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Low Temperature Alters Carbohydrate Metabolism in Easter Lily Bulbs

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Abstract. Easter liliy *(Lilium longiflorum* Thunb. 'Nellie White') bulbs were stored in moist peatmoss for up to 85 days at – 1.0 or 4.5C. Bulbs were periodically removed from storage and analyzed to determine levels of soluble carbohydrates and starch. Storage at – 1.0C induced large accumulations of sucrose, mannose, fructose, and oligosaccharide in both mother and daughter scales. Starch concentration declined substantially during this period. Storage at 4.5C resulted in less dramatic alterations in bulb carbohydrates, although trends toward increased soluble carbohydrates and reduced starch levels were seen. The accumulation of mannose suggests that glucomannan, a secondary storage carbohydrate, was also degraded during – 1.0C storage.

Exposure of vegetative storage organs to low temperatures often induces the accumulation of soluble carbohydrate, a process commonly referred to as "low temperature sweetening" (Tishel and Mazelis, 1966; Burton, 1969; Isherwood, 1973; Davies and Kempton, 1975; ap Rees et al., 1981). This phenomenon has been reported in *Lilium* as well. Stuart (1952) found rapid hydrolysis of insoluble carbohydrates in *Lilium* bulbs stored at 0C, leading to the accumulation of reducing sugars and sucrose. More hydrolytic activity was seen at 0C than at 10C or higher. While starch is the major reserve carbohydrate in *Lilium,* the bulbs are also known to contain glucomannan, a branched polymer containing glucose and mannose residues (Matsuo and Mizuno, 1974; Tomoda et al., 1978). Glucomannan accounts for ≈15% of the total storage carbohydrate in the bulb (Matsuo and Mizuno, 1974). By using HPLC methodology, a more detailed characterization of soluble carbohydrate profiles could be obtained than in earlier work (Stuart, 1952). The objectives of this study were to determine differences in bulb carbohydrate profiles induced by two bulb storage temperatures.

'Nellie White' Easter lily bulbs (20 to 23 cm circumference) were received from a commercial supplier on 27 Oct. 1984 (Expt. 1) and 5 Nov. 1986 (Expt. 2) and immediately repacked into polyethylene bags containing ≈1.5 liters of moist peatmoss per 10 bulbs. Bags were placed either at – 1.0 or 4.5C (± 0.5C). At – 1.0C, the peatmoss surrounding the bulbs was frozen, but actual freezing of bulb tissues did not occur.

At 6- to 20-day intervals (Expt. 1) or 31- to 36-day intervals (Expt. 2), bulbs were removed from storage, dissected into mother and daughter scales, and frozen in liquid nitrogen in preparation for carbohydrate analysis. The techniques used in carbohydrate extraction and analysis were essentially identical to those described previously (Miller and Langhans, 1989). Briefly, freeze-dried tissue was ground in a Wiley mill (20-mesh screen), and 50 mg of tissue was extracted with 12 methanol : 5 chloroform : 3 water (by volume). Soluble carbohydrates were separated and quantitated using an isocratic HPLC system (LKB 2150 pump; Pharmacia-LKB, Piscataway, N.J.) equipped with a Bio-Rad HPX-87C stainless steel column maintained at 85C (Bio-Rad Laboratories, Richmond, Calif.). Degassed HPLC water was used as the mobile phase. Starch was estimated using a glucose oxidase assay following amyloglucosidase (Sigma, St. Louis) hydrolysis of the residue remaining after soluble carbohydrate extraction. The experiment was designed as a split plot, with temperature and time as main plot factors and bulb tissue type as the subplot factor, with four replications per treatment.

Results of the two experiments were nearly identical; therefore, results from the first year (Expt. 1) are presented. Compared to 4.5C, storage at – 1.0C for 86 days induced a 2.6- or 2.9-fold increase in mother and daughter scale sucrose concentration, respectively (Fig. 1A). For the first 49 days, the increase in sucrose concentration was linear at a rate of 3.5 mg sucrose/g of dry weight per day, then leveled off. At the conclusion of both experiments, sucrose represented 20% to 26% of the total bulb dry weight for bulbs stored at – 1.0C. Storage at 4.5C caused a significant, but smaller increase in tissue sucrose concentration, with most of the increase occurring in the first 49 days (Fig. 1A). There was a significant temperature × time × tissue interaction affecting bulb sucrose concentration (Table 1).

Glucose and fructose accumulated in both mother and daughter scales at both temperatures, but the magnitude of change was much less than for sucrose (Fig. 1 B and C). In mother scales, – 1.0C storage caused higher concentrations of each hexose between days 28 and 36, but by the end of each experiment, bulbs stored at 4.5C had the highest concentrations of each hexose. This relationship probably was due to the mother bulb-localized hydrolytic activity and soluble sugar accumulation that is associated with bulb sprouting at 4.5C (Miller and Langhans, 1989). In daughter scales, – 1.0C storage induced nonsignificant accumulation of each hexose during the experiment (Fig. 1 B and C). The lack of massive accumulation of hexose is not surprising, since most of the soluble carbohydrate is sucrose, and hexoses are required for sucrose synthesis.

There was a significant temperature × time interaction affecting mannose concentration, with the difference between – 1.0 and 4.5C storage becoming more pronounced with longer storage durations (Fig. 1D, Table 1). At each temperature, mother scales contained higher levels of mannose than did daughter scales.

The concentration of an unknown oligosaccharide (eluting prior to sucrose) increased more than 25-fold as a result of

Table 1. Analysis of variance for soluble carbohydrate and starch fractions of mother or daughter scales stored at – 1.0 or 4.5C for various durations.

Source of variation	Sucrose	Glucose	Fructose	Mannose	Oligo[z]	Starch
Temperature (T)	***	**	***	***	***	***
Storage duration (D)	***	***	***	***	***	***
Bulb scale type (B)	***	***	***	***	***	**
T × D	***	NS	NS	***	***	***
T × B	NS	NS	NS	NS	***	NS
D × B	***	NS	NS	NS	***	NS
T × D × B	**	NS	NS	NS	***	NS

[z]Unidentified oligosaccharide.
NS,**,***Treatment is nonsignificant, or significant at $0.01 \geq \alpha > 0.001$ or at $\alpha \leq 0.001$, respectively.

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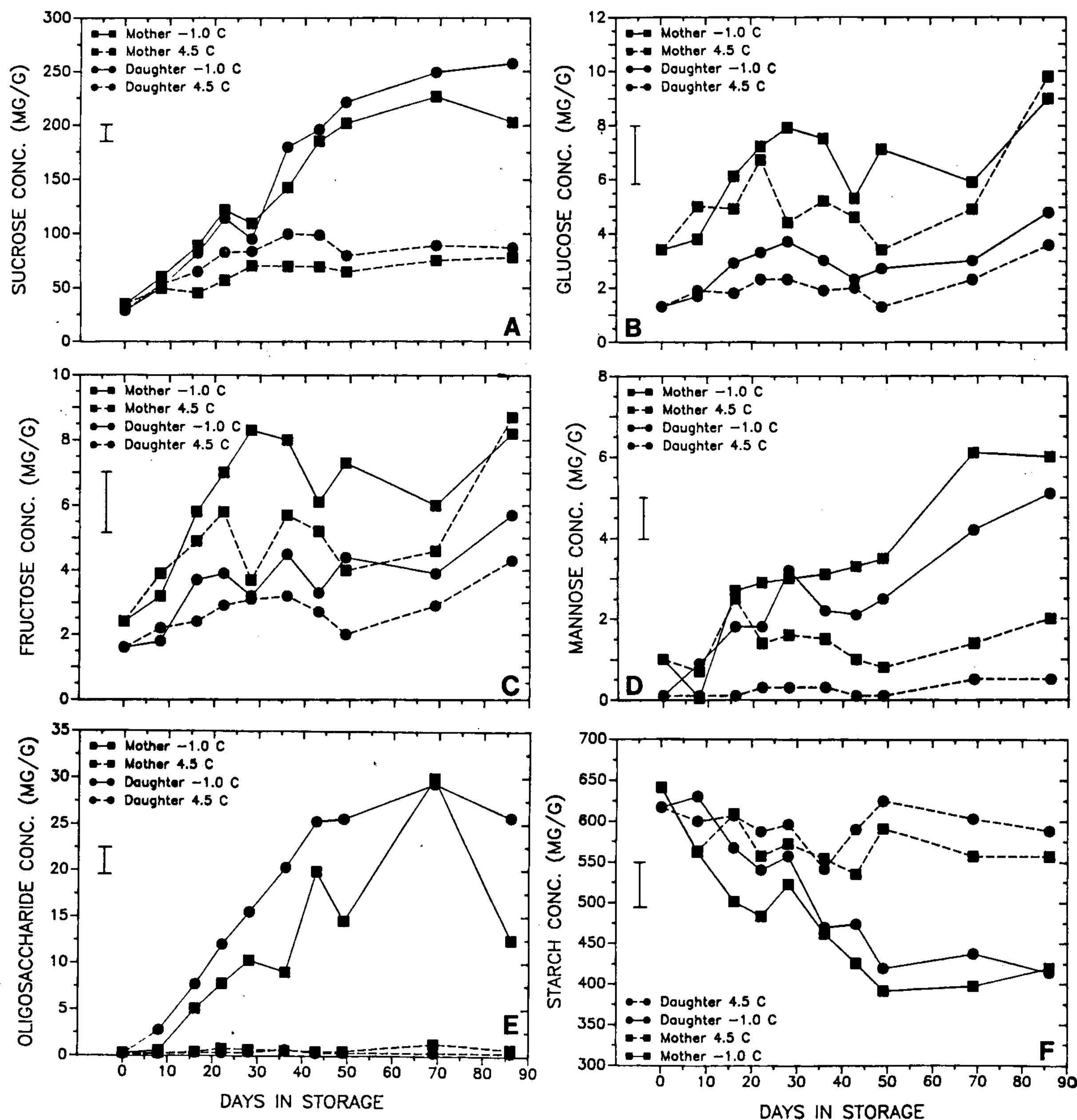

Fig. 1. Effect of long-term storage at – 1.0C (——) or 4.5C (– – –) on the concentration of (**A**) sucrose, (**B**) glucose, (**C**) fructose, (**D**) mannose, (**E**) an unknown oligosaccharide, and (**F**) starch in mother (■) or daughter (●) scales of 'Nellie White' Easter lily. Data points indicate the mean of four bulbs. The bar in each panel represents the 5% LSD value. The analysis of variance for each figure is given in Table 1.

– 1.0C storage (Fig. 1E). Currently, this oligosaccharide is unidentified, although its HPLC retention time suggested it may be a trisaccharide or tetrasaccharide. This oligosaccharide likely arose as a result of starch or glucomannan degradation and was not specifically detected by earlier methods (Stuart, 1952). The magnitude of the increase in concentration of this compound and rapidity of response (Fig. 1E) indicates it may be of practical use as a real-time indicator of unwanted – 1.0C storage of *Lilium* bulbs, e.g., during failure of thermostatic temperature control in commercial storage facilities.

Starch concentration was reduced 20% to 33% in mother and daughter scales as a result of – 1.0C storage (Fig. 1F). In the first 49 days of – 1.0C storage, mother scale starch breakdown was linear at a rate of 5.1 mg starch/g of dry weight per day. The pattern and timing of starch degradation closely coincided with the increase in sucrose. The rate of starch breakdown for the first 49 days was sufficient to account for the rate of sucrose synthesis (5. 1 mg starch/g of dry weight per day vs. 3.5 mg sucrose/g of dry weight per day), providing good evidence that starch was the major source of carbon skeletons for sucrose synthesis.

The present results support earlier experiments on lily storage reported by Stuart (1952). He found rapid starch hydrolysis and accumulations of sucrose and reducing sugar in bulbs stored at 0 to 10C. Stuart (1952) found that storage at 10C induced the most rapid flowering, but 0C storage induced the greatest sugar accumulation in the bulb. The present work has identified the reducing sugars Stuart (1952) reported as glucose, fructose, and mannose.

Starch is widely considered to be the major storage carbohydrate degraded during low-temperature sweetening (ap Rees et al., 1981), and the large reductions in starch concentration (Fig. 1F) support this view for *Lilium* bulbs. We also saw accumulations of free mannose, a hexose that likely arose from glucomannan hydrolysis. Thus, while starch appears to be the major source of soluble sugar during low-temperature sweetening of *Lilium* bulbs, we may infer from our results that glucomannan degradation also occurs at – 1.0C. To the best of our knowledge, no other reports exist concerning the involvement of glucomannan in the low-temperature sweetening of vegetative storage organs.

During forcing, mother scale reserves are preferentially used for providing assimilates to the growing shoot; utilization of daughter

scale reserves is inducible under extended periods of irradiance reduction (Miller and Langhans, 1989). Conversely, the present study indicates both scale types are capable of essentially identical sucrose accumulation and starch degradation during low-temperature storage (Fig. 1 A and F). This difference implies tissue-specific control of enzymes of reserve degradation and sucrose accumulation during forcing, with loss of control in the daughter bulb during low-temperature (– 1.0C) storage. The implication is that these enzymes are capable of activity at – 1.0C and that their continued activity is the first step in the low-temperature-induced accumulation of soluble carbohydrates in *Lilium* bulbs. To fully understand bulb reserve catabolism at low temperature or during normal greenhouse forcing, it will be necessary to identify the enzymes of reserve degradation and determine the regulatory control of their activity.

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