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Fructan Hydrolysis Drives Petal Expansion in the Ephemeral Daylily Flower[1]

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Dry weight, water content, soluble carbohydrate content, and carbohydrate composition of daylily (*Hemerocallis* hybrid cv Cradle Song) flower petals were monitored in the 3 d leading up to full opening and in the first day of senescence. Timing of events was related to the time (hour 0) when flower expansion was 60% complete. Petal dry weight increased linearly from hour −62 (tight bud) to hour 10 (fully developed flower), then fell rapidly to hour 34 as senescence advanced. Increase in water content was proportional to dry weight increase from hour −62 to hour −14, but was more rapid as the bud cracked and the flower opened, giving an increase in fresh weight/dry weight ratio. Soluble carbohydrate was 50% of petal dry weight up to hour 10, then decreased during senescence to reach 4% by hour 34. Up until hour −14, fructan accounted for 80% of the soluble carbohydrate in the petals, whereas hexose accounted for only 2%. Fructan hydrolysis started just prior to bud crack at hour −14, reaching completion by hour 10 when no detectable fructan remained, and fructose plus glucose accounted for more than 80% of the total soluble carbohydrate. The proportion of sucrose remained constant throughout development. Osmolality of petal cell sap increased significantly during fructan hydrolysis, from 0.300 to 0.340 osmolal. Cycloheximide applied to excised buds between hour −38 and hour −14 halted both fructan hydrolysis and flower expansion. The findings suggest that onset of fructan hydrolysis, with the concomitant large increase in osmoticum, is an important event driving flower expansion in daylily.

Modern daylilies are hybrids that have been developed over the past century from crosses involving up to 12 species of the Asian genus, *Hemerocallis*, within the family Liliaceae (Munson, 1989). They are rapidly becoming a feature of gardens in temperate regions by virtue of their large, showy flowers and their tolerance of a wide range of soils and climatic conditions. They have had only limited value as cut flowers because they are among the most markedly ephemeral flowers in regular cultivation. Each bloom is open for less than a day before senescence begins (hence the popular name, daylily), and the subsequent process of senescence is both rapid and dramatic. Work to date has shown the daylily to be a useful model system for flower senescence studies (Bieleski and Reid, 1992; Lay-Yee et al., 1992). The spectacular nature of this senescence process has tended to draw attention away from the equally dramatic bud and flower growth that occurs during the 3 to 4 d leading up to full bloom, with the size doubling about every 30 h to reach a maximum flower weight of 5 to 10 g fresh weight (depending on cultivar), and with the actual opening process from bud to fully expanded flower taking only 5 h (Lay-Yee et al., 1992). This study documented the growth that took place during this rapid flower expansion, and explored some of the underlying physiological events in the petals that were associated with the expansion, in particular the changes in carbohydrate composition and content.

MATERIALS AND METHODS

Plant Material

Hemerocallis hybrid cv Cradle Song plants were grown in a glasshouse (27°C day, 21°C night) under natural light supplemented with incandescent lighting between 4:00 PM and 12:00 AM (Lay-Yee et al., 1992), and flowers were harvested according to Bieleski and Reid (1992). Stages of flower development are shown in Figure 1. Timing of events is reported in relation to the time at which flower expansion is 60% complete (midnight), denoted hour 0 (see Bieleski and Reid, 1992).

Petal Fresh Weight and Dry Weight

At each stage of flower growth, individual petals from 10 flowers were put into separate preweighed glass vials (scintillation vials), which were immediately reweighed. Tissue samples were dried by lyophilization for 40 to 48 h, then vials were reweighed.

Sugar Analyses

At each stage of flower growth, five flowers were sampled. The three petals of each sample were cut into 0.8 to 1.2 cm × 0.5 to 0.8 cm slices (Bieleski and Reid, 1992), which were mixed to provide a uniform subsample of 0.50 g fresh weight. For comparative studies, tissue samples were taken in the same way from finely sliced *Hemerocallis* leaf and root, onion inner bulb, Jerusalem artichoke tuber, and asparagus root. Each sample was killed in liquid nitrogen, transferred to 10 mL of methanol:chloroform:water (12:5:3, v/v) at −25°C and held at −25°C overnight, then extracted in that solvent

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Abbreviation: Cy, cycloheximide.

without grinding, followed by 5 mL of 20% methanol, essentially as described in Bieleski et al. (1992), and finally deionized to give a soluble carbohydrate fraction. The extraction method was shown to recover more than 98% of total petal fructan (and 96% of root fructan).

Separation and quantitation of sugars was by HPLC on a Hewlett-Packard 1084B using an Aminex HPX 87C column at 85°C with water as solvent and using a refractive index detector (Altex, Beckman Industries). Additional information on the oligosaccharides was obtained by substituting an Aminex HPX 42C column for the HPX 87C column. Sugars and oligosaccharides were also separated on Supelco Redicoat G silica gel plates using *n*-butanol:acetic acid:water (50:25:25, v/v) run three times (Spollen and Nelson, 1988), or propyl acetate:formic acid:water (11:5:3, v/v) (Bieleski et al., 1992). Ketoses were detected by urea-phosphoric acid, and other sugars were detected by other specific detection agents (Bieleski et al., 1992).

Extraction of Cell Sap and Measurement of Osmotic Potential

After various standard methods for obtaining cell sap were tested, the following procedure was developed as being superior for soft petal tissues. Several extractors were made by cutting 8-mm discs from fine (0.25-mm mesh) stainless steel wire mesh, each disc making a press fit in the bottom of the barrel of a disposable 3-mL plastic hypodermic syringe from which the plunger had been taken out. The top and bottom one-fifth of each petal were discarded, then the remaining central piece was rolled into a cylinder that could be inserted into the barrel of the syringe. If the petal was too large (hour 10), the petal piece was split longitudinally along the central vein and one-half was used. Then the plunger was replaced and forced down, squeezing the petal wad against the mesh and petal sap out of the tip of the syringe for direct application onto the osmometer sample plate. At the end of a run, the extractors were cleaned and dried for reuse. Sap osmolality was measured using a Wescor 5500 vapor pressure osmometer, which requires a sample volume of only 10 μL. Initial experiments examined the variation in osmolality between different parts of the flower. In studying osmotic change with flower development, sap samples were taken from each petal of 12 flowers, giving 36 samples at each developmental stage.

Inhibition of Flower Opening by Cy

The effect of Cy on carbohydrate changes associated with the flower-opening process was studied as follows. Six flower buds were harvested from the glasshouse at each of three stages in flower development prior to opening (hour −38, hour −26, and hour −14), placed in individual vials containing deionized water, and transferred to a flower evaluation room (20°C, 60% RH, 12 h cool white fluorescent light giving approximately 15 μmol m^{-2} s^{-1}; Bieleski et al., 1992). Three treatments were applied to each set of harvested buds, using two buds per treatment. Control buds had their pedicels in deionized water throughout. A second set was held in 100 μM Cy for 24 h, then transferred to deionized water (100 μM Cy treatment). A third was held in 500 μM Cy for 24 h before transferring to deionized water. Each bud, and each vial with its bud and solution, was weighed at the start, during transfer from Cy to water, and at the end of the treatment so that bud growth, volume of solution taken up, and amount of Cy taken into the tissue could be estimated. All three sets of all three treatments were maintained until hour 10, when the flowers were photographed and petal samples were taken for study of their soluble carbohydrate content, as described above.

RESULTS

Flower Development

During development from a medium-sized bud (about 2 g fresh weight at hour −62) to a fully open flower (about 6.5 g fresh weight at hour 10), the proportion of total flower weight in the petals rose from about 35% to 48%, it remained approximately constant at about 30% in the sepals, and in the gynoecium plus associated corolla tube it fell from 35% to 22%. Thus, development and expansion of the petal was more marked than that of the flower as a whole. The general behavior of the sepals was almost identical to that of the petals (see, for example, Bieleski and Reid, 1992), but this study was limited to petal behavior alone. Petal dry weight increased almost linearly from hour −62 to hour 10 at a rate of about 130 mg per day (Fig. 1). Petal fresh weight, on the other hand, increased much more rapidly between hour −14 and hour 10 than during early stages of development. This was the same period when the flower broke open from a cracking bud 10 mm in diameter to a fully developed bloom 70 mm in diameter (see Lay-Yee et al., 1992). As a consequence, the fresh weight/dry weight ratio rose rapidly over the same period, from 7.5 to 11, showing that part of the petal expansion was due to an increased water uptake (Fig. 1).

During subsequent senescence and collapse of the flower,

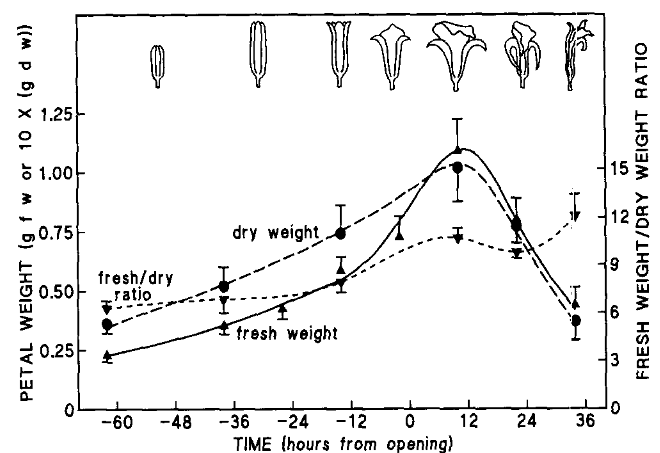

Figure 1. Change in petal fresh and dry weights and in fresh weight/dry weight ratio during daylily flower development. Bars give the SD of each point (mean of values for 10 flowers, each flower value being the average reading for each of the three petals). Sketches give the appearance of flowers at each stage of development (Bieleski and Reid, 1992; Lay-Yee et al., 1992).

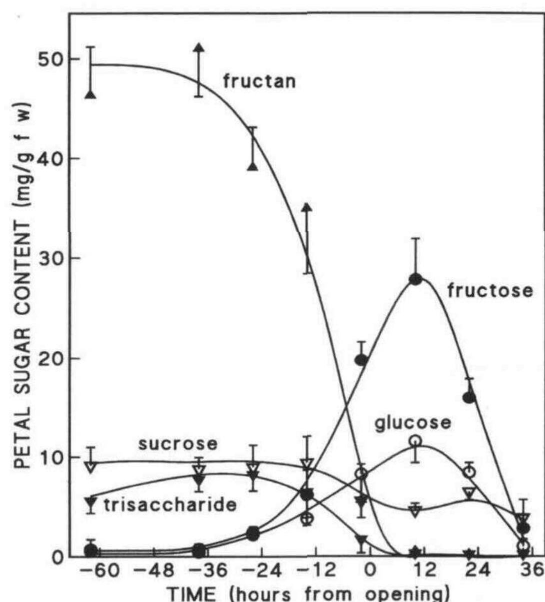

Figure 2. Changes in sugar content of daylily petals during flower development. Bars give the SD of each point (mean of five tissue samples).

from hour 10 to hour 34 (Bieleski and Reid, 1992; Lay-Yee et al., 1992), there was a rapid loss of fresh and dry weights. The fall in fresh weight/dry weight ratio between hour 10 and hour 22 and subsequent rise between hour 22 and hour 34 were both statistically significant ($P < 0.01$ and $P < 0.001$, respectively), the fall corresponding to the wilting that characterizes early senescence (Bieleski and Reid, 1992).

Changes in Petal Carbohydrate Content

With petals taken from developing buds, between hour −62 and hour −14, over 70% of the extracted carbohydrate eluting from the Aminex 87C column was at the solvent front and was identified as oligosaccharide. Another 12% was in an unidentified compound eluting in a position appropriate for a trisaccharide, between raffinose and Suc standards. Suc was present as about 15% of the extracted carbohydrate, whereas Glc and Fru were barely detectable (Fig. 2). No other sugar was detected in any other extract except those at hour 34, which additionally contained a small peak coinciding with the position of Gal and Xyl standards. Between hour −14 and hour −2, there was a dramatic change in the soluble carbohydrate composition, with oligosaccharide and trisaccharide disappearing, Suc remaining more or less constant, and Fru and Glc increasing to become the major carbohydrates present. Finally, between hour 10 and hour 34, the total carbohydrate content declined rapidly, with all but Suc largely disappearing from the petal tissue (Fig. 2).

Identity of the Oligosaccharides

The following data established that a family of oligofructans was present in developing daylily petals. (a) Extracts separated on the Aminex 42C column developed for study of small oligosaccharides contained oligomers ranging from $n = 3$ (the trisaccharide) to $n = 8$ (the limit of separation under the conditions used) and higher. Carbohydrates extracted from asparagus root and onion bulb showed an identical oligomer series, with the daylily petal extracts being intermediate between the two in the relative concentrations of the various oligomers. (b) Standard inulin and petal oligosaccharides were both rapidly hydrolyzed by 0.1 N H_2SO_4 at 100°C (to 80% completion after 1 min and 98.5% after 5 min), the products being Fru and Glc, in a 6.1:1 ratio for the petal oligosaccharide. (c) TLC techniques used by others to separate, identify, and quantify oligofructans (Spollen and Nelson, 1988) gave a series of ketose-containing oligomers ranging from $n = 3$ to $n = 15$ or so (Fig. 3); the oligomers changed in amount and relative concentration, finally disappearing as petals became fully expanded. The trisaccharide band gave somewhat different color reactions in its upper and lower zones, suggesting that two trisaccharide isomers were present.

Carbohydrate extracts from other daylily organs showed that the leaf contained mainly Suc, with traces of fructan having $n = 3$ to 5, and the root with an extended oligomer series from $n = 3$ to at least 30, with the bulk having $n > 15$. Based on these results, it was concluded that daylily petals during their early development contain a series of fructans averaging $n = 6$ to 8, with a maximum of $n = 13$ to 15, and that development of daylily petals involved a complete hydrolysis of the fructans to Fru plus Glc.

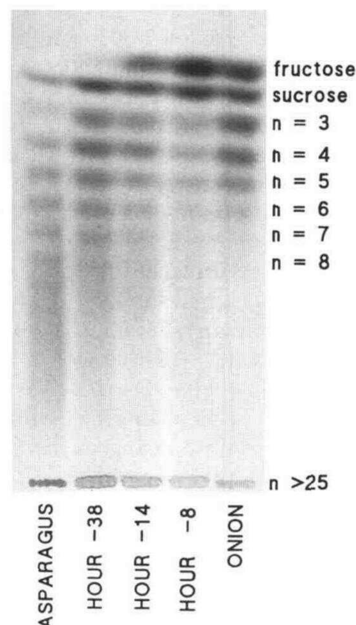

Figure 3. Thin-layer separation (method of Spollen and Nelson, 1988) of soluble carbohydrates from daylily petals at three stages of flower development (hour −38, hour −14, hour −8, with hour 0 being the time when opening is 60% complete), compared with extracts from asparagus root and onion bulb. The spray reagent used was specific for ketose-containing compounds. Numbers at the plate margins give the probable number of hexose units making up the oligosaccharide of the adjacent band (thus, $n = 3$ is the trisaccharide).

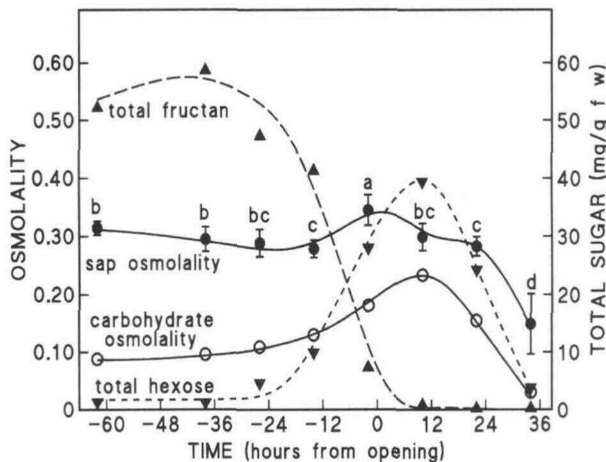

Figure 4. Change in osmolality of petal cell sap during flower development, compared with total fructan (fructan plus trisaccharide) and hexose (Fru plus Glc) concentrations in similar petals at the same stages of development. Osmolality due to carbohydrates present was calculated assuming fructan had an average mol wt of 990. Bars give the SD values of each osmolality reading (mean of values for 12 flowers, each flower value being the average reading for each of the three petals), and points lacking the same letter are significantly different at $P < 0.05$.

Osmotic Changes Associated with Petal Development

The highest osmolality found in the mature flower was of the nectar from the flower base, 1.001 osmolal. Therefore, it was necessary to avoid any contamination of sample tissues with nectar. The gynoecium also had a high osmolality, 0.752 osmolal, perhaps through the presence of nectary tissues. The corolla tube, sepal, and petals were all at about 0.300 osmolal, with equivalent parts of the petals and sepals being almost identical. There was a consistent, small gradient of increasing osmolality from the tip to the base of an individual petal. The middle three-fifths of the petal was used for sampling in the developmental studies.

Osmolality of the petal sap fell from 0.311 to 0.290 osmolal between hour −62 and hour −14, followed by a small, sharp, and significant increase ($P < 0.001$) to 0.340 osmolal between hour −14 and hour −2, which coincided with hydrolysis of fructan to hexose and with opening of the flower (Fig. 4). Osmolality returned to 0.301 osmolal once the flower was fully open (hour 10 to hour 22). Finally, as senescence became marked between hour 22 and hour 34, the osmolality decreased dramatically.

The composition and concentration of the soluble carbohydrate fraction (Fig. 2) was used to calculate the contribution of carbohydrates to the total osmolality of the cell sap. The average fructan was assumed to be of 6 hexose units (mol wt 990), and the water weight of the tissue was calculated from the difference between fresh weight and dry weight. Soluble carbohydrate accounted for about 40% of the total sap osmolality measured for petals from the developing bud, but the contribution rose during the period when fructan was being hydrolyzed and during the rise in cell sap osmolality, to reach approximately 80% at hour 10 when petal size was maximum, then fell to 20% in the senescent petals at hour 34 (Fig. 4). Organic acids, amino acids, and inorganic ions removed in sample preparation presumably accounted for the balance of cell osmolality.

Effect of Cy on Bud Opening

Water uptake of each Cy-treated flower was measured during its time in solution, and the data were used to calculate the final average concentration of Cy in the tissue, assuming that Cy entered passively with water supplying the bud through the cut xylem of the excised petiole and was distributed uniformly. It was 23 to 27 μM in flowers held in 100 μM Cy, and 103 to 122 μM in flowers held in 500 μM Cy. Excised buds not treated with Cy (control) completed their course of development in the same way as matched, nonexcised buds on the stock plants, except that the resulting flowers were somewhat smaller and lighter and had a 32 to 58% lower soluble carbohydrate content depending on the time of excision (data not shown). This is to be expected because excising the buds from the plant stopped the inflow of carbohydrate and other nutrients during the latter part of flower development. In contrast, Cy inhibited or completely halted the opening process, and the effect became more pronounced with 500 μM Cy and earlier time of treatment (Fig. 5). The fresh weight gain of flowers was reduced to less than half by the presence of Cy (Table I).

Hydrolysis of fructan to Fru plus Glc was strongly inhibited by the presence of Cy, to the extent that with the earliest treatment of 500 μM Cy (Table IA), the soluble carbohydrate pattern at hour 10 was the same as that in comparable flowers at the time of excision (hour −38), and totally unlike that in intact flowers at hour 10 or in excised buds held in water until hour 10 (Table I). Buds harvested at hour −14 had already started to open and to convert fructan to hexose (see Fig. 2), but the presence of Cy, after a time lag of about 5 h, still halted further opening and further conversion of fructan (Table IC).

DISCUSSION

This study assigns quantitative values on the rate at which daylily flowers grow during the 3 d prior to full opening.

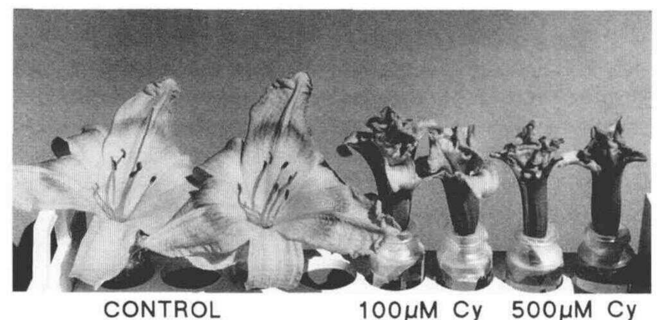

Figure 5. Effect of Cy on opening of daylily flowers excised at hour −26, then held for 24 h in either water (left pair), 100 μM Cy (middle pair), or 500 μM Cy (right pair), and then a further 12 h in water, until hour 10.

Table I. *Flower weight gains and changes in soluble carbohydrates of petals in the absence and presence of Cy during flower development*

Buds were excised and weighed at hour −38 (A), hour −26 (B), or hour −14 (C), held in water, 100 μM Cy, or 500 μM Cy for 24 h, then transferred to water until hour 10, when all buds were reweighed and petal samples were taken for carbohydrate analysis. Weight gain is expressed as percent per day, and carbohydrate composition is expressed as percent total soluble carbohydrate extracted. "Fructan" includes the trisaccharide, which is seen as a separate fraction in HPLC analysis. Data are also given for petals of comparable flowers harvested and sampled for sugar analysis of petals at the time the treatment flowers were excised (hour −38 in A; hour −26 in B; hour −14 in C) or at the time the treatment flowers were sampled for sugar analysis (hour 10). For data within a given row of the three subtables, values lacking a common letter are significantly different at $P < 0.05$.

A Carbohydrate	Excised Buds Treated with			Attached Buds Analyzed at	
	Water	100 μM Cy	500 μM Cy	Hour −38	Hour 10
	% total soluble carbohydrate			*% total soluble carbohydrate*	
Fructan	1.0d	69.8b	89.7a	86.5	1.0
Suc	3.7d	5.6bcd	4.1cd	12.8	10.5
Glc	23.1a	2.0c	0.8c	0.7	27.1
Fru	72.2a	22.7c	5.4e	<0.5	61.4
	% per day				
Weight gain	42.5ab	21.0c	13.6cd		

B Carbohydrate	Excised Buds Treated with			Attached Buds Analyzed at	
	Water	100 μM Cy	500 μM Cy	Hour −26	Hour 10
	% total soluble carbohydrate			*% total soluble carbohydrate*	
Fructan	< 1.0d	76.1b	85.1a	77.7	1.0
Suc	6.6bcd	8.6b	7.3bc	14.8	10.5
Glc	21.9a	2.9c	1.3c	3.6	27.1
Fru	71.5a	12.3d	6.3e	3.9	61.4
	% per day				
Weight gain	36.0b	14.6cd	10.5d		

C Carbohydrate	Excised Buds Treated with			Attached Buds Analyzed at	
	Water	100 μM Cy	500 μM Cy	Hour −14	Hour 10
	% total soluble carbohydrate			*% total soluble carbohydrate*	
Fructan	< 0.2d	37.8c	37.1c	70.5	1.0
Suc	8.6b	24.6a	20.3a	14.0	10.5
Glc	24.4a	9.0b	10.2b	6.9	27.1
Fru	67.0a	28.7b	32.4b	8.6	61.4
	% per day				
Weight gain	48.0a	18.6cd	18.7cd		

Petal dry weight increased 3-fold, almost matching the dry weight increase of another ephemeral flower, *Ipomoea tricolor,* over the same period (Wiemken-Gehrig at al., 1974). Throughout the growth period, soluble carbohydrate accounted for close to half of the total petal dry weight (Fig. 6), again resembling the *Ipomoea* corolla. In each species, the subsequent onset of senescence was accompanied by a rapid loss of dry weight and soluble carbohydrate, with carbohydrate loss being much the greater (Fig. 6). During daylily flower development, petal fresh weight increased more rapidly than dry weight, by 4.6-fold over the 3 d, mainly due to an enhanced water uptake during the 24 h prior to full flower expansion. During the same time there was a quantitative disappearance of fructan, including the trisaccharide, and a corresponding appearance of Fru and Glc, in a ratio of about 3:1, which is consistent with hydrolysis of a fructan of four hexose units. The process was rapid, with fructan content declining from 75% of total soluble carbohydrate to 0 in little more than 24 h. The amount of carbohydrate converted was equally impressive: the maximum hydrolysis rate of fructan was 2.2 mg g^{-1} fresh weight h^{-1}. There are not many examples of such a rapid change in carbohydrate composition.

Thus, fructan metabolism must play an important role in daylily flower development. An active fructan-synthesizing

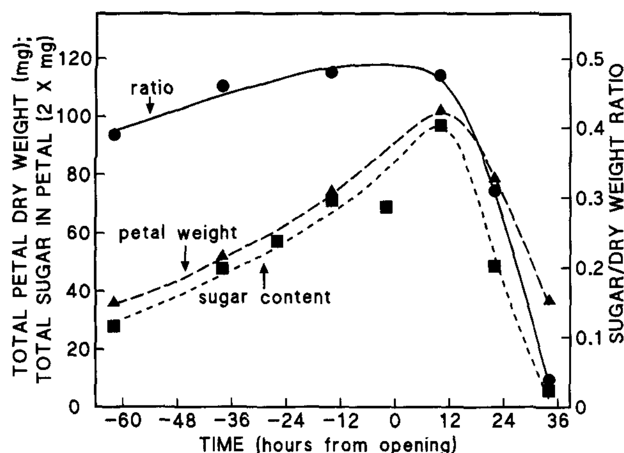

Figure 6. Soluble carbohydrate content of petals, and the ratio of soluble carbohydrate to total dry weight in the petals, as a function of flower development.

enzyme pathway must be operating during early bud development, so that Suc being transported into the bud from the leaves is largely converted into the storage compound, fructan. At about hour −24, the net fructan synthesis stops and a hydrolytic pathway becomes dominant, either through the synthesis of a new hydrolytic enzyme (presumably a fructan exohydrolase) or its activation from a previously inactive state (Simpson and Bonnett, 1993). Preliminary studies have shown the presence of Suc-Suc-fructosyltransferase activity in developing daylily petals, and fructan exohydrolase activity in expanded petals (data not presented). Finally, once senescence becomes apparent after hour 16, hexose and water start to decrease from the petals, the rate of hexose loss again being rapid at 1.9 mg g^{-1} fresh weight h^{-1}. A small amount of unidentified sugar appearing in the senescent petals at hour 34 probably arises from breakdown of wall polysaccharides and pectins (cf. Weimken-Gehrig et al., 1974) and may be Gal.

The extent of fructan hydrolysis is such that it must cause a major increase in osmoticum in the petal (Schnyder and Nelson, 1987). In the absence of any change in tissue volume, hydrolysis of 50 mg fructan g^{-1} fresh weight of an average chain length of six hexose units to free hexose should increase the component of cell sap osmolality due to that carbohydrate from 0.059 mosmoles g^{-1} to 0.352 mosmoles g^{-1}, or by 0.293 osmolal. The actual change is not as great as this because over the period of hydrolysis the tissue volume increases by 2 to 2.5 times; but even so, this change in carbohydrate composition causes the contribution of sugars toward total sap osmolality to increase from 27% at hour −62 and 46% at hour −14 to 79% at hour 10 (Fig. 4). There can be little doubt that this change in carbohydrate composition is intimately linked with the process of petal expansion. The main question is which process is the cause and which is the effect?

One possibility is that the flower starts rapid expansion because an increase in wall extensibility allows water to enter the cell, and the resulting drop in cell osmolality then triggers mechanisms to restore the osmolality. Alternatively, hydrolysis of fructan may be the initiating event, with the resulting increase in osmolality being the force that then drives expansion. Measurements of petal sap osmolality (Fig. 4) support the second possibility, in that a rise in cell sap osmolality coincided with the maximum hydrolysis rate of the fructans, the increase in fresh weight/dry weight ratio, and the expansion of the petal (Fig. 1). Compared with most biological properties, the cell sap osmolality was remarkably consistent from petal to petal within a flower and, to a lesser degree, between different flowers at the same stage of development. SE values for osmolality measurements were, on the whole, much smaller than those for carbohydrate measurements (compare Fig. 4 and Fig. 2). Further, except for the change between hour −14 and hour −2 (for which $P < 0.001$), there was very little change in osmolality of the petal sap with flower development (Fig. 4), suggesting that this aspect of petal physiology was generally under tight control. A similar observation has been made regarding the osmolality of rose petal sap (Evans and Reid, 1988). This stability makes the rise in osmolality observed in daylily petals at the time of flower expansion all the more noteworthy.

The effect of Cy provided additional evidence that fructan hydrolysis was directly associated with petal expansion (Table I, Fig. 5). Both the weight gain and fructan hydrolysis were most markedly inhibited by 500 μM Cy applied to flowers excised at hour −38 (Table IA), less affected by 100 μM Cy applied to flowers excised at hour −14, and largely unaffected by excision alone (Table IC). Despite the dramatic effect of the inhibitor on bud development, there were no overt signs of tissue damage, and the bud appeared to be halted at the state of development that existed at the time the Cy was first applied, much as was observed when Cy was applied to excised fully open flowers (Lay-Yee et al., 1992). Cy can be expected to affect any process in the developing bud that requires the translation of new mRNA. Nonetheless, the strong parallel between the effects of Cy concentration and application time on the separate processes of bud opening and fructan conversion provides additional evidence that fructan conversion carried out by newly synthesized enzyme is an initiating event that then osmotically drives petal expansion. This does not exclude the likelihood that there is also an increase in wall extensibility. Thus, there is still some expansion of excised, treated buds, even though there has been a complete interruption of sugar import as a result of excising the bud from the plant and a complete inhibition of fructan hydrolysis through the action of Cy (Table I, A and B, data for 500 μM Cy).

It seems that one major effect of Cy in this system may be to block the synthesis or activation of fructan exohydrolase that is required to bring about hydrolysis of the fructan (and that has been shown in preliminary studies to occur). If so, there is a pertinent conclusion to be drawn from the results observed when Cy was applied to buds that had already started to open and hydrolyze fructan (hour −14; Table IC). Although the fructan hydrolysis must have continued for a short time after Cy application (estimated at about 5 h by comparing carbohydrate composition data in Table IC with those in Fig. 2), the processes of hydrolysis and opening were then inhibited well before they reached their conclusion. The implication is that fructan exohydrolase activity did not persist even after fructan hydrolysis had been initiated, and that

this enzyme may not be stable in the cell but may turn over completely within a period of a few hours, at least in daylily. The proposition is relevant to ideas about the control of fructan exohydrolase activity in plants (e.g. Simpson and Bonnett, 1993).

There are other cases reported where rapid changes in fructan content are associated with some phase in plant organ development. They include decreases during the sprouting of tulip and *Lycoris* bulbs (Ohyama et al., 1988; Nagamatsu et al., 1991), enhanced synthesis in the cell-elongation zone of grass leaves (Schnyder and Nelson, 1987), storage in maturing grass internodes (Bonnett and Incoll, 1993) with subsequent remobilization into developing grass and cereal grains (Blacklow et al., 1984; Schnyder et al., 1988; Borell et al., 1989; Mehrhoff and Kühbauch, 1990), and rapid changes in response to altered source/sink relationships (Prud'homme et al., 1992). Some studies have suggested an osmotic role of fructan-sugar interconversions, whether as a cryoprotectant (Pollock et al., 1988), a desiccation protectant (Pressman et al., 1989), or a factor contributing to cell expansion (Schnyder and Nelson, 1987; Pontis, 1989). However, the changes in daylily give the most clear-cut example to date of fructans serving an osmotic function.

The finding that fructan-to-Fru conversion, with its resulting effect on cell osmolality, is linked with petal expansion in the daylily flower is a new mechanism to be added to the short list of processes already believed to be involved in flower expansion. Rose flower expansion is the one most directly comparable to daylily expansion. Ho and Nichols (1977) suggested that soluble sugars in the rose corolla are important as osmotically active substances in driving flower expansion, with starch acting as a buffer. However, Evans and Reid (1988) directly measured the osmotic potential of cell sap from expanding rose petals and found an increase from −790 to −690 kPa during the time of rapid petal expansion (equivalent to a change from 0.340 to 0.304 osmolal), leading them to propose that an increase in cell wall extensibility was triggering the change, with starch hydrolysis occurring as a secondary response to the decrease in osmotic potential gradient brought about by inflow of water. Despite the different osmotic relationships, there are several similarities between rose and daylily in their petal-expansion behavior. It seems likely that an increase in cell wall extensibility and an increase in osmoticum are both important, with cell wall extensibility playing the dominant role in the rose, and fructan hydrolysis playing the dominant role in the daylily.

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