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Onset of Phloem Export from Senescent Petals of Daylily[1]

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During senescence, petals of attached daylily (*Hemerocallis* hybrid cv Cradle Song) flowers lost 95% sugar and 65% dry weight over the first 24 h, with 30% of dry weight loss coming from nonsugar components. Detaching flowers did not delay senescence, but halted loss of carbohydrate and amino acid, suggesting that loss in the intact state was due to phloem export. Petal autolysis occurred mainly in the interveinal parenchyma, causing vascular strands to begin separating from the petal mass. Such vascular strands still stained with tetrazolium and accumulated sucrose, indicating a retained viability. Their sucrose accumulation rates were high in comparison with those of other plant tissues, and the accumulated product was mainly sucrose. Sucrose synthesis took place in the senescent petal, and sucrose was the principal sugar in phloem exudate, whereas hydroxyproline and glutamine were the main transport amino acids. [^{14}C]Sucrose applied to attached senescent flowers was rapidly translocated to other parts of the plant, particularly developing flower buds. Thus, onset of phloem export allowed most of the soluble carbohydrate and amino acid in the senescing flower to be retrieved by the plant. Additional salvaged material came from proteins and possibly from structural carbohydrate. Over a 12-h period, the flower switched from acting as a strong carbohydrate sink during expansion to become a strong source during senescence. This rapid reversal offers potential for phloem transport studies.

The daylily, *Hemerocallis* hybrid cv Cradle Song, has been useful for studying development and senescence processes in flowers. First, its ephemeral flower has been shown to have several features of senescence common to other, slower-declining plant tissues (Bieleski and Reid, 1992). When the first physical signs of senescence begin to appear (wilting, then onset of autolysis in the petal tips, later extending over the whole petal), there is a small but well-defined respiration climacteric, an increase in apparent free space, an increase in efflux of sugars and ions, and a change in the phospholipid pattern. Rather surprisingly, the ability of petals to accumulate phosphate and to maintain phosphate ester synthesis (along with a high energy charge) survives well into senescence. Second, the daylily does not respond to ethylene nor to ethylene inhibitors in its senescence and produces only traces of ethylene, leading to the conclusion that it is an ethylene-insensitive flower (Lay-Yee et al., 1992). There is also a rapid, large loss of protein from the petals during senescence, with some changes to the protein pattern. Third, major dry weight and soluble carbohydrate changes occur during petal development and senescence (Bieleski, 1993). During bud growth carbohydrate accumulated in the petal as fructan until the time when the bud started to open.

In the next 24 h, as the petals expand rapidly and the flower opens to its full size, the fructan is hydrolyzed rapidly and completely to hexose, giving a large increase in petal osmoticum and a small increase in cell osmolarity. The hydrolysis is seen as the prime event driving petal expansion. The various developmental processes continue without interruption when the flowers are excised, but are halted by cycloheximide. Cycloheximide treatment completely inhibits fructan hydrolysis and flower expansion (Bieleski, 1993), the respiration climacteric associated with senescence (Bieleski and Reid, 1992), the process of senescence itself, and the loss of protein and tissue dry weight as senescence developed (Lay-Yee et al., 1992; Bieleski, 1993). It was concluded that in the daylily flower, the process of development and senescence involves sequential reading of genomic messages, and that senescence is a programmed dismantling process.

A striking feature of senescence in daylily petals and flowers is their marked loss of soluble carbohydrate, dry weight, and protein, approximately 95, 65, and 75%, respectively, during the first 24 h (Lay-Yee et al., 1992; Bieleski, 1993). Only 8 to 9% of the dry weight loss can be attributed to respiration, since the average respiration rate during this time, 170 μL CO_2 g^{-1} fresh weight h^{-1} (Bieleski and Reid, 1992), equates to only 5.9 mg of the total loss, 64.9 mg carbohydrate per petal (Bieleski, 1993). This paper studies the physiological nature of the losses, their significance to the senescence process, and their function in overall plant behavior of *Hemerocallis*.

MATERIALS AND METHODS

Plant Material

Daylily plants (*Hemerocallis* hybrid cv Cradle Song) were grown as described by Bieleski and Reid (1992). Flowers and petals were sampled as described by Bieleski (1993), and timing of events is given in relation to the time at which flower expansion was 60% complete (midnight), denoted h 0 (Bieleski and Reid, 1992).

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Passive Losses

In the later stages of senescence, the limp petals glisten with free liquid, which can be so copious that it sometimes drips to the ground. Any such passive loss was measured by placing aluminum foil squares under eight senescing flowers from h 10 to 34, washing each foil with distilled water, and assaying the washings for sugars according to Bieleski (1993).

Effect of Excising Flowers on Weight Loss

At h 10, six matched flowers were tagged and left on the plant (attached flowers), whereas a second lot of six flowers was excised (excised flowers) and held, also in the glasshouse, in individual small vials containing 12 mL of water. At h 34, the attached flowers were harvested, liquid in each vial holding the excised flowers was taken for sugar analysis, each of the 12 flowers was weighed and the petals were detached and reweighed, and then the petals were sampled for sugar analysis and the balance of the petal tissue was lyophilized for 48 h to obtain its dry weight/fresh weight ratio. Measurements on the flowers at the time of tagging, h 10, were as described by Bieleski (1993). Samples for amino acid estimation were taken from the lyophilized tissue after dry weight measurement. The experiment was repeated twice more with the excised flowers being held in a vase life room (Bieleski and Reid, 1992) and yielding almost identical results (data available on request).

Sugar and Amino Acid Quantitation

To study soluble carbohydrates, the petal tissues were sampled and extracted, and then extracts were purified and analyzed for sugars as described by Bieleski et al. (1992) and Bieleski (1993). Phloem exudates were handled according to Bieleski (1994). Amino acids from tissue samples equivalent to 0.5 g fresh weight were extracted by the procedure of Bieleski and Turner (1966) and then estimated colorimetrically by the procedure of Lee and Takahashi (1966).

Tetrazolium Staining of Senescent Petal Tissue

Petals at various stages of senescence from h 30 to 42 were excised and floated in 0.5% (w/v) phenyl tetrazolium chloride for 4 h at room temperature. This reagent was used to test for seed viability, where formation of a red formazan indicated the presence of active dehydrogenases.

Sites of Suc Uptake in Senescent Petals

Four petals were taken at each of four stages of senescence (h 28, 31, 34, and 37) and floated in two changes of distilled water with gentle rocking for 15 min to remove loose tissue and solutes released by the autolytic process of senescence. Because the petals were very fragile and sticky, wash water held on the tissue mass was not removed by blotting. In taking the unblotted tissue, about 0.7 mL of free wash water was transferred with a petal sample weighing approximately 0.6 g, and this was allowed for in the procedures. Washed petals were placed in 20 mL of 2 mM [^{14}C]Suc (4.6 kBq mL^{-1}) for 3 h at 20°C. At the end of the uptake period, [^{14}C]Suc solution was removed by suction and the petal was washed in two changes of 50 mL of unlabeled 2 mM Suc over 20 min and then in 100 mL of water to remove all freely diffusible ^{14}C-containing material. Each petal was then floated onto a rectangle of filter paper and lifted from the water, such as in mounting algal specimens. The paper plus tissue was drained from underneath by blotting with paper towels, and then dried and autoradiographed as described by Mitchell and Bieleski (1977) to reveal sites of Suc accumulation.

Rate of Suc Uptake into Senescent Petals

Nine flowers were harvested at h 31, the petals were excised and split longitudinally in half with a needle, and the 54 pieces were washed as above. Thirty uniform pieces were selected for this experiment, 4 were selected for the experiment on composition of accumulated sugar, and the remainder were discarded. Five washed halves were put into each of the six beakers, 20 mL of 2 mM [^{14}C]Suc at 1.55 kBq mL^{-1} were added, and the beakers were gently rocked on a low-speed shaker. At 40-min intervals a beaker was chosen, the tracer solution was sucked off, and the tissue was washed in two 40-mL changes of unlabeled 2 mM Suc over 15 min. Each petal piece was laid on filter paper to remove free liquid, weighed, and then extracted with 2 mL of 1% (v/v) formic acid and measured for radioactivity as described by Bieleski (1977).

Composition of Accumulated Sugar

Four petal halves were prepared as described above, allowed to accumulate from 10 mL of 2 mM [^{14}C]Suc at 18.5 kBq mL^{-1} for 3 h, and washed in unlabeled Suc as above. Each petal piece was individually weighed and extracted for soluble carbohydrates according to Bieleski (1994). The residue was hydrolyzed (6 N HCl, 100°C, 30 min) to solubilize any starch or protein synthesized during the uptake period. ^{14}C assays were carried out on the various fractions: final residue, hydrolysate (starch and protein), chloroform phase from the extraction step (lipid and phospholipid), eluates from the Sephadex SP column (amino acids) and Sephadex QAE column (organic acids), and the neutral aqueous fraction (sugars) (Redgwell, 1980). ^{14}C-sugars were separated by TLC on Macherey-Nagle (Darmstadt, Germany) MN 300 cellulose plates with propyl acetate:formic acid:water (11:5:3, v/v) (Bieleski and Young, 1963; Bieleski and Redgwell, 1985). Autoradiography, recovery of individual radioactive compounds, and their scintillation counting were as described by Redgwell et al. (1974).

Composition of Phloem Sugars

Twelve flowers were harvested at h 10, placed immediately in vials containing distilled water, carried to the laboratory where they were weighed, had their peduncles

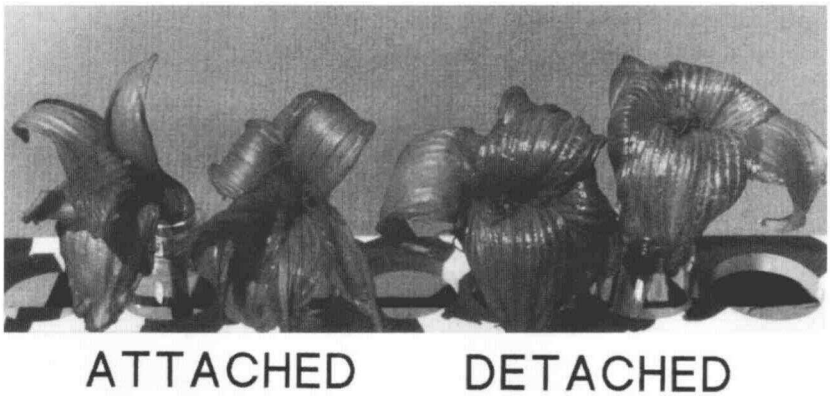

Figure 1. Appearance at h 34 of attached daylily flowers (left) and daylily flowers excised at h 10 and then allowed to senesce for 24 h (right).

recut under water, and placed in fresh distilled water for 45 min to remove solutes released by the cutting. At h 11, six flowers were placed in individual vials containing 4 mL of distilled water, and the remaining six flowers were placed in vials containing 4 mL of 4 mM EDTA at pH 6.0 (see Costello et al., 1982). At h 31, after 20 h, the flowers were removed and reweighed, and each 4 mL of vial liquid was processed to obtain sugar and amino acid fractions as described by Redgwell (1980) and Bieleski (1994). The procedure was repeated with 10 flowers harvested at h 31 and conducting efflux between h 32 and 36. Separation and quantitation of sugars was as described by Bieleski (1994) and that of amino acids was as described by Walton et al. (1991).

Translocation by Intact Senescent Flowers

Two representative h-32 flowers on separate plants were chosen. A thin strip of Parafilm was wound around the pedicel of the flower and pressed firmly onto it to provide a barrier against any potential surface flow of liquid from one side to the other. The top two sepals of the flower were then excised to provide open access to the top petal. Over 30 min, 0.25 mL of 2 mM [^{14}C]Suc at 370 kBq mL^{-1} was applied to the surface of the exposed petal in one 25-μL aliquot at a time, using the tip of the micropipette to spread the liquid. An open plastic bag was then put over the flower, but away from its surface, to reduce drying in the glasshouse environment. After 4 h, the treated petal plus adjacent corolla segments were excised to prevent any accidental transfer of tracer to the stem, and then the flowering stem was harvested and cut into segments that were weighed, extracted with 1% (v/v) formic acid, and measured for radioactivity as above.

RESULTS

Passive Losses

No foil taken from under the senescing flowers showed any detectable deposits arising from the flowers above, and no detectable sugar was recovered. Thus, any passive loss of carbohydrate through this route would have been below 0.01 mg flower^{-1} or 0.002 mg g^{-1} fresh weight.

Effect of Excising Flowers on Senescence Changes

In outward appearance, excised flowers followed the same time course of senescence as attached flowers (Fig. 1). A slightly greater fullness in the petals of detached flowers was equivalent to them lagging about 4 h behind attached flowers in a 24-h span. In contrast, there were major effects on flower composition. Petals from detached flowers, compared with those of attached flowers, had significantly higher fresh weight, dry weight, percentage dry weight,

Table I. *Fresh and dry weights, percentage dry weight, and sugar and amino acid contents of petals from daylily flowers that were either held on the plant until h 34 (attached) or excised at h 10 and held in vials containing water until h 34 (detached)*

At h 34, petal samples were taken from both sets and assayed. Values given are on a per petal basis: "dry wt minus solutes" gives the petal dry weight minus the measured sugar and amino acid components. Values of petals from attached flowers taken at h 10 in another experiment indicate the state of the flowers at the time when detached flowers were excised. Significance of difference of the attached versus detached value for each feature is given.

Sample	Petal Feature					
	Fresh wt	Dry wt	Percent dry wt	Total sugar	Total amino acid	Dry wt minus solutes
	mg	*mg*	*%*	*mg*	*mg*	*mg*
Hour 34						
Attached	373 ± 74	34.0 ± 5.1	9.3 ± 1.82	3.7 ± 2.8	1.7 ± 1.1	28.7 ± 2.8
Detached	807 ± 146[c]	95.9 ± 16.6[c]	12.0 ± 1.51[a]	38.3 ± 8.3[c]	12.1 ± 1.9[c]	45.5 ± 8.2[b]
Hour 10						
Attached	1089 ± 135	101.7 ± 14.1	9.4 ± 0.54	46.9 ± 6.9	9.4 ± 4.2	45.4

[a] $P < 0.05$. [b] $P < 0.01$. [c] $P < 0.001$.

and sugar and amino acid contents (Table I). Loss of sugars from excised flowers into the vial water was minuscule (107 ± 57 μg sugar g^{-1} fresh weight flower, equivalent to 0.093 mg $petal^{-1}$ or 0.22% of the total sugar). Percentage sugar composition was also very different: in the detached flowers at h 31 it was essentially the same as at the time of excision, h 10 (apart from appearance of an unidentified sugar chromatographing in the position of Gal); whereas attached flowers, besides losing most of their sugar, showed a marked fall in the proportion of hexose, particularly Fru (Table II). Their loss in dry weight was significantly greater than that accounted for by sugar plus amino acid (Table I, right column), suggesting that there was also loss of structural materials as a consequence of the autolysis. Thus, detaching the flowers stopped the carbohydrate and dry weight changes occurring on the plant almost completely, suggesting that the loss in flower carbohydrate during senescence noted by Bieleski (1993), including some loss of structural material, was the result of a retranslocation process.

Tetrazolium Staining of Senescent Petals

With flowers harvested between h 31 and 42, parenchyma in the thick petal base sometimes became stained with tetrazolium, but this did not occur in the blade itself. There, staining was confined to the vein system, but was present even in vein ends that had become detached from the surrounding parenchyma tissue by the autolytic processes (Fig. 2). In hand sections, stain was concentrated in the region of the phloem. Through the day, veins progressively lost their staining ability, so that by the time the flowers had dried out (around h 44) any staining was confined to veins in the petal base, which was still moist. Flowers excised at h 10 and held in vials until h 31 to 44 showed much of the same staining behavior as intact flowers, and any staining differences appeared due to small differences in the rate of drying.

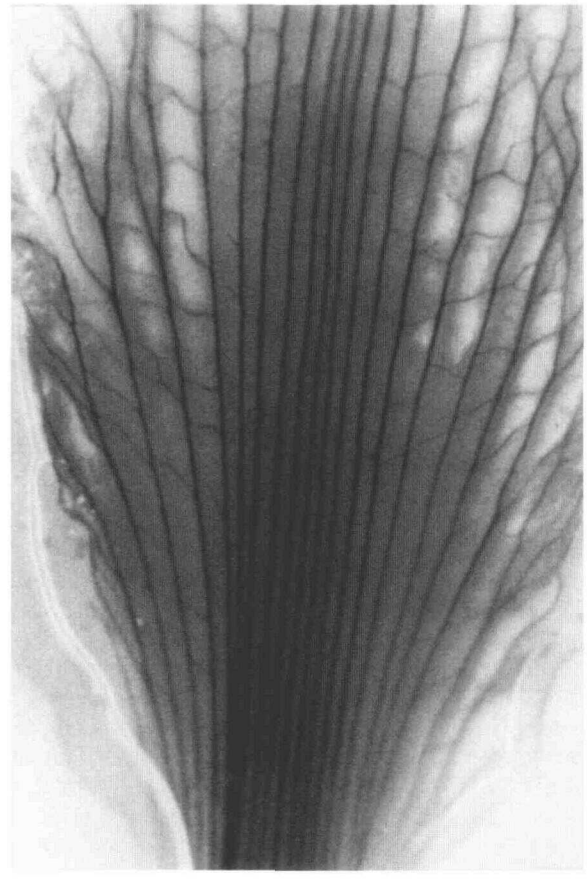

Figure 2. Formazan staining of veins in a senescent daylily petal harvested at h 34 and reacted for 4 h with 0.5% (w/v) phenyl tetrazolium chloride. Note the loss of some interveinal tissues by autolysis.

Table II. *Proportion of individual sugars in sugar fractions from petals of daylily flowers either held on the plant until h 34 and then harvested (attached) or excised at h 10 and held in vials containing water until h 34 (detached)*

At h 34, all petal samples were taken for sugar analysis. The individual sugar values are percent of total sugar in extract, and the total content per petal is as given in Table I. Control values from attached flowers taken at h 10 in another experiment show the approximate sugar composition of flowers at the time when the detached sample was excised. An unidentified sugar, possibly Gal, was present in h 34 samples but not in earlier samples. Fructan and other sugars were below the limit of detection. Significance of difference of attached versus detached values is given.

Sample	Sugar	Attached Petals	Detached Petals
		% total sugar	
Petals at h 34	Suc	43.0 ± 8.06	22.5 ± 2.92[b]
	Glc	13.4 ± 4.36	29.7 ± 0.80[b]
	Gal?	6.8 ± 2.21	2.3 ± 0.48[a]
	Fru	36.9 ± 4.66	45.5 ± 3.19[a]
Petals at h 10	Suc	10.4 ± 0.60	
	Glc	25.8 ± 2.21	
	Fru	63.8 ± 2.70	

[a] $P < 0.01$. [b] $P < 0.001$.

Sites of Suc Accumulation

Flowers in this experiment were obtained from a more humid glasshouse atmosphere than those used above and were still moist at h 40. Senescent petals harvested between h 28 and 37 actively accumulated [^{14}C]Suc (Fig. 3A); radioactivity was exclusively confined to the veins, with any activity present in the interstitial tissue being below the detection capability of the x-ray film (Fig. 3B). Veins that had become detached from the surrounding parenchyma as the result of autolysis still accumulated [^{14}C]Suc actively.

Rate of Suc Accumulation

Suc was progressively accumulated into isolated halves of senescent daylily petals, and this was still continuing at h 37 (Fig. 4). There was a small but significant fresh weight loss resulting from a continuation of autolysis and some shedding of parenchyma into the uptake medium. An increase in sample SD with time reflected an increased variability in the physical appearance of individual petal

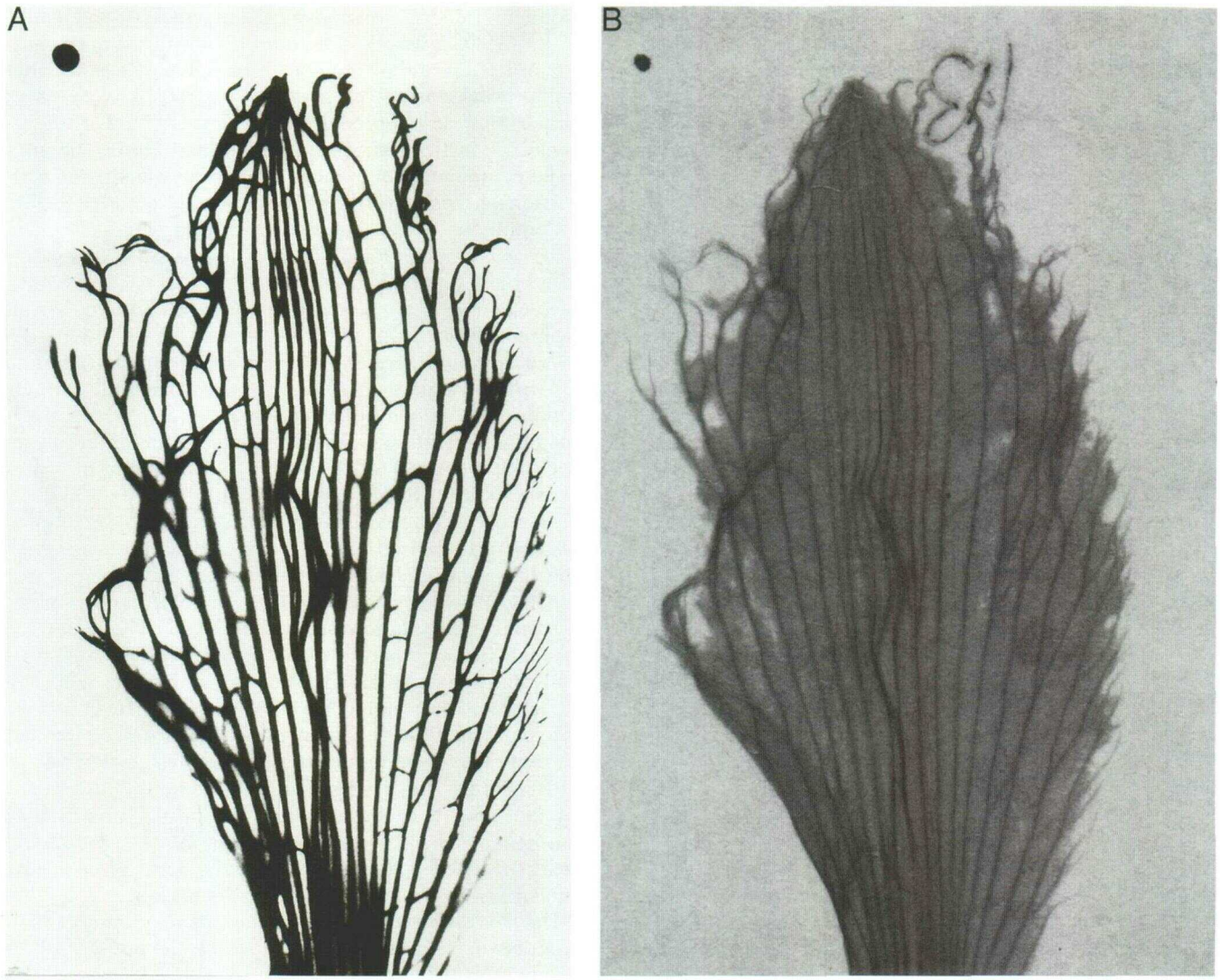

Figure 3. Autoradiograph (A) and photograph (B) of a senescent daylily petal harvested at h 31 and allowed to accumulate [^{14}C]Suc for 3 h. Note the loss of some interveinal tissue by autolysis.

pieces. The initial rate of Suc accumulation was 585 nmol Suc g^{-1} fresh weight h^{-1}.

Products of Suc Accumulation

During the 3-h uptake period, 1500 to 1750 nmol Suc g^{-1} fresh weight was taken up into the petal pieces (Fig. 4) and recovered in the tissue extract. About 85% of the radioactivity recovered was in the sugar fraction, with the amino acid fraction being the next most important (7.5%). Smaller proportions were in the organic acid (4.5%), protein plus starch (2.6%), and lipid (0.45%) fractions (Table III). Within the sugar fraction, 85% of the radioactivity was present as Suc, with 11.5% as Glc and 3.5% as Fru (Table III). No significant amount of any other sugar or of fructan was detected.

Nature of Phloem Contents

From h 11 to 31, EDTA increased the amount of sugar leaking from the cut ends of the peduncles 13-fold in comparison to flowers in water (Table IV). The composition of the extra sugar that leaked as the result of EDTA treatment was taken to reflect the composition of the sieve tube contents: Suc was the major sugar present, with somewhat lesser amounts of Glc and Fru (Table IV). Late in senescence (h 32–36) flowers still released sieve tube sugars, but at about one-tenth the rate, in keeping with a similar decrease in sugar content of the petals (Table I). There was also a 14-fold difference in amino acid release under the two different treatments. Gln and Hyp were identified as the two major amino acids in the phloem exudate (Table V). The rate of sugar release (10.98 μg g^{-1} fresh weight h^{-1}) was about 5 times the rate of amino acid release (2.04 μg g^{-1} fresh weight h^{-1}).

Sinks for Suc Transport

Substantially the same results were obtained with the two flowering stems. Between h 32 and 36, over 60% of the Suc applied to the senescent petal was transported out of

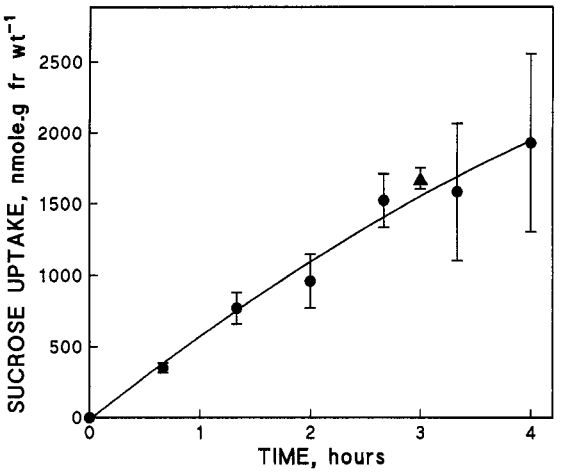

Figure 4. Rate of uptake of [^{14}C]Suc by half-petals from senescent daylily flowers harvested at h 31 and allowed to accumulate from 2 mM Suc. Points shown as ● were obtained from the main uptake experiment (rate of sugar uptake), and the point shown as ▲ is the value from the identical tissue used in studying products of sugar accumulation.

the flower into other parts of the plant, during which time it traveled over 95 cm and beyond the base of the flowering stem (Fig. 5). The most active sinks were the developing flower buds, whether on the same or an adjacent branch. Only small amounts entered an adjacent open flower (Fig. 5).

DISCUSSION

Initial results established that a large loss of metabolites from senescing flowers on the intact plant was not due to respiration or passive leakage. Between h 10 and 34, detached flowers lost only 5.8 mg dry weight petal^{-1} and 8.6 mg sugar petal^{-1}, and gained 2.7 mg amino acid petal^{-1} (from Table I). The dry weight loss equates to that lost in respiration, whereas the increased amino acid is accounted for by hydrolysis of protein, which should have produced about 3.7 mg amino acid petal^{-1} over the period (Lay-Yee et al., 1992). In comparison, attached flowers lost 67.7 mg dry weight petal^{-1}, 43.2 mg sugar petal^{-1}, and 7.7 mg amino acid petal^{-1}. The difference between the two sets of data, 61.9 mg dry weight, 34.6 mg sugar, and 10.4 mg amino acid per petal, gives the amount of each component that disappeared from the petal through being joined to the plant. Because there was only 0.1 mg sugar petal^{-1} found in vial water holding the excised flowers, neither passive diffusion nor return flow through the xylem can account for the movement, leaving phloem transport as the likely path.

The pattern of localization of tetrazolium staining (Fig. 2) and Suc uptake (Fig. 3) both showed that after 24 h of senescence (by h 34) the petal parenchyma was completely inactive, in keeping with its autolytic breakdown, whereas the vein system and particularly the phloem behaved as if it was fully alive and functional, losing viability only around h 45. Suc taken up was partly turned into other products (Table III), demonstrating metabolic functionality in the vascular tissues; whereas the significant incorporation of label into the amino acid fraction suggests that the amino acids arising from protein hydrolysis were being turned over and reprocessed into forms suitable for retranslocation (later shown to be Gln and Hyp). The only major component not to incorporate label was the cell wall fraction—hardly surprising in an organ at the end of its life.

Continuous active Suc transport into senescent petal pieces was demonstrated (Fig. 4). The rate can be compared with others reported in the literature. Leaf slices accumulated Glc from 1 mM solution at 100 to 900 nmol g^{-1} fresh weight h^{-1} (Bieleski, 1977), and excised parenchyma tissues accumulated Suc from 1 mM solution at 10 to 40 nmol g^{-1} fresh weight h^{-1} (Bieleski, 1966). Excised vascular bundles and phloem tissues have shown particularly high Suc transport rates against strong concentration gradients (Bieleski, 1966): 500 to 700 nmol g^{-1} fresh weight h^{-1} from 1 mM Suc into freshly excised vascular bundles, 370 into freshly excised apple phloem (Bieleski, 1969), and up to 2000 in aged, excised phloem tissues (Bieleski, 1966, 1969). The rate measured here for senescent petals using 2 mM Suc (Fig. 4) corresponds to about 420 nmol g^{-1} fresh weight h^{-1} from 1 mM solution (see Bieleski, 1966, 1977). But if we take into account that 75 to 85% of the tissue was metabolically inactive (see Fig. 3), the bundles themselves will have accumulated Suc at 1600 to 2800 nmol g^{-1} fresh weight vascular bundle h^{-1}, equaling the highest rates recorded for excised phloem tissues to date. A translocation rate of 4000 nmol g^{-1} fresh weight petal h^{-1} is needed to account for the amount of sugar exiting the petal (Table I). The conclusion is that the vascular bundles in senescent daylily petals are loading Suc effectively by the standard of normal phloem tissues in other plants.

The next step was to discover the nature of translocation products in the senescent flower using the approaches of Wiemken et al. (1976) and Costello et al. (1982). Here EDTA is used to chelate Ca^{2+}, preventing callose formation that normally blocks the sieve plates and allowing osmotic forces to extrude sieve tube contents into the surrounding

Table III. *Distribution of ^{14}C in various cell fractions from senescent daylily petal segments (extracted at h 34) following uptake of [^{14}C]Suc from 2 mM Suc solution for 3 h (mean total uptake = 1681 ± 68 nmol g^{-1} fresh weight over the 3 h)*

Cell Fraction	Total of ^{14}C
	%
Insoluble residue	0.14 ± 0.05
Lipid and phospholipid	0.56 ± 0.08
Protein and starch	2.56 ± 0.30
Organic acids	4.45 ± 0.21
Amino acids	7.55 ± 0.71
Sugars	
Total	84.71 ± 0.89
Suc	70.86 ± 0.47
Glc	10.55 ± 0.47
Fru	3.07 ± 0.32
Trisaccharide	0.20 ± 0.03
Fructan	<0.05

Table IV. *Amount (in μg g^{-1} fresh weight h^{-1}) of sugars released from cut peduncle ends of senescing daylily flowers placed in either distilled water or in 4 mM EDTA at pH 6.0*

The third and sixth rows in the table give the difference between efflux in EDTA and efflux in water, to isolate the EDTA-specific component of efflux.

Treatment of Flower	Suc	Glc	Fru	Other
Water[a]	0.411 ± 0.140	0.248 ± 0.177	0.280 ± 0.211	0.023 ± 0.014
4 mM EDTA	8.359 ± 4.744	1.585 ± 0.564	1.915 ± 0.819	0.082 ± 0.091
Difference	7.948	1.337	1.635	0.060
Water[b]	<0.010	0.257 ± 0.182	0.390 ± 0.286	0.160 ± 0.109
4 mM EDTA	0.460 ± 0.236	0.750 ± 0.252	0.650 ± 0.226	0.177 ± 0.059
Difference	0.460	0.493	0.260	−0.017

[a] 20 h between h 11 and 31. [b] 4 h between h 32 and 36.

solution. Sugar coming from cut tissue surfaces is detected as loss into water, whereas the increased loss due to EDTA is taken to come from the phloem. With daylily, loss of sugars and amino acids from peduncles of excised senescing flowers was increased 13-fold by 4 mM EDTA, confirming that the phloem was still active late into senescence (Table IV). Although Suc was a minor sugar in the petal tissues (Bieleski, 1993), it was the dominant sugar in the exudate, suggesting that active synthesis of transport compounds was occurring.

Finally, when [^{14}C]Suc was applied to a senescent petal on an intact plant, its export was extensive (over 50% exported in 4 h) and rapid (a speed of more than 25 cm h^{-1}). Both the pattern of movement (Fig. 5) and the rate are typical of what has been observed in many studies in which $^{14}CO_2$ has been fed to a source leaf and subsequent movement of photosynthetic products has been followed. However, in this case the source is a senescing organ that a short time previously had been a powerful sink. The developing flower bud on the stem depicted in Figure 5, which is the prime sink, would in 3 d reach the same state of senescence as the senescent source flower. In fact, the switch from sink to source occurs over a much shorter time span, on the order of 12 h. In the 24 h leading up to full-flower opening at h 10, the petal dry weight and sugar content increased by 42 and 45%, presumably through the normal inward phloem transport process. In the subsequent 12 h, to h 22, dry weight and sugar contents decrease by 26 and 51%, respectively (Lay-Yee et al., 1992; Bieleski, 1993). These new results show that the loss occurred through outward phloem transport.

It is unlikely that the switch from sink to source is caused by a sudden change in the plant itself, which is in a form of steady state. During most of the 3-month flowering period, there are about 10 flowers in all stages of development present on the scape, and as 1 flower abscises at the end of its life, another is being initiated at the start. Developmental changes in the flower are not halted by excising the flower, but are stopped by cycloheximide. It seems that the triggering event has to occur within the flower itself. One obvious candidate would be an increase in total soluble solids or sugar content up to the point at which mass action forced movement out instead of movement in. This is not supported by measurements on sap osmolarity, which show a maximum well before full opening, or by the measured changes in sugar concentration, which are too small and in the wrong direction to explain the reversal of flow (Bieleski, 1993). Instead, the data strongly suggest that the phloem is programmed to develop a loading capability at the start of senescence, in the same way that the phloem is excluded from the autolytic changes occurring in the adjacent parenchyma tissue. There is no evidence as to what form this loading capability takes, but it could well be the synthesis of Suc carrier protein located in the companion cell or sieve tube membranes. A change in hormone level may play a part, since GA_3 has been shown to enhance phloem loading when applied to a source leaf of bean (Aloni et al., 1986).

One event that would be expected to occur would be increased Suc synthesis, to convert Fru and Glc (the main carbohydrates in the mature flower), plus other carbohydrates released during autolysis, into the transport form Suc (cf. Winkenbach and Matile, 1970). There is evidence that this is happening. The Suc concentrations in petals of the excised flowers can be calculated from (total sugar per petal)/(petal fresh weight), in Table I, times (fraction of Suc in total sugar),

Table V. *Proportion of different amino acids, as a percentage of total amino acid weight, in the amino acid fraction of material released from cut peduncle ends of senescing daylily flowers held in 4 mM EDTA at pH 6.0 for 20 h between h 11 and 31*

The amount released was 40.74 ± 17.8 μg g^{-1} fresh weight (at a rate of 2.04 ± 0.89 μg g^{-1} fresh weight h^{-1}), and minor amino acids contributing to the "other" fraction were (in descending order) Thr, Val, Lys, Ile, Arg, Pro, Phe, Tyr, Gly, and His. With comparable flowers placed in water (see text), only Hyp, Asp, and Ser were consistently found above the minimum level of detection for an individual amino acid (corresponding to approximately 0.2 μg g^{-1} fresh weight), so that the total amino acid released by flowers standing in water was estimated to be less than 3 μg g^{-1} fresh weight.

Amino Acid	Total of Amino Acid
	%
Gln	21.3 ± 1.22
Hyp	20.5 ± 2.16
Asp	11.6 ± 1.41
Asn	6.7 ± 0.55
Ser	6.2 ± 1.52
Ala	4.4 ± 0.82
Leu	4.2 ± 0.72
Glu	3.8 ± 0.87
Other amino acids	21.4 ± 2.56

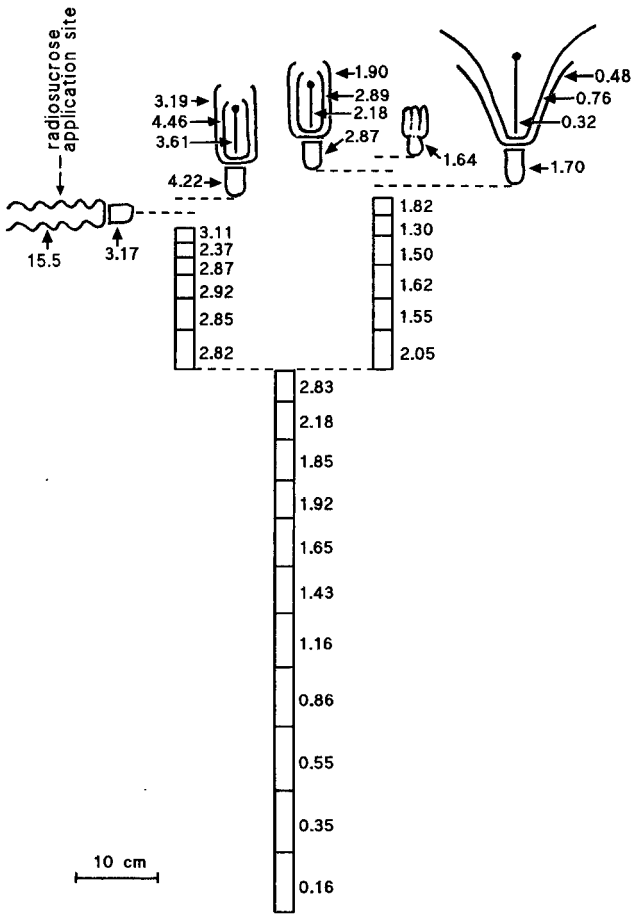

Figure 5. Distribution of radioactivity in a daylily flower stem supplied at h 32 with 92 kBq [^{14}C]Suc, applied to one petal of a senescent flower (dashed arrow), then allowed to carry out transport for 4 h. Values given are kBq g^{-1} fresh weight recovered at h 36. The diagram depicts a single scape branching near the top. The left branch carries the treated senescent flower (far left, wavy lines) plus an h −12 bud (above and to the right). The right branch carries (from left to right) an h −12 bud, a young (approximately h −132) bud in the middle, and an open (h 12) flower on the far right. The various flower parts are indicated diagrammatically. Taking tissue weight into account, 91% of the nominal amount of tracer supplied was recovered in the extracts, and 57% of the recovered activity had been transported beyond the treated petal and adjacent corolla to more distant organs.

in Table II. At h 34, detached petals had a much higher Suc content and concentration (20.4 mg g^{-1} fresh weight) than petals of attached flowers at h 34 (4.2 mg g^{-1} fresh weight) (Tables I and II), or at h −14, −2, and 10 (8.7, 6.1, and 4.8 mg g^{-1} fresh weight) (Bieleski, 1993). It is clear that there is active synthesis of Suc in the senescing petal, which is revealed when its removal has been blocked.

The picture that emerges is this. At about h 10, several programmed senescence events are set in train: these are tissue specific, so that what occurs in the parenchyma is entirely distinct from what occurs in the phloem and associated cells. In the parenchyma, hydrolytic enzymes are synthesized, leading to hydrolysis of protein and some structural carbohydrate, and then autolysis of the cells. It is not clear whether phloem tissues escape autolysis by being geographically removed from the production of the hydrolases, whether they are protected in some way, or whether a process such as ubiquitination makes the parenchyma cells much more susceptible. In the phloem and its associated cells there is synthesis of phloem-loading carriers, particularly a Suc transporter, and activation of pathways for Suc and amide synthesis, followed by rapid loading of the phloem and outward translocation of solutes, particularly to the next developing flower, until at the end of 24 h over 80% of the soluble carbohydrate, tissue N, and tissue P have been salvaged and removed. Up to half of the water loss from the petal during this time may be to support the phloem stream, rather than a loss by evaporation to the atmosphere.

These results help explain an anomaly from an earlier study. Bieleski and Reid (1992) supplied ^{32}Pi to senescing petal tissues in the expectation that the tissue would accumulate less Pi as the cells became more leaky. Instead, Pi uptake increased and was accompanied by active P-ester turnover. It now seems probable that Pi was behaving like the sugars and was being actively loaded into the phloem for retranslocation. We can go even further. In many environments the nutrients N and P are in very short supply and limit growth, and plants that have evolved for such conditions are slow growing and have luxury photosynthetic capacity (Chapin, 1980). The daylily is adapted to poor soils (Munson, 1989), and its effective mechanism for recovering flower carbohydrates may well have evolved, not to conserve carbohydrate per se, but to provide the transport vehicle for conserving P and N.

Retranslocation from leaves has been studied for over 50 years (Williams, 1955; Thrower, 1967; Thimann, 1980). However, the process described here is different in three important respects. First, although the leaf shifts from sink to source behavior during early development (e.g. Bieleski and Redgwell, 1985), it is a carbohydrate source for most of its life and there is no sudden shift associated with onset of senescence (Hopkinson, 1964; Thrower, 1967). When progressive hydrolysis of protein and P-containing compounds begins, the P, N, and K are removed in a well-established carbohydrate stream. Second, the leaf continues synthesizing carbohydrate until it falls from the plant and there is no marked decline in carbohydrate content. There is no marked structural breakdown of the tissues (Thimann, 1980), and the persistence of high phloem activity in an autolytic environment has not been reported. Third, the process is much slower, taking from 1 to several weeks instead of being complete in 24 h (Williams, 1955; Thrower, 1967). The results presented here for daylily give the most dramatic example of retranslocation reported to date.

Studies on retranslocation from senescing flowers are more restricted in scope and number (see Borochev and Woodson, 1989). The role of ethylene has attracted the most attention, as in the study by Nichols and Ho (1975) in which ethylene enhanced carbohydrate export from senescing carnation petals into the nectar, gynoecium, and stem. In contrast, senescence of daylily appears unaffected by ethylene (Lay-Yee et al., 1992). The closest parallel is in the excellent work of Matile and co-workers (Winkenbach

and Matile, 1970; Wiemken-Gehrig et al., 1974; Wiemken et al., 1976), in which senescence of another ephemeral flower, *Ipomoea tricolor*, has been studied. Our findings with daylily (Bieleski and Reid, 1992; Lay Yee et al., 1992; Bieleski, 1993) support theirs, even though the two plants differ in many respects (monocotyledon versus dicotyledon, tussock form versus vine, a fructan carbohydrate economy versus a Suc one, and flowers on a scape versus flowers along a stem). It appears that the common theme of a rapidly senescing ephemeral flower is what determines the close similarities. Where the present study traverses new ground is in concentrating on the critical role of the phloem, on the marked persistence of its activity in a background of autolyzing cells, and on the rapid reversal in source-sink behavior. Given the other parallels, it seems likely that the same phenomenon will be operating in *Ipomoea* (Matile and Winkenbach, 1971).

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