allowed to cure for 16 h. Each chamber was flushed with CO₂-free air and flow control was achieved using glass capillary tubes under a head pressure of 5 kPa using a low-pressure line regulator (Model 3701; Matheson Gas Products, Montgomeryville, PA). Flow was measured before and after the experimental run for each chamber and averaged $\approx 1.2 \text{ mL} \cdot \text{min}^{-1}$. The CO₂ concentration of a chamber-exit gas line was determined on a

Fig. 1. Experimental apparatus allowing the measurement of respiration rate of uncut asparagus spear sections where Section 1 was the tip and Section 5 the most basal. Only four sections shown here to simplify the graphic. Temperature was maintained at 0 °C and the flow rate for each chamber was $\approx 1.2 \text{ mL} \cdot \text{min}^{-1}$. Gas samples were withdrawn from latex tubing sections attached to the ports in the section chambers.

100- μL gas sample taken with a 500- μL insulin-type syringe. CO₂ analysis was performed using an infrared gas analyzer (ADC 225-MK3 Servomex Series 1100; Analytical Development Co., Crowborough, U.K.) with N₂ as the carrier gas at a flow rate of $\approx 100 \text{ mL} \cdot \text{min}^{-1}$ (Beaudry and Gran, 1993). Spear sections are referred to as 1, 2, 3, 4, and 5 where Section 1 was the tip and 4 to 5 were successive, increasingly basipetal spear sections, with Section 5 being the most basal section cut at the time of harvesting. Respiration rates were determined after 16 h when the silicone sealant had cured and after an additional 1, 2, 3, 4, 8, 10, 16, and 23 d. Data are the average of three single-spear replications. Given the nature of the apparatus, the weight of individual sections of the spears could not be determined before the experiment, so weight loss of the spear sections could not be measured.

EXPT. 2. A second, parallel experiment was performed to determine changes in respiratory activity and in sugar profiles during storage at 0 °C. In this experiment, six replicate lots of six spears each were used for respiratory analysis over a 33-d period. An additional six lots of 14 spears each was used for sugar analysis, and from each replicate, two spears were selected at each time point. One spear was left intact and the other was cut into sections to mirror the sections designated in Expt. 1. In addition, a more detailed respiratory analysis was conducted on spears from a second harvest; in this case, there were four replicate lots of six spears each held for 36 d at 0 °C.

SOLUBLE SUGAR DETERMINATIONS. For sugar measurements, spears were selected from each of the six replicate lots after 0, 1, 7, 9, 13, 16, and 23 d. Spears were either left intact or cut into sections. The spear sections were cut at 45-mm-long intervals, measuring from the tip. These sections were referred to as Sections 1 (tip), 2, 3, 4, and 5. Section 5, wounded at the time of harvest, was discarded and excluded from the study. Spear sections and whole spears were frozen in liquid N₂ on removal from the respirometer and freeze-dried using a lyophilizer (Model H3248; VirTis Research Equipment, Gardner, NY) at -18 °C and 4 Pa. Tissue samples for Time 0 were frozen in liquid N₂ in the field immediately after harvest and stored at -80 °C until lyophilization and analysis. Samples were weighed before and after lyophilization to record fresh and dry weight and calculate percent dry weight. Lyophilized samples were powdered using a mortar and pestle.

Sugars were extracted from freeze-dried asparagus powder by immersion in 3.5 mL of an 80:20 ethanol:water (volume/volume) solution for 15 min at room temperature (Everard et al., 1994). The insoluble material was pelleted using a centrifuge (Model RG-5C; Sorval Instruments, DuPont Co., Newtown, CT) run at 2000 g_n for 5 min and the supernatant decanted into a 50-mL centrifuge tube. The pellet was resuspended and extracted two additional times using the same procedure. The pellet and container was then washed with 5 mL of water, which was

combined with the ethanol/water extract. To remove traces of chlorophyll, 5 mL of chloroform was added to the combined 15.5-mL ethanol/water mixture. The tubes were capped, shaken vigorously, and then centrifuged at 1000 g_n for 3 min. The upper, clear aqueous phase containing the soluble sugars was transferred to a test tube and evaporated to dryness using a vacuum desiccator equipped with a refrigerated condensation trap maintained at -103 °C (Speedvac Model SC200; Savant Instruments, Farmingdale, NY). Fructans were extracted from the pellet by boiling in distilled, deionized H₂O for 30 min. The extracted fructans were hydrolyzed by 0.1 N H₂SO₄ for 30 min and subsequently neutralized with 1 N NaOH (Smith, 1981). The resulting soluble sugars were assayed by high-performance liquid chromatography (HPLC) as described below.

Fructose, glucose, and sucrose were separated and quantified using an HPLC (Series 4000i; Dionex Corp., Sunnyvale, CA) equipped with gradient pump, guard column, CarboPack PA1 column (4 × 250 mm), autosampler, and pulsed amperometric detector connected to an integrator (Model 4270; Dionex Corp.) according to Layne and Flore (1995). The mobile phases used were ultrapure water, which was sonicated and degassed with

Fig. 2. Respiration rate (fresh weight basis) of the tip (Section 1), three successively basipetal 4.5-cm-long sections (Sections 2 through 4), and the butt (Section 5) of intact asparagus spears as a function of time when stored at 0 °C for 23 d and measured using the apparatus shown in Figure 1. (A) Relative respiration rates of an individual spear section in nanomoles per kilogram per second, whereas B shows absolute rates of respiration in nanomoles per second. Each point represents the average of measurements for three spears. The bars are the SE of the sample.

Fig. 3. Proportion of total CO₂ production contributed by the tip (Section 1), three successively basipetal 4.5-cm-long sections (Sections 2 through 4), and the butt (Section 5) of intact asparagus spears as a function of time when stored at 0 °C for 23 d in the apparatus shown in Figure 1. Data are derived from Figure 2. The equation for the fitted line for the respiration rate of each spear section is given in Table 1 and the values of the variables for these equations are given in Table 2.

Table 1. Empirical equations for whole spear respiration in nanomoles per kilogram per second and the proportion of the whole spear respiratory carbon flux emitted by the tip (Section 1), three successively basipetal 4.5-cm-long sections (Sections 2 through 4), and the butt (Section 5) of intact asparagus spears as a function of time when stored at 0 °C for 23 d.^z

Spear portion	Equation no.	Expression
Whole spear	8145	$y = a + b \times \exp(-c \times x) + d \times \exp(-e \times x)$
Section 1 (tip)	8006	$y = a + b \times \exp\{-\exp[-(x - c)/d] - [(x - c)/d] + 1\}$
Section 2	8063	$y = a + b\{1 + \exp[(x + d \ln(e) - c)/d]\}^{-(e + 1)/e} \times \{\exp[(x + d \times \ln(e) - c)/d] \times (e + 1)^{(e + 1)/e}\}/e$
Section 3	7003	$y = (a + c \times x + e \times x^2)/(1 + b \times x + d \times x^2)$
Section 4	2154	$y = a + b \times x + c \times x^{0.5} + d \times 2.718^{-x}$
Section 5	7005	$y = (a + c \times x + e \times x^2 + g \times x^3)/(1 + b \times x + d \times x^2 + f \times x^3)$

^zEquation number references mathematical functions for TableCurve 2D (Version 2.0; Jandel Scientific, San Rafael, CA). The variable *x* refers to time in days and values for remaining variables are provided in Table 2.

Table 2. Variable values for equations in Table 1 describing respiratory CO₂ emissions in nanomoles per kilogram per second for the whole asparagus spear (wet weight basis) and for the proportion of whole spear respiration contributed by the tip (Section 1) and four successively basipetal 4.5-cm-long spear sections as a function of time when stored at 0 °C for 23 d.

Variable	Spear portion					
	Whole spear	Section 1 (tip)	Section 2	Section 3	Section 4	Section 5 (base)
<i>a</i>	121.8	0.1578	0.1606	0.2068	0.1240	0.1991
<i>b</i>	238.9	0.07372	0.06471	-0.4771	-0.001891	-0.4932
<i>c</i>	6.560	1.872	8.429	-0.1079	0.01975	-0.08941
<i>d</i>	250.3	2.229	9.593	0.1048	0.04144	0.06194
<i>e</i>	0.1370	—	1.565E-08	0.02352	—	0.008550
<i>f</i>	—	—	—	—	—	0.001618
<i>g</i>	—	—	—	—	—	0.0007247
<i>r</i> ²	0.78	0.97	0.93	0.94	0.67	0.99

helium, and 200 mM NaOH, also sonicated and degassed, and the flow rate was 1 mL·min⁻¹. The analyses were run using a combination of gradient elution and isocratic elution techniques by programming the gradient pump as follows: 1) before sample injection, the column was eluted with 65% water and 35% NaOH; 2) at time = 0 min, the sample (20 µL) was injected and the gradient was adjusted to 75% water and 25% NaOH by time = 0.1 min; the elution remained at this ratio until time = 1.5 min; 3) from time = 1.5 min to 9.0 min, the gradient was adjusted to end at 0% water and 100% NaOH; 4) from time = 9 min to 28 min, the column was isocratically eluted with 100% NaOH. The column was maintained at room temperature. Analytical-grade fructose, glucose, and sucrose (Sigma Chemical Co., St. Louis, MO) were used as standards, which were run at the beginning and after every 12 samples. Identity of the eluted material was confirmed by comparing retention times of the samples with those of standards. Each standard and sample was tested three times and the average response of the HPLC detector was used for further calculations.

WHOLE SPEAR RESPIRATORY MEASUREMENTS. Spears were held in 1.92-L glass jars (Ball Corp., Broomfield, CO) whose lids were equipped with inlet and outlet sampling ports and ventilated with humidified, CO₂-free air at ≈80 mL·min⁻¹. Respiratory analysis was performed after 1, 2, 3, 4, 6, 7, 8, 10, 11, 12, 16, 18, 24, and 32 d for one harvest and after 15 and 30 min and 1, 4, 12, and 16 h, and 1, 6, 12, 18, 24, 30, and 36 d for a second harvest. For those spears used in the latter analysis, the respirometry jars were placed on ice and transferred to the laboratory within 15 min of harvest where they were immediately placed in a cold room at 0 °C and attached to the ventilation system. The first measurement (15 min) took place after approximately one volume change. Four replications were used.

Fig. 4. Respiration rate of whole asparagus spears (Expt. 2) and sections (summed, Expt. 2) held 33 and 23 d, respectively, at 0 °C. Spears were trimmed to 180 mm before storage. For whole spears, each point corresponds to a jar containing six spears; for spear sections, each point corresponds to the average of three spears. The bars represent the SE of the sample. The fitted line for the respiration rate of whole spears is given in Table 1 and the values of the variables for this equation are given in Table 2.

CARBOHYDRATE BALANCE. Carbon flux was calculated for the respiratory carbon emission by assuming that six CO₂ molecules constituted one hexose equivalent. Because the respiratory activity of the five sections of the spear (Expt. 1) had to be

measured on different spears from which samples were obtained for sugar analysis (Expt. 2), we bridged the two sets of data by calculating the proportion of the total respiratory carbon emitted by each of the five sections of the spear. The data were described using empirical equations obtained from and fitted by a commercial software package (TableCurve 2D Version 2.0; Jandel Scientific, San Rafael, CA). Because we

measured the respiratory rate of the intact spears in the sugar analysis study, we could then estimate the rate of carbon emission for each spear section and, at the same time, track changes in soluble carbohydrate for that section. The difference in the rate of carbon flux through CO₂ emission and sugar depletion was calculated for each section and the data were again described using a single empirical equations obtained from and fitted by a commercial software package (TableCurve 2D). Any excess in the rate of sugar depletion relative to carbon lost as respiration was evaluated with respect to possibly supporting respiratory activity in adjacent spear sections or supporting flux into other carbon sinks (e.g., cell wall formation or protein formation) within the tissue.

Results and Discussion

The respiration rate in the tip (Section 1) of asparagus spears was ≈40% to 50% higher than the section immediately basal to it (Section 2) for the first 24 to 48 h of storage (Fig. 2A). Throughout storage, respiration rate was highest at the tip and decreased progressively toward the base with the exception of the cut end of the spear (Section 5), which respired slightly more rapidly than Sections 3 and 4 (Fig. 2A). The elevated respiration of the basal section may have been the result of a wound response, an elevated rate of moisture loss, enhanced CO₂ diffusion, or microbial breakdown. However, it is unlikely that a wound response would persist for the 23 d of the study and, although no visible evidence of shrivel or decay was noted, the impact of the moisture loss and microbial activity cannot be dismissed. Nevertheless, the uncertainty associated with the impact of wounding on the cut end caused us to exclude it from sugar determinations and subsequent modeling. The time-dependent respiratory profiles depicted in Figure 2A were similar in form with that reported previously for whole spears and spear sections held at 0 through 20 °C (Lill et al., 1990; Saltveit and Kasmire, 1985). The advantage of performing measurements on intact spears, and a novel feature of the present study, is that the respiration rate of the upper four sections of the spear was measured without the confounding effect of wounding.

Because the respiration rate of the spear sections changed relative to one another over time, it was necessary to calculate the proportion of the total respiration of the spear that could be ascribed to each segment during the storage period. This was done first by calculating the absolute rate of CO₂ production in

Fig. 5. Time-dependent changes in hexose equivalents of glucose (top panel), fructose (upper middle panel), and sucrose (lower middle panel) and total sugar (bottom panel) content (dry weight basis) of the tip (Section 1) and three successively basipetal 4.5-cm-long sections (Sections 2 through 4) of intact asparagus spears as a function of time when stored at 0 °C for 23 d. Each point represents the average of measurements for sections from six spears. The bars are the SE of the sample. Best-fit lines for total sugars are derived from empirical equations and fitted variables given in Table 3.

Table 3. Variable values for an empirically derived equation [$y = a + b \times \exp(-c \times x) + d \times \exp(-e \times x)$, where x refers to time in days] describing total sugar content (micromoles per gram in hexose equivalents) for the tip (Section 1) and three successively basipetal 4.5-cm-long sections (Sections 2 through 4) of intact asparagus spears as a function of time when stored at 0 °C for 23 d.^z

Variable	Spear portion			
	Section 1 (tip)	Section 2	Section 3	Section 4
<i>a</i>	−55.34	−6425	−17310	−16350
<i>b</i>	359.7	8040	878.1	673.0
<i>c</i>	4.499	5.880E-3	2.052	2.000
<i>d</i>	931.1	302.0	19660	1950
<i>e</i>	0.07280	2.400	2.868E-3	4.690E-3
<i>r</i> ²	0.98	0.98	0.99	0.99

^zFitted lines are depicted in Figure 5.

nanomoles per second for each segment at each point in time (Fig. 2B). The rate of respiratory carbon flux for each segment differed as much as 2-fold but tended to be less variable than the rate of respiration expressed on a per-gram basis. The proportion of total spear respiratory carbon flux ascribed to each segment was then calculated (Fig. 3). The respiratory carbon flux was roughly 20% for each of the five sections with the proportion ascribed to Section 4 tending to be lower than that of the others. The proportion of respiration changed over time for each segment, but none of the changes amounted to more than 10% of the total for the whole spear. The resulting data were fitted empirically with various equations chosen for their ability to describe the data rather than for characterizing an enzymatic or physiological function per se (Tables 1 and 2).

The respiration rate of the spears in Expt. 2 declined during storage in a manner very similar to that recorded for the spear sections (Fig. 4) and were, again, in keeping with previously

published findings (Brash et al., 1995; Papadopoulou et al., 2001). Although the spears were harvested early in the morning and put immediately in an ice chest, the rapid initial rate of respiration is likely attributable, in part, to incomplete cooling, which, under the conditions imposed, required ≈ 30 min to remove field heat (data not shown). The respiratory data for Expt. 2 was fitted empirically with a simple exponential equation selected for its accuracy in depicting the data (Fig. 4; Tables 1 and 2). According to this expression, respiration declined to $\approx 60\%$ of its initial value after 24 h. The respiratory rate for the spears of Expt. 2 tended to be slightly higher than those in Expt. 1 initially (Fig. 4), but the time-dependent changes were similar in form, suggesting the time-dependency of changes in metabolic processes for spears in both experiments was similar.

In Expt. 2, fructose, glucose, and sucrose contents were lowest at the tip and increased as distance from the tip increased

Fig. 6. Percent depletion of fructose, glucose, and sucrose of the tip (Section 1, top panel) and three successively basipetal 4.5-cm-long sections [Sections 2 (upper middle panel), 3 (lower middle panel), and 4 (bottom panel)] of intact asparagus spears as a function of time when stored at 0 °C for 23 d. Data are derived from Figure 5.

Fig. 7. Best fit of the relationship between fructose [top panel (tip: $r^2 = 0.98$; Section 2: $r^2 = 0.75$; Section 3: $r^2 = 0.78$; Section 4: $r^2 = 0.88$)], glucose [middle panel (tip: $r^2 = 0.98$; section 2: $r^2 = 0.88$; section 3: $r^2 = 0.55$; section 4: $r^2 = 0.50$)], and sucrose [lower panel (tip: $r^2 = 0.82$; Section 2: $r^2 = 0.88$; Section 3: $r^2 = 0.73$; Section 4: $r^2 = 0.77$)] consumption (dry weight basis) and respiration rate in Sections 1 (tip), 2, 3, and 4 (base), of asparagus spears stored at 0 °C for 23 d. Data are derived from Figures 2 and 5.

(Fig. 5), which is consistent with previous findings (Hurst et al., 1993a; Irving and Hurst, 1993). Although total hexose equivalents for all three sugars declined during storage, this pattern from tip to base was maintained throughout the study. The rate of sugar use, and most especially sucrose, was highest for the first day of storage for all spear sections. The extensive decline in sucrose in the first day of cold storage may be linked to the high initial respiration that followed harvest (Brash et al., 1995) or perhaps was the result of the incorporation of sucrose into other cellular constituents. The amount of depletion in millimoles of hexose equivalents of total sugars per gram on a dry weight basis was similar for Sections 1 through 3 and most rapid for the basipetal section (Fig. 5, bottom panel). The depletion in total sugars for the Sections 1 through 4 for could be depicted empirically using a single equation (Table 3). The slope of the fitted line was used to calculate the rate of sugar loss (nanomoles hexose equivalents per gram dry weight per second) for each spear section as a function of time.

In terms of percent carbohydrate lost, depletion was most pronounced for the spear tip (Fig. 6), a phenomenon reported

previously for spears held at non-refrigerated temperatures (Irving and Hurst, 1993; McKenzie et al., 2004; Saltveit and Kasmire, 1985). The decline in sucrose was markedly more rapid than other sugars in the first 24 h after harvest, decreasing 50% in the tip, 20% in Section 2, 43% in Section 3, and 42% in Section 4. For all spear sections except the tip, the rate of depletion of all sugars tended to be relatively constant after Day 1. After 23 d storage, the sucrose level in the tip was reduced 96% from harvest levels, whereas glucose and fructose were reduced $\approx 80\%$ and 50%, respectively. More basipetal sections had extensive but less complete sugar depletion than the tip when expressed on a percentage basis.

Fructans were not detected in harvested spears (data not shown), supporting the findings of Pressman et al. (1989) in expanded shoots of asparagus. It is not entirely clear if sucrose is the sole phloem-transported sugar, but it is the major translocated carbohydrate derived from the fructans in the roots and is, therefore, the primary source of the energy and carbon skeletons for spear growth and development (Bhowmik et al., 2001; Irving and Hurst, 1993; Robb, 1984; Wilson et al., 2008). The content of sucrose and its products, fructose and glucose, represent the main carbohydrate store for the harvested spear tissue to draw on to support metabolic processes during storage (Irving and Hurst, 1993).

Fig. 8. Changes in the ratios of the concentrations (moles per gram dry weight) of sucrose relative to fructose (A), and glucose (B), and in fructose relative to glucose (C) of the tip (Section 1) and three successively basipetal 4.5-cm-long sections (Sections 2 through 4) of intact asparagus spears as a function of time when stored at 0 °C for 23 d. Data are from derived from Figure 5.

Fig. 9. Changes in percent dry weight (A) and in absolute dry weight with soluble sugars subtracted (B) for the tip (Section 1) and three successively basipetal 4.5-cm-long sections (Sections 2 through 4) of intact asparagus spears as a function of time when stored at 0 °C for 23 d. Each point represents the average of measurements for sections from six spears and vertical bars are the SE of the sample.

Fig. 10. Mass of respired hexose equivalents of carbon dioxide (dark gray), sugar content (medium gray), and dry matter [light gray], dry weight with sugars subtracted] for the tip (Section 1, top panels) and three successively basipetal 4.5-cm-long sections [Section 2 (upper middle panels), Section 3 (middle panels), and Section 4 (lower middle panels)] and whole asparagus spears (bottom panels) in absolute amounts (left panels) and relative amounts (right panels) during storage at 0 °C for 23 d. Data are derived from Figures 2 to 5 and Tables 1 to 3 as described in the text.

The temporal patterns for sugar depletion were similar to those for respiration in that both declined with time (compare Figs. 4 and 5). From these data, it is not clear if respiratory demand depleted sugar supplies or if the sugar supplies restricted respiratory activity, because both patterns are time-dependent and are, therefore, confounded. Paradoxically, the positional pattern in sugar content was the reverse of that for respiration such that the rate of respiration was highest in the tip, where sugar levels were lowest, calling into question any direct link between sugar levels and respiratory activity. That being said, there was a strong correlation found, for all sections, between the rate of respiration and the content of each of the sugars (Fig. 7). Interestingly, each spear section differed in the relationship between respiratory substrates (sugars) and the rate of metabolic activity (as measured by respiration). The spear tip appeared to be the most responsive of the sections evaluated in that it had the highest rates of respiration at low sugar levels and the decline in respiration was most rapid with declining sugar availability. Although these data support the assertion by King et al. (1990) that carbohydrate availability may be limiting to metabolic activity, our data would suggest that any linkage between respiratory activity and sugar content is complex and highly tissue-dependent. Indeed, sugars in vascular plants are

hypothesized to be the long-distance messengers of whole-organism carbohydrate status as well as substrates for both cellular metabolism and local carbohydrate-sensing systems (Koch, 1996). Enzyme activities up-regulated by carbohydrate availability in plants include many that would lead to enhanced sucrose use through storage, respiration, and biosynthetic processes (McKenzie et al., 2004). In general, carbohydrate depletion enhances expression of genes for reserve mobilization and import/export processes (Koch et al., 1996).

In our experiment, the ratio of sucrose to fructose was highest for the tip for the first 2 weeks of storage, after which Section 2 maintained the highest ratio (Fig. 8). There was a somewhat similar pattern for the ratio of sucrose to glucose, which was high in the tip and in Section 2. These data suggest that glucose and fructose generated in the apical tissues of the spear were rapidly metabolized, a conclusion consistent with the observed high rates of respiration for these tissues. Cleavage of sucrose in plant tissue occurs by either invertase or sucrose synthase. The activity of sucrose synthase is twice that of invertase in asparagus tips (Irving and Hurst, 1993; McKenzie et al., 2004), whereas that of acid invertase is highest in the subtending tissues (Hurst et al., 1993a). An elevated sucrose synthase activity might be

expected to yield a relatively higher fructose to glucose ratio compared with invertase because sucrose synthase yields uridine diphosphate (UDP)-glucose rather than glucose (Coperland, 1990). The high level of fructose relative to glucose in Section 2 (45 to 90 mm from the tip) would be consistent with this region being one of higher sucrose synthase activity. In addition, UDP-glucose is the precursor for cellulose microfibril biosynthesis (Giddings et al., 1980), so it is also reasonable that the relatively high fructose/glucose ratio in Section 2 may reflect the diversion of glucose and UDP-glucose to cell wall synthesis. This is consistent with the expectation that cell wall synthetic activity is expected to be greatest in the region of Section 2 (Robb, 1984), but it was not measured in this study. The rise in fructose relative to glucose for Sections 1, 3, and 4 in the last 2 weeks of the study suggests a shift in metabolism favoring the catabolism of glucose or some limit in its formation. Irving and Hurst (1993) noted that hexokinase activity tends to be preserved in senescing asparagus, whereas sucrose synthase, fructokinase, and invertase are not. It may be that the more stable hexokinase activity tends to promote a more rapid phosphorylation of glucose relative to fructose, thereby enriching fructose compared with glucose.

The percent dry weight was $\approx 40\%$ higher for the tip compared with the more basipetal sections throughout the storage

Fig. 11. Difference between hexose consumption by respiration and hexose depletion from sugar loss in the tip (Section 1) and three successively basipetal 4.5-cm-long sections (Sections 2 through 4) of intact asparagus spears as a function of time when stored at 0 °C for 23 d. Negative numbers indicate the amount to which sugar depletion outstrips respiratory carbon loss; positive numbers indicate the amount to which respiration exceeds sugar depletion for a given spear section. Data are derived from Figures 2 to 5 and Tables 1 to 3 as described in the text.

period (Fig. 9), most likely because the tip contains a large number of smaller meristematic and unexpanded cells. The percent dry weight tended to increase over time for the tip and decline for the more basal sections of the spear, suggesting allocation of resources from the more basipetal portions of the spear. It is also possible that the spear tip had dehydrated at a greater rate over the course of the experiment. However, no signs of dehydration were evident. As noted previously, there is no way to determine weight loss in a particular portion of the spear over time. When the weight of soluble sugars was subtracted from the absolute dry weight, there was a tendency for an increase in non-hexose dry weight as storage duration increased (Fig. 9B), suggesting conversion of sugars to other cellular constituents. It is possible, however, that this trend is artifactual in that the spears were randomized and hence not sorted to select for uniform size or weight at each time point such that we may have inadvertently used slightly smaller spears in the first couple weeks of the study.

The data were used to calculate the mass of respired CO₂ (hexose equivalents), sugar content, and dry matter (dry weight with sugars subtracted) for sections of asparagus spears and whole spears in absolute amounts as a function of time (Fig. 10, left). These data, in turn, were converted to mass fractions for the spear as a function of time (Fig. 10, right). There was a tendency for absolute mass to be higher for the later sampling dates, likely an artifact of randomization as previously noted. The loss in respiratory mass was similar in magnitude to the decline in sugar mass, clearly demonstrating that the primary avenue for sugar depletion is through respiration. Using the fitted empirical equations of Tables 1 and 2, we were able to calculate the rates of carbon flux for both respiration and sugar depletion (both as hexose equivalents) (Fig. 11). When the rate of sugar depletion is subtracted from the rate of sugar depletion through respiratory activity, the sign of the resulting difference tells us the whether carbohydrate loss outstrips respiratory losses or vice versa:

a negative number indicates sugar depletion was greater than could be accounted for by respiratory carbon use. Our data suggest that for the first several hours, sugars were converted more rapidly to cellular constituents such as proteins, lipids, and cell wall materials (Hurst et al., 1994; Hurst and Clark, 1993; King et al., 1990) than the respiratory gas, CO₂. However, after the first day in storage, the two fluxes largely balanced one another in each section for the remainder of the 23-d storage period. Interestingly, the carbon balance for Section 4 was consistently negative throughout the storage period, suggesting that sugar export may have occurred to the more acropetal tissues. If carbohydrate of this section were being transported to the more acropetal sections of the spear, the data would suggest that it helps to support the formation of non-hexose cellular constituents in addition to respiration. This interpretation is supported by the observed increase in the proportion of non-hexose dry weight found throughout storage (Fig. 10).

The data highlight the dynamic nature of carbon allocation and use in asparagus spears after harvest. They indicate that most of the

carbohydrate held in the harvested spear as sucrose, glucose, and fructose is eventually respired in the maintenance of the spear tissues. Given that the rates of respiratory carbon loss were similar to the rates of sugar depletion for Sections 1 through 3 for the greater part of the storage period, it appears that regulation of both phenomena may be linked. The apparent apical movement of carbohydrate from the most basal section of the spear suggests the spear is actively using the greater carbohydrate reserves of the basal section in lieu of the root storage supplies from which the spear has been separated. This suggests that high or low sugar levels in the harvested spear, and especially in the spear base, may influence the potential for quality retention of the spear tip.

The very high rate of sugar depletion relative to the respiratory carbon flux in the first few hours after harvest is intriguing. It may reflect the period during which the harvested spear is adjusting to its new situation in which a carbohydrate supply for continued growth and development is initially being perceived. One interpretation is that at harvest, the spear is engaged in rapid interconversion of sugars to numerous cellular constituents and, on detection of a decline in carbohydrates supply, rapidly shuts down nonessential pathways for carbohydrate allocation (e.g., those associated with cell division and cell expansion and other aspects of plant growth). It would be interesting to determine if this period could be abbreviated, thereby preserving a significant portion of the carbohydrates pool in the harvested spear.

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