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Changes in Carbohydrates and Osmotic Potential during Rhythmic Expansion of Rose Petals

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Abstract. **Rhythmic pulses of irreversible petal expansion in rose (*Rosa hybrida* L. 'Sonia') petals cause diurnal changes in the rate of flower opening. Time-lapse cinematography revealed a transient increase in the rate of rose flower opening that commenced shortly before the onset of a light period and lasted for a few hours. Petal expansion, which occurred sequentially from the outer to the innermost whorl, involved rhythmic increases in fresh and dry weights. The amount of expansion was greatest in the distal portion of each petal and least near the petal base. Periods of rapid expansion were accompanied by decreases in starch and increases in soluble sugars in the petals, but the total carbohydrate content of the petals remained constant during a light–dark cycle. During expansion, the osmotic potential of the outer petal increased from −790 to −690 kPa. Starch hydrolysis during petal growth appears to be important for maintenance of cell size, but it is not the factor controlling cell expansion.**

Higher plants display diverse mechanisms permitting rapid opening of their flowers. Opening may involve several phenomena, including growth of subtending tissues (as in iris), growth of petals (roses), reorientation of flower parts (cyclamen), and abscission of ensheathing organs (poppy) (17). Flower opening is sensitive to temperature (12) and light (10), plant water relations (7, 15) and growth regulators (6, 13, 16, 20, 21).

There have been few published studies of either the biochemistry or the physiology of flower opening. Weinstein (22) reported that the fresh weight of 'Better Times' rose petals increased until the flower was fully open, but that petal dry weight decreased. These data are consistent with an opening mechanism involving cell expansion without an increase in cell number. Ho and Nichols (9) suggested that the increase in soluble sugar content of rose petals is derived largely from starch hydrolysis, and that a major function of low-molecular-weight carbohydrates is to lower the osmotic potential in petal cells, thereby promoting an influx of water to drive cell expansion. This argument was amplified by Hammond (8), who suggested that rose flower opening is driven by a concomitant increase in amylase activity.

A good model for examining the control of flower opening would be one of the many flowers that exhibit diurnal variations in the rate of opening (4, 19). The diurnal opening of Kalanchoë flowers involves reversible expansion and contraction of petal cells in response to photoperiodic stimuli (18). This mechanism of reversible ion fluxes is likely to be different from that governing flower opening involving irreversible petal growth. The rose presents a particularly intriguing example of this type of flower opening. Alternating periods of light and darkness trigger pulses of irreversible growth of the petals, resulting in rhythmic changes in the rate of flower opening (5). Here we have examined the changes in starch and sugar contents and osmotic potential of the petals of opening rose flowers in relation to the rhythm of petal growth and position of the petals in the corolla to investigate the role of carbohydrates in rose flower opening.

Materials and Methods

Plant material. 'Sonia' rose flowers were obtained from a commercial grower at normal harvest maturity (sepals starting to reflex) and transported dry to Davis, Calif. at 5°C, or were obtained form plants grown under normal daylength conditions in a greenhouse in the Environmental Horticulture Dept., Univ. of California, Davis (18° minimum and 27° maximum greenhouse temperatures). Flowers were harvested in the early morning and handled uniformly before treatment.

Postharvest treatment. Flower stems were trimmed under water to 30 cm (for time-lapse cinematography) or 5 cm (for other experiments) and placed in test tubes containing distilled deionized water (DI) in a room kept at 20°C and 50% to 60% RH. Lighting, when needed, was 13 $\mu mol \cdot s^{-1} \cdot m^{-2}$, provided by cool-white fluorescent tubes (General Electric F96T12-CW-1500) controlled by a 24-hr time clock. Flowers were entrained to the desired photoperiod for 24 hr before experiments started.

Time-lapse filming. Flower opening was recorded by 16-mm time-lapse cinematography; single frames were exposed at 10-min intervals synchronized with 0.6-s flashes of light from an electronically controlled photoflood apparatus. Flower diameter was determined by measuring the screen image of the flower when the film was projected. The diameters of 10 flowers were recorded.

Location of areas of expansion in petals. The outermost petal of each of five flowers was marked with dots of ink along the midvein and along the perpendicular to the midvein, midway up the petal, using a dressmaker's pattern-tracing wheel, which placed dots 2.1 mm apart on the adaxial side of the petals. After 2 days, the marked petals were detached and the distance between marks was determined.

Measurement of petal growth. In rose flowers, the petal whorls open sequentially, starting with the outer whorl (5); we therefore used petals from the non-expanding inner whorls as controls in studies of petal growth. Four replicate flowers were removed at intervals, their petals were detached, and the fresh weights of the outer petal whorl (petals 1 to 5) and of an inner whorl (petals 11 to 15) of each flower were recorded. Dry weight measurements were made after petals were frozen in liquid nitrogen and lyophilized.

Extraction and analysis of starch and soluble sugars. The lyophilized petals were ground to a fine powder with a mortar and pestle and 100 mg was extracted in ethanol for 6 hr in a Soxhlet apparatus. The ethanol-insoluble residue was removed from the extraction thimble and dried in air before starch analysis. The ethanol-soluble fraction was evaporated to dryness under vacuum and redissolved in 5 ml of glass-distilled water.

The ethanol-insoluble residue was prepared for starch analysis

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by a modification of the method of MacRae (14). The residue was wetted with 80% ethanol, then suspended in 4 ml of glass-distilled water and heated for 3 hr in a boiling water bath while being shaken continuously to solubilize the starch. After solubilization, the suspension under test was cooled to room temperature. Water lost by evaporation was replaced, and 1.5 ml of buffer solution (0.2 M sodium acetate–acetic acid, pH 4.6) and 0.5 ml of a 1 mg·ml^{-1} solution of amyloglucosidase (Sigma) was added. The suspension was incubated in a 55°C water bath with continuous shaking for 4 hr, after which glucose released by hydrolysis was determined using a colorimetric assay based on the hexokinase-catalyzed conversion of glucose to glucose-6-phosphate (2). Losses during extraction, which were found to average 5%, were determined from the recovery of known amounts of ethanol-washed corn starch added to representative samples of the petal residue.

Total soluble sugar content was estimated by the following modification of the anthrone method of Yemm and Willis (23). The anthrone reagent comprised 200 mg of anthrone and 100 ml of H_2SO_4 (made by adding 500 ml of concentrated acid to 200 ml of water). A 40-µl aliquot of the ethanol-soluble fraction under test was layered on cold anthrone reagent, cooled for 5 min, heated in a boiling water bath for 10 min, and then cooled for an additional 5 min prior to determining absorbance at 600 nm. A standard curve was prepared by measuring the absorbance of glucose solutions over a concentration range of 10 to 100 mg·liter^{-1}. Estimates of total glucose equivalents in each sample were corrected for a 2% loss during extraction and analysis, as determined by measuring the recovery of a known amount of glucose subjected to the entire extraction procedure.

Determination of petal osmotic potential. For measurement of petal osmotic potential, the outermost petal was detached from each of 12 flowers at intervals. Detached petals were sealed individually in air-tight containers, frozen immediately in liquid nitrogen, then stored at −20°C. The petals were thawed by placing the sealed containers for 6 min in a 25° stirred water bath. Each petal was then removed and its sap expressed with a hand press. An aliquot of ≈10 µl of sap was collected on a filter paper disk and its osmotic potential was determined using a vapor pressure osmometer (Wescor 5100C, Logan, Utah) that had been calibrated against NaCl solutions of known osmolality.

Results

Diurnal changes in flower diameter. Roses maintained in 12:12 hr cycles of light and darkness exhibited a diurnal rhythm during opening (Fig. 1). Flower diameter increased rapidly for a few hours, beginning shortly before the lights came on, then remained nearly constant for the remainder of the day and night. This pattern continued for several days, with petal expansion occurring sequentially from the outer to the innermost whorls (data not shown).

Location of areas of expansion in petals. Expansion was not uniform across the petal surface (Fig. 2). The increase in petal length, measured along the midvein, was smallest near the petal base and was greatest in the distal half of the petal (Fig. 2A). The gain in petal width was smallest near the midvein and increased toward the petal margins (Fig. 2B). During expansion, the length to width ratio of the petals remained constant.

Changes in petal fresh and dry weight. Rhythmic increases in petal fresh and dry weight began late in the dark period (2 hr before the onset of the light cycle) and continued through the early portion of the light period (to 4 hr after "dawn") (Fig. 3A and B). No fresh or dry weight gains occurred during other

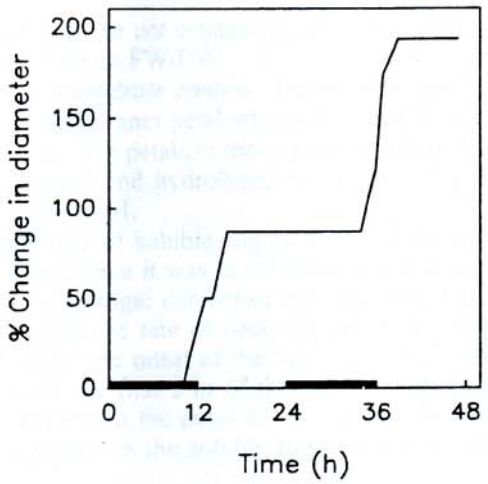

Fig. 1. Change in diameter of a rose flower opening in a 12:12 hr cycle of light and darkness. Data show changes for a typical flower during the second and third day of opening. Heavy bars on the abscissa = dark periods.

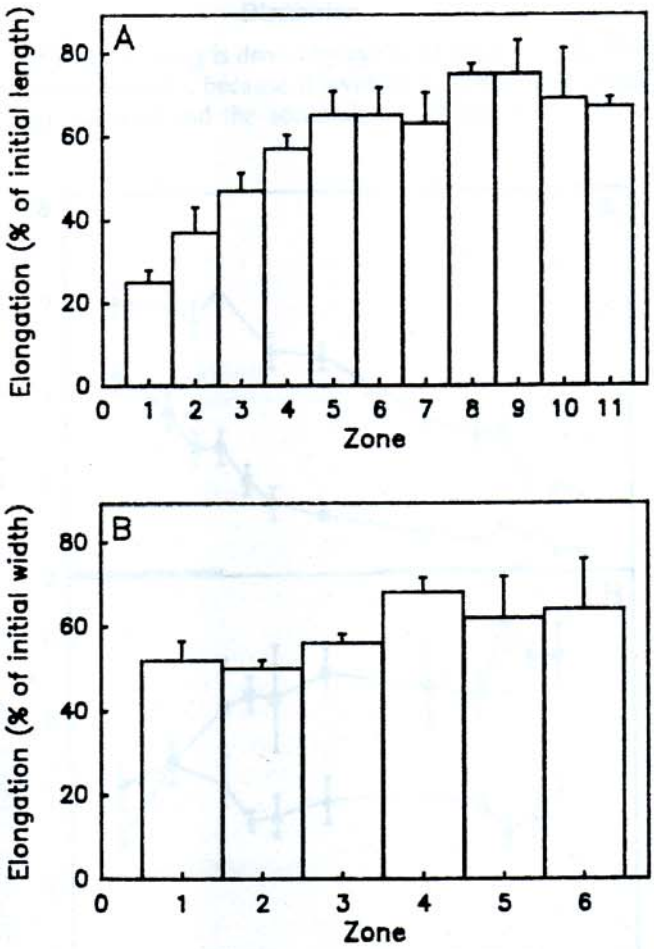

Fig. 2. Distribution of areas of expansion of petals from the outer whorl of rose flowers exposed to a 12:12 hr cycle of light and darkness for 2 days. (**A**) Expansion along the midvein: Zone 1 was at the base of the petal; Zone 11 was ≈4 mm from the petal tip. (**B**) Expansion perpendicular to the midvein, midway up the petals: Zone 1 represented the first 2.1 mm out from the midvein; Zone 6 was ≈4 mm from the petal margin. Values are means of five flowers ± SE.

portions of the light or dark cycles. Concurrent, but much smaller, fresh and dry weight gains occurred in the inner whorl.

The fresh weight : dry weight ratio (FW/DW) of each petal whorl increased over time (Fig. 4). FW/DW increased rapidly as the petals in the outer whorl expanded (from 6 hr before the onset of the light cycle until 4 hr after dawn). Petals from the inner whorl, which were not expanding during that time, showed only a slow increase in FW/DW.

Changes in carbohydrate content. The starch content of both the outermost and the inner petal whorls declined during flower opening (Fig. 5A). The petals in the outermost whorl, however, contained less starch and hydrolyzed starch more rapidly than those in the inner whorl.

The concentration of soluble sugars in the outer whorl was substantially higher than it was in the inner whorl (Fig. 5B). A sharp rise in soluble sugar content of the outer whorl coincided with the time when the rate of petal expansion increased, beginning 4 hr before the onset of the light cycle and continuing to increase during the first 2 hr of the light period. Other than a transient increase near the onset of the light period, there was no substantial change in the soluble sugar content of the inner whorl.

Changes in osmotic potential of the outermost petals. The osmotic potential of the outermost petal increased from −790 to −690 kPa during the time of rapid petal expansion (Fig. 6). Nearly all of the increase in osmotic potential (from −790 to −710 kPa) associated with petal expansion occurred during the dark period; there was no significant change in osmotic potential during the light period after petal expansion ceased.

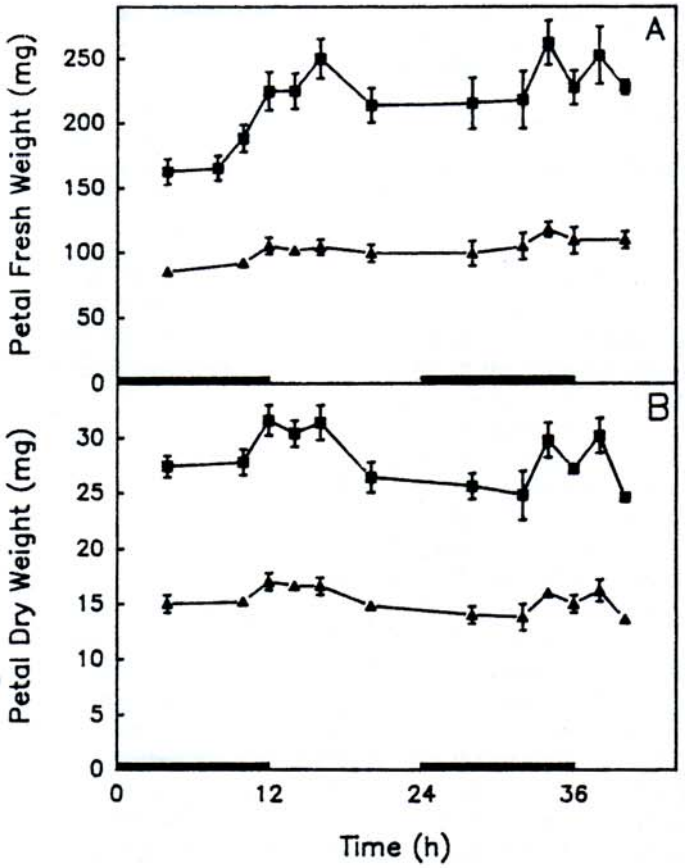

Fig. 3. Fresh weight (**A**) and dry weight (**B**) of petals from the outer whorl (■) and an inner whorl (▲) of rose flowers opening in a 12:12 hr cycle of light and darkness. Values are means of four flowers ± SE. Heavy bars on the abscissa = dark periods.

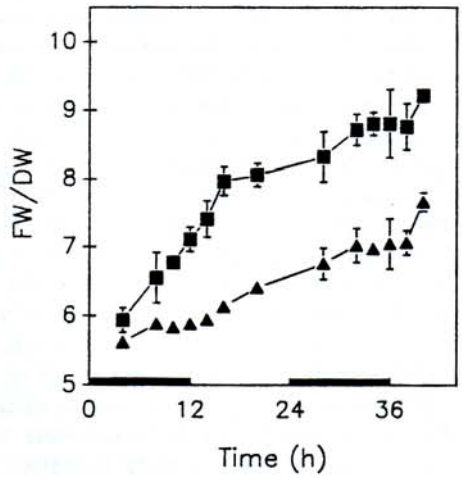

Fig. 4. Fresh weight/dry weight ratio of petals from the outer whorl (■) and an inner whorl (▲) of rose flowers opening in a 12:12 hr cycle of light and darkness. Values are means of four flowers ± SE. Heavy bars on the abscissa = dark periods.

Discussion

Rose flower opening is driven by cycles of petal growth. This growth is irreversible, because it involves a permanent increase in flower diameter and the accumulation of dry matter in the

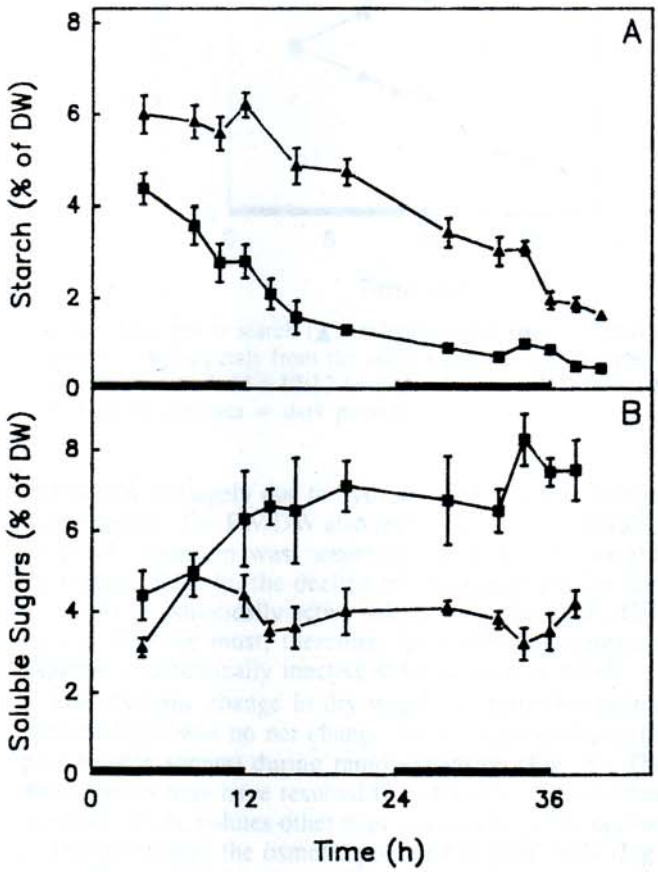

Fig. 5. Starch (**A**) and soluble sugar (**B**) content of petals from the outer whorl (■) and an inner whorl (▲) of rose flowers opening in a 12:12 hr cycle of light and darkness. Values are means of four flowers ± SE. Heavy bars on the abscissa = dark periods.

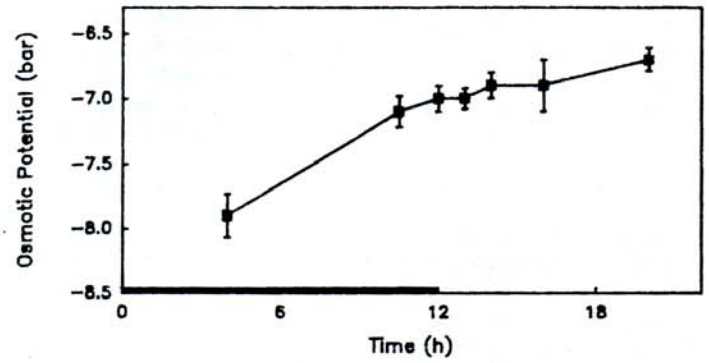

Fig. 6. Osmotic potential of petals from the outer whorl of rose flowers opening in a 12:12 hr cycle of light and darkness. Values are means of petal from 12 flowers ± SE. Heavy bars on the abscissa = dark periods. One bar = 100 kPa.

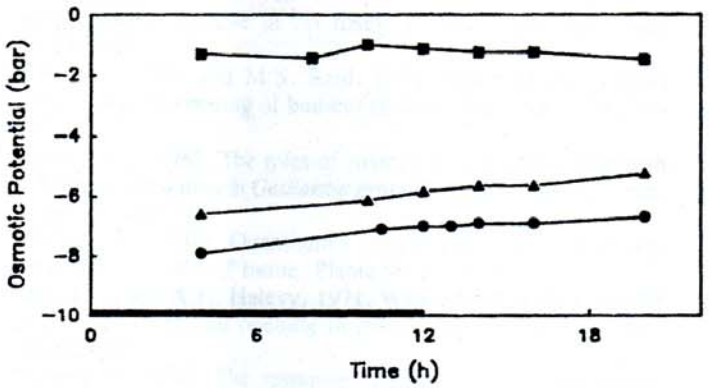

Fig. 7. Contribution of soluble sugars (■) and other solutes (▲) to the osmotic potential (●) of petals from the outer whorl of rose flowers opening in a 12:12 hr cycle of light and darkness. For calculation of the osmotic potential due to soluble sugars, the molecular weight of the sugars was assumed to be 180 and the volume of the solution was calculated from the difference between fresh and dry weight of the petals. The osmotic potential due to other solutes was calculated from the difference between the measured osmotic potential of the petal sap and the calculated osmotic potential due to soluble sugars. Heavy bars on the abscissa = dark periods. One bar = 100 kPa.

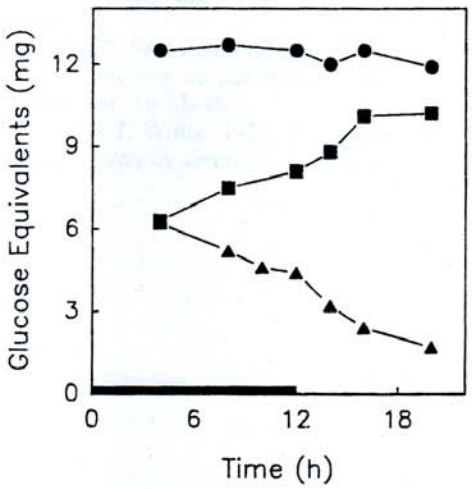

Fig. 8. Changes in starch (▲), soluble sugars (■), and total carbohydrates (●) in petals from the outer whorl of rose flowers during one opening cycle in a 12:12 hr cycle of light and darkness. Heavy bars on the abscissa = dark periods.

petals. Growth is distributed unevenly across the petal: the regions of the most active growth are distal to the petal base. Variation in the extent of expansion in different regions of the petal probably explains the cupping of the growing petals and may contribute to the reflexing of petals during flower opening. These characteristics distinguish the process of rose opening from that of flowers such as Kalanchoë, where cycles of reversible petal expansion are caused by a temporary redistribution of ions between the petal mesophyll and epidermis (18).

The steady-state growth rate of plant cells and tissues is thought to be governed by four factors: wall extensibility, hydraulic conductivity, osmotic pressure gradient across the plasma membrane, and the yield threshold (1, 3). The cyclical change in the growth rate of rose petals is presumably caused by a change in one or more of these factors. The proposal (8, 9) that petal expansion is caused by an increase in water uptake following starch hydrolysis would require that the osmotic pressure gradient across the plasma membrane increase at the time petal expansion begins. The data presented here are not consistent with this proposal. The osmotic potential of petal cells increased during petal expansion, so that the osmotic pressure gradient decreased, rather than incrased, at the time of petal expansion. Factors other than water uptake, for example cell wall extensibility, probably control rose petal expansion.

Starch hydrolysis, although probably not the controlling factor, undoubtedly plays an important role in rose petal growth. If no additional osmoticum had formed during petal growth, the influx of water would have increased the osmotic potential from −790 kPa to about −500 kPa. This would have caused a substantial reduction in the osmotic potential gradient. Maintenance of the osmotic potential at −690 kPa during petal growth can be explained by the increased quantity of sugar in the petals (Fig. 7). Because the total carbohydrate content of the petals changed little during petal growth (Fig. 8), the sugar increase is largely the result of hydrolysis of starch in the petals.

The FW/DW ratio is an indirect measure of the portion of the day weight that consists of osmotically active solutes. A large value indicates that a relatively high percentage of the dry matter in the tissue consists of low-molecular-weight solutes. The rise over time in the FW/DW ratio of the petals indicated that the contribution of osmotically active solutes to the dry weight increased relative to that of other components of the dry weight. Indeed, the change in the FW/DW ratio is nearly the inverse of the change in starch during the same time period (compare Figs. 4 and 5A). Thus, it seems likely that the increase in FW/DW is largely due to hydrolysis of starch to osmotically active sugars. The FW/DW also increased during intervals when no petal expansion was occurring and petal dry weight was decreasing. Clearly, the decline in dry weight did not result in a net loss of osmotically active solutes from the petals. Cell size during this time must, therefore, have been maintained at the expense of osmotically inactive solutes, such as starch.

The rhythmic change in dry weight is somewhat perplexing because there was no net change in total carbohydrates (starch plus soluble sugars) during rapid expansion (Fig. 8). The dry weight gains may have resulted from translocation of other solutes into petals; solutes other than sugars apparently account for a large portion of the osmotic potential of petal cells (Fig. 7).

Finally, it should be noted that the discovery of a gradient of starch and soluble sugar content across the petal whorls of the rose corolla points to the need to consider more carefully the

developmental changes that occur during flower opening. Important aspects of flower development and senescence may be masked by the tendency to regard the entire corolla, or worse the whole flower, as a single entity, as has been done in other studies (8, 9, 11, 22).

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