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Patterns of Assimilate Production and Translocation in Muskmelon (*Cucumis melo* L.)[1]

I. Diurnal Patterns

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ABSTRACT

Continuous monitoring of steady-state carbon dioxide exchange rates in mature muskmelon (*Cucumis melo* L.) leaves showed diurnal patterns of photosynthesis and respiration that were translated into distinct patterns of accumulation and phloem export of soluble sugars and amino acids. Leaf soluble sugar patterns in general followed the pattern of photosynthetic activity observed in the leaf, whereas starch accumulated steadily throughout the light period. Sugar and starch levels declined through the dark phase. Phloem exudate analysis revealed that diurnal levels of the major transport sugars (stachyose and sucrose) in the phloem did not appear to correlate directly with the photosynthetic activity of the leaf but instead were inversely correlated with leaf starch accumulation and degradation. The amino acid pool in leaf tissues remained constant throughout the diurnal period; however, the relative contribution of individual amino acids to the total pool varied with the diurnal photosynthetic and respiratory activity of the leaf. In contrast, the phloem sap amino acid pool size was substantially larger in the light than in the dark, a result primarily due to enhanced export of glutamine, glutamate, and citrulline during the light period. The results indicate that the sugar and amino acid composition of cucurbit phloem sap is not constant but varies throughout the diurnal cycle in response to the metabolic activities of the source leaf.

Rates of assimilate production in source leaves are determined both by biochemical regulation of key biosynthetic and degradative enzymes and by regulation of compartmentalization events that distribute assimilates between storage (chloroplast, vacuole) and transport (phloem) compartments (3, 22). Export of photosynthetically fixed carbon from a source leaf is, therefore, dependent on the functioning of many complex metabolic events that control the production of phloem-mobile assimilates such as sucrose and delivery of these solutes to the phloem (3, 6, 18). In some plants, a direct correlation can be found between sucrose levels in the leaf and the rate of carbon export from the leaf, suggesting that the rate of phloem transport is controlled directly by the rate of sucrose synthesis (3, 6, 18). In still other plants, leaf sucrose levels have no correlation with rates of export, suggesting possibly that it may not be the synthesis of sucrose but rather its delivery to the phloem system that is important in determining export rates (3, 22). This may indicate a role for vacuolar transport processes in the control of export (3).

At present, virtually all of our current information concerning biochemical regulation of carbon partitioning comes from plants that translocate sucrose exclusively (18). Recently, a renewed interest has been shown in carbon partitioning in those species that, in addition to sucrose, also synthesize and export the raffinose oligosaccharide, stachyose. The biochemistry of carbon partitioning between phloem-mobile and storage metabolites is far more complicated in these plants because of the additional mechanisms that must be invoked to account for the diversion of carbon between sucrose and galactinol, the two major precursors of stachyose (5, 13, 16). In addition, however, there is increasing evidence that stachyose synthesis itself may be part of the phloem loading process in stachyose-translocating plants.

Immunological evidence indicates that stachyose synthesis can occur within the intermediary cells of the minor vein phloem (8). Physiological studies have indicated that an apoplastic loading step may not be required for delivery of assimilates to the phloem of stachyose-translocating plants (8, 17, 19, 20). The present models, therefore, conjecture that synthesis of the raffinose oligosaccharides may take place within the intermediary cells from mesophyll-derived galactinol and sucrose. These precursors are thought to be delivered to the intermediary cells via the abundant plasmodesmata that interconnect these cells and the mesophyll (19, 20). If these models prove to be correct, then an additional spatial component will have been added to the carbon-partitioning story that probably does not exist for sucrose-translocating species.

The picture that is emerging, therefore, is that carbon partitioning in stachyose-translocating species is going to be more complicated than simply the addition of more biochemical pathways to the current mesophyll cell models that exist at present for sucrose-translocating species (18). However, at present, very little is known about the carbon-partitioning patterns within source leaves of these species. For example, although it has been well established that stachyose is a primary export product formed by photosynthesis in the light (5, 13, 15-17), the relative contributions of stachyose and

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sucrose to the phloem export pool throughout the light cycle is not known. Similarly, the form of carbon synthesized from stored reserves and exported in the dark has not been determined. Therefore, the aim of the present study was to determine the leaf levels and export profiles of the principle phloem-mobile products (raffinose oligosaccharides, sucrose, and amino acids) in muskmelon (*Cucumis melo* L.) leaves throughout a normal diurnal cycle.

MATERIALS AND METHODS

Chemicals

All chemicals, enzymes, and reagents were obtained from Sigma Chemical Co. (St. Louis, MO). All HPLC solvents were HPLC grade and purchased from Fisher Scientific.

Plant Material

Muskmelon plants (*Cucumis melo* L. cv Honeybush) were grown from seed in 1-gallon containers in a soil:sand:peat mix (1:1:1, v/v) in a growth chamber under a diurnal regimen of 16 h light/8 h dark and 30°C day/20°C night temperatures. Maximum PPFD was 0.325 mmol photons m^{-2} s^{-1} (PAR) at pot level provided by fluorescent and incandescent lighting. Plants were watered daily with half-strength Hoagland solution. At 5 to 6 weeks of age (plastochron index, 17–22 [11]), when the plants were in flower, plants were moved to a similar growth chamber in the laboratory set for similar growth conditions. The onset of the 16-h photoperiod was manipulated to allow collection of samples at different points in the diurnal cycle. The plants were allowed to adjust to the new light/dark cycle for a period of 1 week before experiments.

Leaf Gas Exchange Measurements

Fully expanded leaves (average leaf plastochron index, 8 [11]) were used for all experiments. At the beginning of the experimental period, a 20- to 30-cm^{-2} area at the tip of the leaf (about 25% of the total leaf area) was fastened into a Plexiglas leaf chamber (30 mL internal volume) that enclosed both adaxial and abaxial surfaces of the leaf. A gas stream containing 320 μL L^{-1} CO_2 was mixed using a Matheson Dynablender mass flow controller system and brought to 40% RH and 25°C (10). The gas stream was passed simultaneously over the upper and lower leaf surfaces at a flow rate of 1 L min^{-1} and CER[2] was determined using an IRGA (model 225 MK3; Analytical Development Co., Hoddesdon, United Kingdom) operated in the differential mode. Transpiration rates were determined from dew point hygrometry readings (Dew-all digital humidity analyzer; EG&G Environmental Equipment, Waltham, MA). Leaf and chamber temperatures were monitored (10) with K-type thermocouples (Cole-Parmer Industries, Chicago IL).

[2] Abbreviation: CER, carbon exchange rate; PITC, phenylisothiocyante.

Leaf Tissue and Phloem Sap Analysis

Leaf tissue samples were collected at 2-h intervals throughout the diurnal cycle. To facilitate sampling and to reduce potential wounding artifacts caused by the sampling techniques, no more than four samplings were collected from any one plant. At each sampling time, six leaf discs were randomly punched from the regions of the leaf blade outside the leaf chamber using a ¼- inch diameter paper punch. The leaf discs were weighed and quickly frozen in aluminum foil envelopes on dry ice within 1 min following excision. The discs were then lyophilized for 48 h and reweighed to obtain dry weights.

Phloem sap samples were obtained at the same time intervals by making a single shallow incision into the petiole of the enclosed leaf and and two widely spaced incisions into the stem internodes directly above and below the leaf (4, 11, 15). A measured volume of exudate (20–50 μL) was collected in precalibrated 20-μL capillary tubes and transferred to microfuge tubes containing 450 μL 80% ethanol. The tubes were placed on dry ice and stored frozen until analysis.

Leaf Carbohydrate Analysis

After lyophilized leaf tissue samples were weighed, they were extracted in 80% ethanol and the extracts partitioned by ion exchange chromatography into neutral and basic fractions as described previously (10). The starch content of the extracted residue was determined following digestion with amyloglucosidase (9) by measuring the released glucose using a commercially available glucose detection kit (Sigma HK 10).

The neutral fractions were taken to dryness in a vacuum centrifuge (Savant Instruments, Farmington, NY) and resuspended in 60 μL water, and the sugar content of a 20-μL sample was determined by HPLC as described previously using a Waters SugarPak 1 column held at 90°C (9).

Leaf Tissue Amino Acid Analysis

The basic fractions were taken to dryness, resuspended in 100 μL of a drying reagent consisting of triethylamine:absolute ethanol:water (1:1:1, v/v), and dried again. The amino acids were then converted to their PITC derivatives essentially as described by Yang and Sepulveda (23). Briefly, 200 μL PITC reagent (phenylisothiocyanate:absolute ethanol:triethylamine:HPLC grade water, 1:7:1:2, v/v) was added to each sample, and after 10 min the samples were dried to remove excess reagent. Samples were resuspended in 4.0 mL resuspension buffer (15 mM sodium acetate, 3% [v/v] acetonitrile, and 0.025% [v/v] triethylamine, adjusted to pH 7.4 with phosphoric acid). After filtration through 0.2-μm syringe filters, a 20-μL sample was injected onto a Rainin Dynamax ODS column (4.6 × 25 mm) held at 48°C. The column was connected to a Beckman binary gradient chromatography system, and injections were performed by a Spectraphysics SP8780 autosampler.

Separation of PITC-amino acids was effected using a binary gradient buffer system essentially as described by Yang and Sepulveda (23) with some modifications to the gradient program. Briefly, buffer A consisted of 30 mM sodium acetate,

6% (v/v) acetonitrile, and 0.05% triethylamine, adjusted to pH 6.4 with phosphoric acid; buffer B consisted of 60% acetonitrile in HPLC grade water. The most effective program for separation of PITC-amino acids on this HPLC system was as follows: first linear gradient, 0 to 20% B over 4 min; isocratic at 20% B for 9 min; second linear gradient, 20 to 50% B over 4 min; isocratic at 50% B for 12 min; third linear gradient, 50 to 95% B over 1 min; isocratic at 95% B for 8 min; fourth linear gradient, 95 to 0%B over 9 min; isocratic at 100% A for 10 min. This program separated all standard protein amino acids in about 28 min. Individual PITC-amino acids were identified and quantified by UV absorption at 254 nm by comparison to PITC-amino acid standards prepared from known quantities of protein and nonprotein amino acids.

Phloem Sap Analysis

Before ion exchange, phloem sap samples were centrifuged for 1 min in a microcentrifuge to pellet the precipitated p-protein. A 50-μL aliquot of the supernatant was removed and used for potassium determination by atomic absorption spectroscopy. Another 400-μL aliquot of supernatant was diluted with 1 mL of water and subjected to ion exchange chromatography as described above. All subsequent derivatization and HPLC procedures were performed as described above for leaf tissues for determination of phloem sap sugars and amino acids.

RESULTS

Photosynthesis and Assimilate Partitioning

Photosynthetic rates (CER) reached a maximum (approximately 5.0 mg CO_2 dm^{-2} h^{-1}) within the first hour of light and remained steady throughout the first half of the photoperiod (Fig. 1A). Toward the latter half of the photoperiod, photosynthetic rates declined (Fig. 1A) to approximately 1 mg CO_2 dm^{-2} h^{-1}. The reasons for this decline in photosynthesis are not clear but may have been a function of the on-off (square wave) light regimen experienced by the plants. Declines in photosynthesis, although more gradual than those seen here, in response to rapid on-off (square wave) light regimens have been reported previously for bean (2) and sugar beet (1, 2).

Leaf dry weight increased slightly toward the later stages of the light period and then slowly declined throughout the dark period (Fig. 1B). Leaf starch levels increased during the light phase from 25 mg to 75 mg dm^{-2} and then decreased back to 25 mg dm^{-2} during the dark period (Fig. 1C).

Leaf Soluble Carbohydrate Patterns

In general, leaf sugar levels (Fig. 2) showed similar diurnal patterns to that seen for starch (Fig. 1C). Levels of stachyose (Fig. 2A), raffinose (Fig. 2B), galactinol (Fig. 2C), sucrose (Fig. 2D), and hexoses (Fig. 2E) all showed a slow increase as the light period progressed, followed by a more rapid decline during the dark period (Fig. 2A). Leaf verbascose levels remained basically unchanged throughout the diurnal period (Fig. 2A). Galactinol, sucrose, and hexoses (glucose and fructose/myo-inositol) made up the bulk of the soluble carbohydrates in the leaf, particularly during the later hours of the light period (Fig. 2, C–E). Galactose was found only in trace quantities (<2 mM, if at all) and showed no distinct diurnal patterns (data not shown).

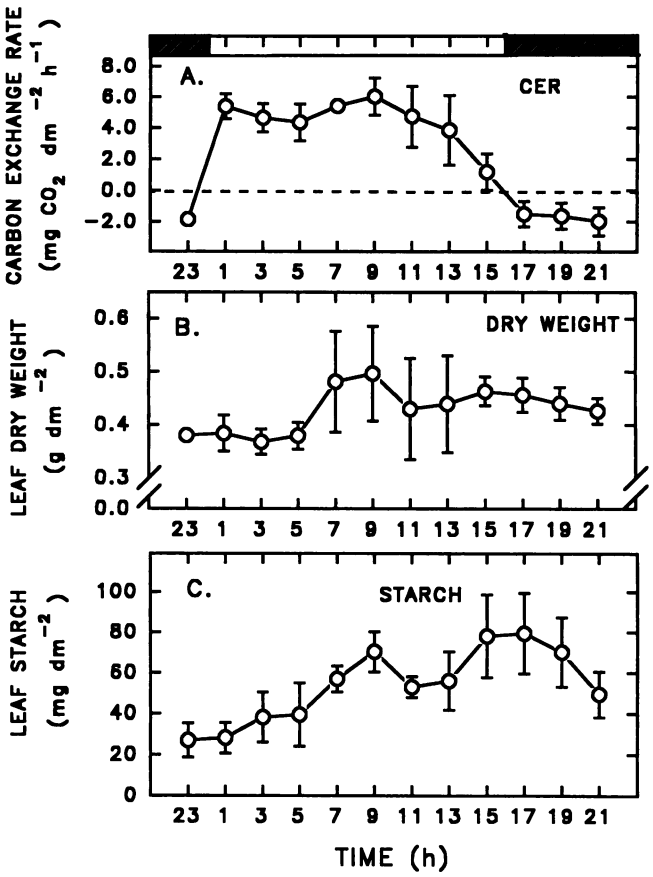

Figure 1. Diurnal CERs (A), dry weight changes (B), and leaf starch levels (C) in mature muskmelon leaves. Plants were maintained for 1 week before experimentation in a growth chamber under a diurnal regimen of 16 h light/8 h dark and 30°C day/20°C night temperatures. Maximum PPFD was 0.325 mmol photons m^{-2} s^{-1} (PAR) at pot level provided by fluorescent and incandescent lighting. Data represent the means of three samples per time interval (±SE).

Phloem Sap Sugar and Potassium Patterns

Diurnal fluctuations in concentrations of various sugars were also seen in phloem exudates obtained from cucurbit plants (Fig. 3). Levels of stachyose (Fig. 3A) and raffinose (Fig. 3B) increased rapidly during the early part of the light period, achieving maximal levels of about 50 and 10 mM, respectively, and then declined rapidly during the later stages of the light period. During the dark period, levels of these two sugars showed a lesser increase, to about 3 and 25 mM, respectively (Fig. 3A and B). Verbascose concentrations remained at about 1 mM throughout the diurnal cycle (Fig. 3A). Levels of sucrose increased from about 20 to 60 mM during the first 5 h of light and then decreased to 20 mM by the end

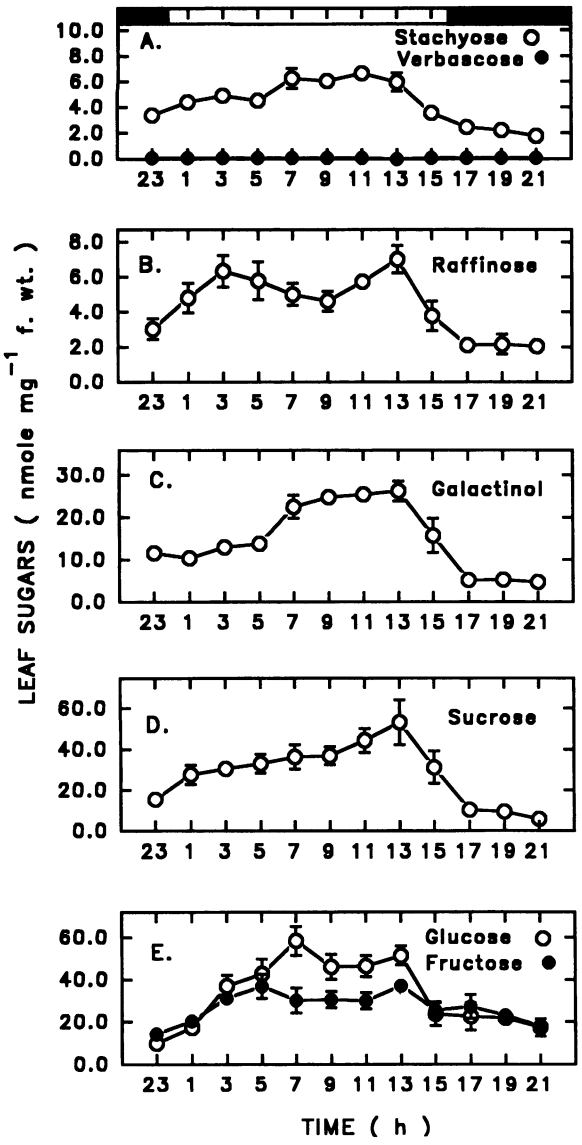

Figure 2. Diurnal changes in soluble sugars in mature muskmelon leaves. Data represent the means of three samples per time interval (±SE).

of the light period (Fig. 3C). During the first 3 h of dark, sucrose levels increased to 80 mM and then decreased to 20 mM by the end of the dark phase.

Levels of hexoses (glucose, galactose, and fructose/myo-inositol, Fig. 3D) were found only in trace quantities in phloem saps (≤2 mM) and showed no distinct diurnal patterns. Potassium levels remained unchanged at about 75 mM until the later stages of the light period, when a slow decline to 50 mM was observed (Fig. 3E). Phloem potassium levels returned to 75 mM by the end of the dark period (Fig. 3E).

Leaf Amino Acid Patterns

In general, amino acids constituted a much smaller metabolite pool than did sugars (Fig. 4A), and the pool size of total amino acids tended to be more stable (Fig. 4A). However, the contribution of specific amino acids to the total pool varied significantly during the diurnal cycle (Fig. 4). All major protein and some nonprotein amino acids were detected in muskmelon leaves (data not shown), but the principal amino acids were citrulline, glutamate, aspartate, glutamine, glycine, proline, alanine, arginine, and threonine (Fig. 4). In the early part of the photoperiod, citrulline was a principal component of the leaf amino acid pool (Fig. 4D). Toward the later stages of the light period, citrulline levels declined, and aspartate (Fig. 4B) and glutamate (Fig. 4C) became increasingly more important. In the dark, citrulline, aspartate, and glutamate were the principal contributors to the amino acid pools (Fig. 4, B–D). Levels of glycine (Fig. 4B), glutamine (Fig. 2C), proline (Fig. 2D), and arginine (Fig. 2E) showed no significant changes in response to light or dark periods. Levels of alanine and threonine (Fig. 4E) were higher in the light than in the dark.

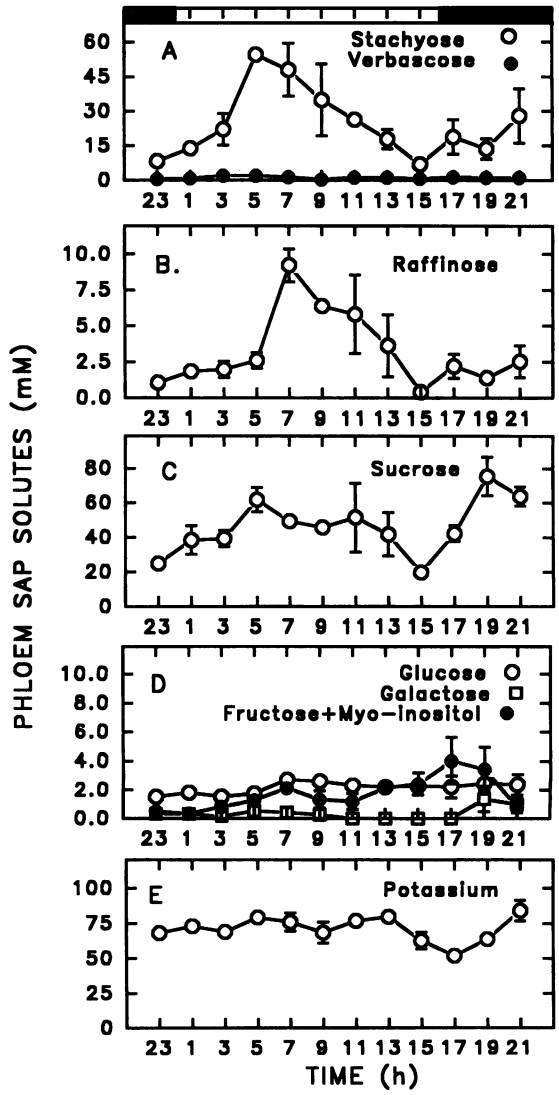

Figure 3. Diurnal changes in sugars and potassium in phloem sap from muskmelon plants. Data represent the means of three samples per time interval (±SE).

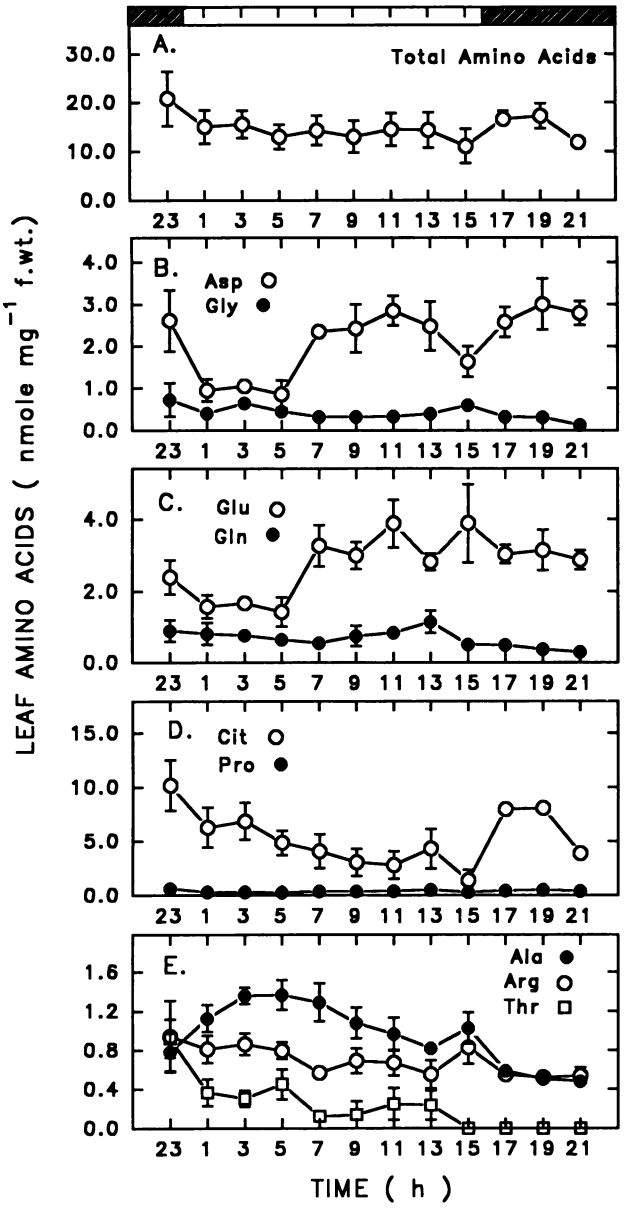

Figure 4. Diurnal changes in soluble amino acids in mature muskmelon leaves. Data represent the means of three samples per time interval (±SE).

Phloem Sap Amino Acid Patterns

In the phloem, the same amino acids that predominated in the leaf were also those that predominated in the phloem sap (Fig. 5). However, significantly higher levels of total amino acids were found in the phloem in the light (approximately 75 mM) than in the dark (approximately 25 mM) (Fig. 5A). The major amino acids in the phloem were glutamate, glutamine, and citrulline (Fig. 5, C and D). Diurnal fluctuations in individual amino acid levels in the phloem (Fig. 5) showed no distinct correlation with patterns seen in the leaf (Fig. 4).

DISCUSSION

As reviewed by Pate (12), the stem incision method for collection of phloem sap for analysis can be problematic because it allows the possibility of contamination and dilution of phloem sap samples by solutes and water from cut cell surfaces and xylem elements. That this can occur was demonstrated in a previous study (15) by the presence of low amounts of sugars in predominantly monosaccharide form in cucurbit phloem sap. The levels of monosaccharides were found to increase as the site of sampling neared young, easily damaged sink tissue and was, therefore, considered an artifact of the sampling method (15). For this reason, care was taken in the present study to sample mature stem and petiole tissues. The phloem sap samples collected had a pH of 7.5 to 8.0 as measured using narrow range pH paper (data not

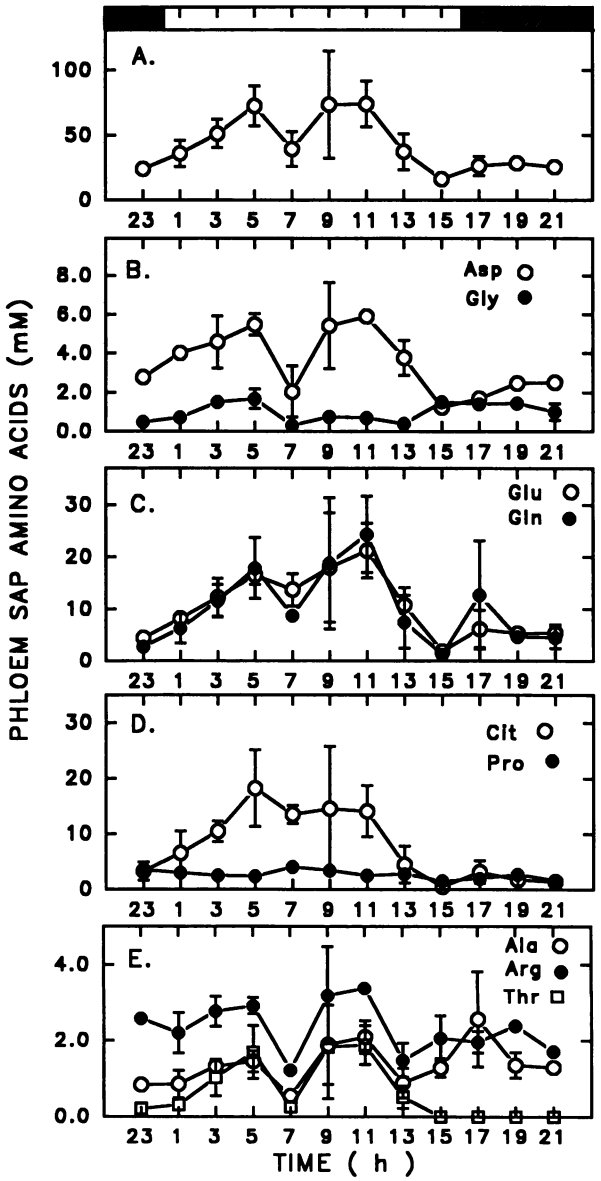

Figure 5. Diurnal changes in amino acids in phloem sap from muskmelon plants. Data represent the means of three samples per time interval (±SE).

shown) and had high potassium levels (Fig. 3E), suggesting a phloem origin for the samples. They also contained only low levels of monosaccharides (Fig. 3D), suggesting very little contamination from surrounding cut cells. In addition, the patterns seen for phloem sap stachyose and raffinose levels, with their characteristic midday peak (Fig. 3A; 3B), were very similar to those reported previously for diurnal sucrose levels in *Nicotiana* phloem exudates (7). These findings, coupled with the good reproducibility of the samplings from plant to plant, indicate that the diurnal increases and decreases observed in muskmelon phloem sap solutes in the present study were not likely to be simple dilution artifacts. Nonetheless, because the actual degree to which dilution by xylem water may have occurred during sample collection is not known, the values reported here are probably underestimates.

The data from this study indicate that phloem sap sugar and amino acid levels appear to depend on the metabolic status of the source leaf and that considerable variation in export patterns can be expected during a diurnal cycle. The highest phloem sap solute levels were seen in the light, which is in agreement with recent histochemical (14) and plasmolytic (21) studies of cucurbit leaf phloem. The highest sugar levels were seen close to midday, a time when photosynthesis was maximal but starch accumulation in the chloroplast and hexose accumulation in the vacuole were relatively slow. However, soon after this midday peak, as starch synthesis and hexose began to accumulate in the leaf, there was a corresponding decrease in sugar levels in the phloem even though photosynthesis continued unchanged. It appeared, therefore, that the amounts of sugar available for phloem loading could be modulated by diversion of fixed carbon to starch and vacuolar storage pools within the leaf.

The data indicate that, in the muskmelon leaf, appreciable accumulation of carbohydrate (starch, Fig. 1C; galactinol, Fig. 2C; sucrose, Fig. 2D; hexoses, Fig. 2E) occurred, particularly toward the latter half of the light period. Because this experiment was performed under growth chamber conditions that provided only low light levels, these sugar accumulations may not represent levels that are actually achievable in these tissues under field conditions. It was notable, however, that the pool size of stachyose was always significantly (nearly 10-fold) smaller than pools of galactinol, sucrose, or hexoses (Fig. 2) and showed very little accumulation in the leaf compared to these other sugars. This strongly suggests that a differential compartmentation of stachyose occurs within the muskmelon leaf compared with that which occurs for sucrose or hexoses. This finding may provide indirect support for current models for stachyose synthesis (17, 19, 20) which suggest that stachyose synthesis occurs in the intermediary cells of the phloem. If stachyose was being made within the phloem, then accumulation of stachyose would not have been expected to occur in the present study because stachyose would have no access to the mesophyll vacuolar compartment. The leaf levels of stachyose would, therefore, be limited by the capacity of the phloem system itself. Sucrose and hexoses, on the other hand, which are thought to be produced in the mesophyll, would have access to the vacuolar storage compartment and, therefore, would be expected to accumulate as observed in the leaf. As a result, the data presented here support the idea that the stachyose "pools" seen in the leaf tissues actually represent stachyose that was present in the leaf phloem.

In summary, the research presented here demonstrates that the composition of cucurbit phloem sap (sugars, amino acids, potassium) has a great deal of plasticity and reflects to a large extent the metabolic and compartmentation activities of the source leaf. Although the data presented here cannot allow exact determination of the biochemical mechanisms controlling carbon partitioning within the cucurbit leaf, useful inferences can be drawn from the patterns observed concerning the fluxes of carbon between major compartments within the leaf, namely, the chloroplast, the vacuole, and the phloem. The data presented here, therefore, provide an important foundation for future biochemical investigations of assimilate partitioning in the Cucurbitaceae.

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LITERATURE CITED

1. **Fondy BR, Geiger DR** (1985) Diurnal changes in allocation of newly fixed carbon in exporting sugar beet leaves. Plant Physiol **78:** 753–757
2. **Fondy BR, Geiger DR, Servaites JC** (1989) Photosynthesis, carbohydrate metabolism, and export in *Beta vulgaris* L. and *Phaseolus vulgaris* L. during square and sinusoidal light regimes. Plant Physiol **89:** 396–402
3. **Geiger DR, Fondy BR** (1991) Regulation of carbon allocation and partitioning: status and research agenda. *In* JL Bonnemain, S Delrot, J Dainty, WJ Lucas, eds, Recent Advances in Phloem Transport and Assimilate Compartmentation. Ouest Editions, Nantes, France, pp 1–9
4. **Hall SM, Baker DA** (1972) The chemical composition of *Ricinus* phloem exudate. Planta **106:** 131–140
5. **Handley LW, Pharr DM, McFeeters RF** (1983) Relationship between galactinol synthase activity and sugar composition of leaves and seeds of several crop species. J Am Soc Hortic Sci **108:** 600–605
6. **Hendrix DL, Grange RI** (1991) Carbon partitioning and export from mature cotton leaves. Plant Physiol **95:** 228–233
7. **Hocking PJ** (1991) The composition of phloem exudate and xylem sap from tree tobacco (*Nicotiana glauca* Grah.). Ann Bot **45:** 633–643
8. **Holthaus U, Schmitz K** (1991) Distribution of stachyose synthase in *Cucumis melo* L. Planta **185:** 479–486
9. **Madore MA** (1990) Carbohydrate metabolism in photosynthetic and nonphotosynthetic tissues of variegated leaves of *Coleus blumei* Benth. Plant Physiol **93:**617–622
10. **Madore MA, Grodzinski B** (1984) Effect of oxygen concentration on ^{14}C-photoassimilate transport from leaves of *Salvia splendens* L. Plant Physiol **76:** 782–786
11. **Madore MA, Grodzinski B** (1985) Effects of CO_2 enrichment on growth and photoassimilate transport in a dwarf cucumber (*Cucumis sativus* L.) cultivar. J Plant Physiol **121:** 59–71
12. **Pate JS** (1976) Nutrients and metabolites of fluids recovered from xylem and phloem: significance in relation to long-distance transport in plants. *In* JF Wardlaw, JB Passioura, eds, Transport and Transfer Processes in Plants. Academic Press, New York, pp 253–281
13. **Pharr DM, Sox HN** (1984) Changes in carbohydrate and enzyme levels during the sink to source transition of leaves of *Cucumis sativus* L., a stachyose translocator. Plant Sci Lett **35:** 187–193
14. **Pristupa NA** (1983) Distribution of ketosugars among cells of

collecting bundles of the *Cucurbita pepo* leaf. Sov Plant Physiol **30:** 372–378

15. **Richardson PT, Baker DA, Ho LC** (1982) The chemical composition of cucurbit vascular exudates. J Exp Bot **33:** 1239–1247
16. **Robbins NS, Pharr DM** (1987) Regulation of photosynthetic carbon metabolism in cucumber by light intensity and photosynthetic period. Plant Physiol **85:** 592–597
17. **Schmitz K, Holthaus U** (1986) Are sucrosyl-oligosaccharides synthesized in mesophyll protoplasts of mature leaves of *Cucumis melo*? Planta **169:** 529–535
18. **Stitt M, Quick WP** (1989) Photosynthetic carbon partitioning: its regulation and possibilities for manipulation. Physiol Plant **77:** 633–641
19. **Turgeon R** (1991) Symplastic phloem loading and the sink-source transition in leaves: a model. *In* JL Bonnemain, S Delrot, J Dainty, WJ Lucas, eds, Recent Advances in Phloem Transport and Assimilate Compartmentation. Ouest Editions, Nantes, France, pp 18–22
20. **Turgeon R, Beebe DU** (1991) The evidence for symplastic phloem loading. Plant Physiol **96:** 349–354
21. **Turgeon R, Hepler PK** (1989) Symplastic continuity between mesophyll and companion cells in minor veins of mature *Cucurbita pepo* leaves. Planta **179:** 24–31
22. **Wardlaw IF** (1990) The control of carbon partitioning in plants. New Phytol **116:** 341–381
23. **Yang CY, Sepulveda FI** (1985) Separation of phenylthiocarbamyl amino acids by high-preformance liquid chromatography on Spherisorb octadecylsilane columns. J Chromatogr **346:** 413–416