

Transport of Organic Solutes in Phloem and Xylem of a Nodulated Legume¹

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ABSTRACT

Collections of xylem exudate of root stumps or detached nodules, and of phloem bleeding sap from stems, petioles, and fruits were made from variously aged plants of *Lupinus albus* L. relying on nodules for their N supply. Sucrose was the major organic solute of phloem, asparagine, glutamine, serine, aspartic acid, valine, lysine, isoleucine, and leucine, the principal N solutes of both xylem and phloem. Xylem sap exhibited higher relative proportions of asparagine, glutamine and aspartic acid than phloem sap, but lower proportions of other amino acids. Phloem sap of petioles was less concentrated in asparagine and glutamine but richer in sucrose than was phloem sap of stem and fruit, suggesting that sucrose was unloaded from phloem and amides added to phloem as translocate passed through stems to sinks of the plant. Evidence was obtained of loading of histidine, lysine, threonine, serine, leucine and valine onto phloem of stems but the amounts involved were small compared with amides. Analyses of petiole phloem sap from different age groups of leaves indicated ontogenetic changes and effects of position on a shoot on relative rates of export of sucrose and N solutes. Diurnal fluctuations were demonstrated in relative rates of loading of sucrose and N solutes onto phloem of leaves. Daily variations in the ability of stem tissue to load N onto phloem streams were of lesser amplitude than, or out of phase with fluctuations in translocation of N from leaves. Data were related to recent information on C and N transport in the species.

Transport of organic solutes in the nodulated legume depends on four major physiological activities: the export of fixed N from nodules via the xylem (1, 6); the export of sugar and other photosynthetic products from leaves in phloem (10, 11); the cycling of transpirationally attracted N through leaves via phloem (2, 3); and the transfer in stems of N solutes originally obtained from xylem to upward and downward moving streams of phloem translocate (9, 12). Interrelationships of these components during growth of the legume *Lupinus albus* L. have recently been expressed quantitatively using an empirical modeling technique for deriving net fluxes of C and N between plant parts in xylem and phloem (9). This paper describes the solutes present in various segments of the plant's transport system and shows how changes in proportions and amounts of individual solutes relate to the partitioning of C and N during growth and daily functioning of the plant. The study complements recent autoradiographic investigations (3) on the fate of xylem-fed ¹⁴C-labeled amino compounds in *L. albus*.

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MATERIALS AND METHODS

Plant Material. Effectively nodulated white lupin (*L. albus* L. cv. Ultra) plants were grown in nitrogen-free sand culture in a naturally lit glasshouse during July to November in Perth, Western Australia.

Collection of Xylem Sap. Bleeding sap (xylem exudate) was obtained from root stumps of 12 to 16 decapitated plants at intervals over the period 46 to 100 days after sowing, and from large (>20 mg fresh weight) detached nodules over the period 60 to 100 days. Collection techniques were as described previously (4-6). The first drop of exudate to form was discarded and exudate then collected for 15 to 20 min. Samples were deep frozen immediately after collection.

Collection of Phloem Sap. Phloem exudates were collected from shallow incisions in stems, petioles, and fruit stalks at the locations indicated in Figures 1 and 2. As indicated in a recent publication (9), phloem sap from petioles was used to identify the mixture of photosynthetic products and of cycled N products translocated from leaves. Phloem sap from base and top of stems was used to indicate the composition of the downward and upward streams of translocate after passage of leaf-derived translocate through stems.

Collection techniques were as described earlier (10). A series of collections from 46 to 100 days after sowing were made during daytime (11.00-16.00 h), the population of plants sampled being identical to that used in construction of a model for C and N flow in *L. albus* (9). A detailed study was also made of petiole phloem sap composition for four age groups of leaves and the data related to changes in N content of the leaves. A study of diurnal changes in phloem sap composition involved sampling phloem sap from stem base, stem top, and petioles from 56-day-old plants at 6-h intervals over a period of 48 h. All studies involved replicate samples of 12 to 16 uniform plants, and phloem sap collections were restricted to a 10-min period after incision. Sampling errors for phloem sap analyses and N determinations of plant parts were as detailed in an earlier publication (9).

Analyses of Sap Samples. Levels of sucrose (95% of the soluble carbohydrate of phloem), K⁺ (the major cation), and individual amides and amino acids in the sap samples were determined as detailed elsewhere (10, 11). Fuller analyses of phloem and xylem sap, including determinations for organic acids and mineral elements, were recently published for *L. albus* (7, 8).

RESULTS

Changes in Composition of Phloem Sap with Plant Age (Fig. 1). Data for levels of sucrose, K⁺, total amino acids, and amides (Asn + Gln) were as shown in Figure 1. Sucrose levels in phloem sap of petioles and stem base were consistently higher than in phloem sap of stem tops, inflorescence stalks, and fruits, whereas the reverse was true for concentrations of amide in phloem. These differences held for all times of sampling and for both primary and secondary axes of the shoot, and were interpreted as showing

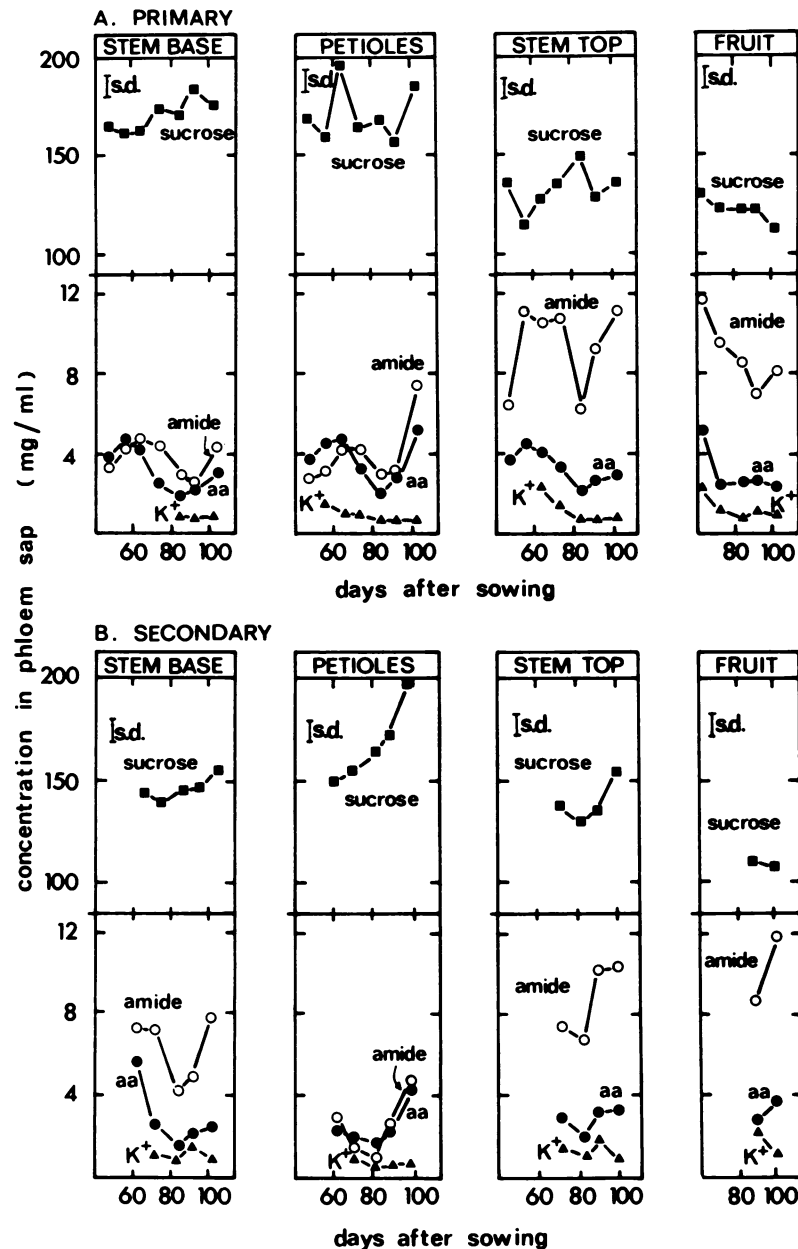


FIG. 1. Changes with plant age in concentrations of major solutes in phloem sap collected from stems, petioles, and fruits of primary (A) and secondary (lateral) (B) shoots of nodulated plants of *L. albus* depending solely on nodules for their N supply. Collection sites for phloem sap are depicted in Figure 2. Amide: Asn + Gln; aa: range of amino acids as listed in Figure 2. Standard deviations are shown for the data.

that sucrose was removed from and amides added to phloem as the translocate from leaves moved upward through the stem toward fruits or vegetative apices. These solute exchanges were reflected in substantial differences in the weight ratios of the major classes of organic solutes in phloem at different sampling locations. Thus, for phloem sap of petioles and stem base the weight ratios for sucrose to amino acids + amides were high (18–59) in comparison with values for stem tops and fruits (7–15), whereas the amide to amino acid weight ratio of phloem sap was higher for stem tops (1.9–3.8) and fruits (2.2–3.8) than for petioles (0.5–1.5) and stem base (0.9–2.0).

Concentrations of amino acids did not differ substantially between sampling points at most stages of plant growth but petiole phloem sap showed elevated levels of amino acids at 100 days, when most leaves were senescent and losing N. Fluctuations of K^+ in phloem during plant growth were not well correlated with

variations in levels of amino acids, amides, or sucrose in phloem (Fig. 1).

Relative Composition of Amino Fraction of Phloem and Xylem Sap (Fig. 2). Changes with age in sap composition at the different locations on the plant were expressed on a molar basis (Fig. 2). Proportions of the sap amino fraction as amide (Asn + Gln) were 83 to 90% for xylem sap from nodulated roots and detached nodules, 60 to 77% for phloem sap of fruits and top of stems, 43 to 70% for phloem sap of stem base, and 33 to 54% for phloem sap of petioles. The molar ratio (Asn to Gln) in phloem was relatively constant (2 to 3:1) despite considerable variation in total amide levels and in amide content relative to other solutes (Figs. 1 and 2). Thus, Asn and Gln were involved to an equal extent in enriching the upward moving phloem stream in stems with N. Xylem sap was relatively more rich in Asp than phloem sap, less rich in Val, Ser, Phe, Ile, and Leu. Amino acid composition of

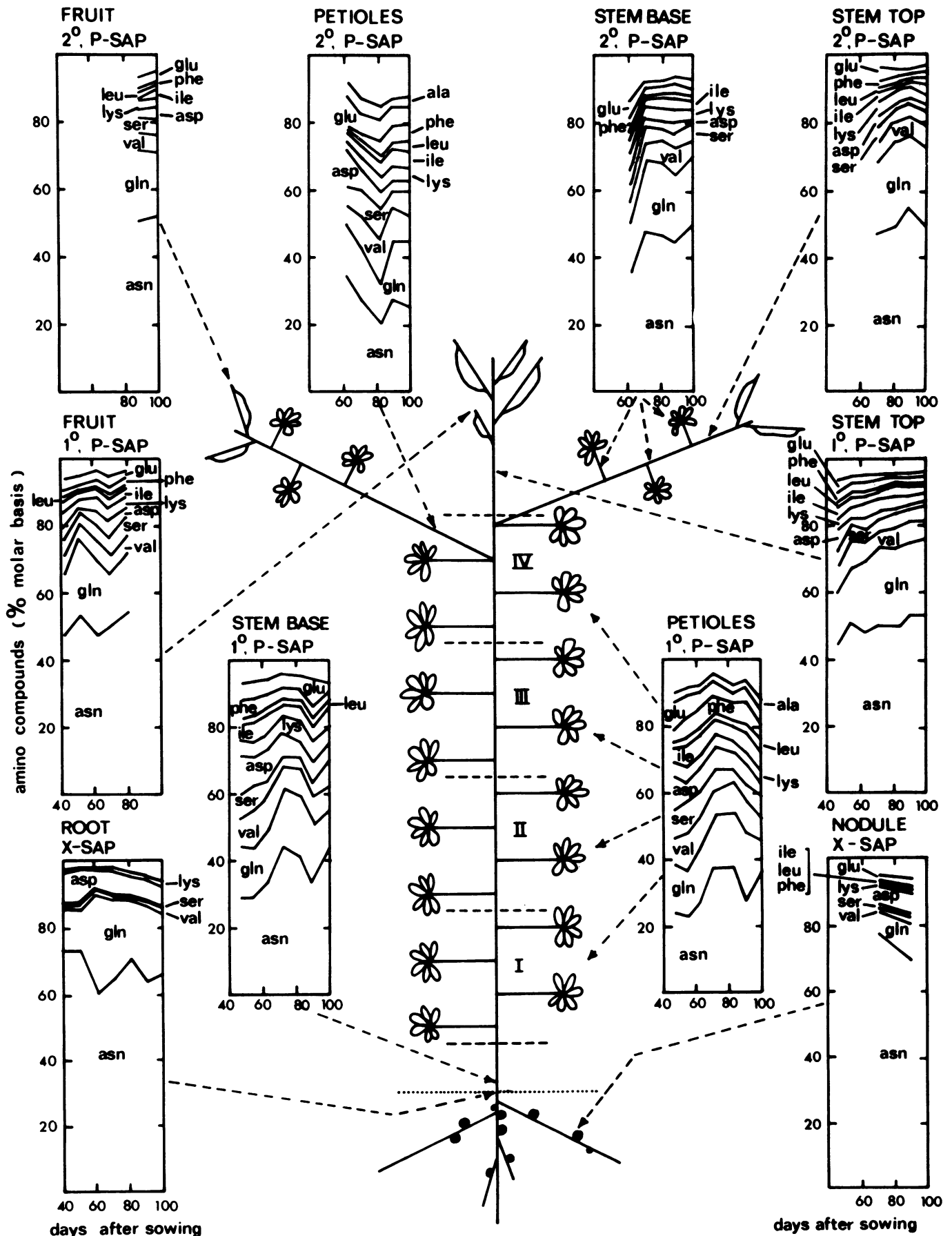


FIG. 2. Composition of the amino fraction of xylem (X-) and phloem (P-) sap of nodulated *L. albus* plants relying on symbiotic N fixation for their N supply. Changes in composition at each sampling site during growth of the plant were expressed on a % molar basis. Petiole phloem sap composition refers to the pooled sample from all leaves on the primary or secondary axes of the shoot. (See Fig. 1. for concentrations of major solutes of the phloem sap samples.)

phloem sap was very constant with age and sampling point.

Changes in Petiole Phloem Sap Composition with Age of Leaves (Fig. 3). Four groups of leaves on the primary stem were sampled for N content over the period 40 to 120 days after sowing, and their petioles cut for phloem sap collection over the period 46 to 100 days. The oldest group of leaves (nodes 1 to 4, marked I in Figs. 2 and 3) lost N throughout the study period; leaf group II behaved similarly, whereas groups III and IV exhibited phases of increase, maintenance, and fall in N content (Fig. 3A).

The phloem sap analyses showed no consistent variations with age and between age groups of leaves in sucrose level or in relative composition of the amino fraction of the sap samples, but significant differences were evident in the weight ratio of sucrose to

amino acids + amides (Fig. 3B). Values for the ratio were high whereas leaves were still increasing in N content, fell slightly during the period of leaf development when N content was at or near maximum, rose to a second higher peak value at 80 days when all leaves were still green and photosynthetically active but had commenced to decline in N content, and then fell rapidly as the leaves senesced and lost the bulk of their N. At each time of sampling, values for the ratio were higher for upper than for lower leaves, suggesting differences with position on a stem in export of sucrose relative to translocation and cycling of N. These differences were significant ($P < 0.05$) between leaf groups I and IV for all but the last sampling times.

Diurnal Fluctuations in Composition of Phloem Sap (Figs. 4 and 5). Changes in composition of phloem sap from petioles of mature leaves and from base and top of primary stem were followed in 56-day-old plants over a 48-h period (Fig. 4). As indicated from the life cycle study (Fig. 2) sucrose levels were higher in petiole phloem sap than in stem top phloem sap, and amide (Asn + Gln) levels consistently higher in stem phloem sap than in sap of petioles. This suggested that removal of sucrose from the upward moving phloem stream and loading of amide onto the upward and downward moving phloem streams occurred in stem tissue throughout day and night. Levels of amino compounds in phloem tended to rise during the day and fall at night and diurnal fluctuations in sucrose levels in petiole phloem sap were also evident (13).

Percentage composition of the amino fraction of phloem sap varied only slightly at each sampling location over the 48-h period, but a rise in levels of Asn relative to other amino acids at night was evident in petiole phloem sap, but not in stem phloem sap.

Substantial variations in the weight ratio of sucrose to amino compounds were recorded for petiole phloem sap (Fig. 5A), suggesting diurnal changes in the availability of sucrose relative to N solutes at sites of phloem loading in the leaf. At night, when transpirational delivery of newly fixed N to the leaf via xylem was likely to be much reduced, a sap rich in sucrose relative to amino compounds was produced, indicating readier access by the loading process to the carbohydrate pools of the leaf than to its pools of N. During the day, the situation changed to one of greater availability of N compounds and a corresponding decrease in the ratio of sucrose to amino compounds in the sap. At this time much of the N translocated from the leaf would have consisted of amino compounds recently acquired through transpirational activity (see ^{14}C -labeling studies described in refs. 3 and 12).

Diurnal fluctuations in the weight ratio of sucrose to amino compounds were less marked in stem phloem sap samples than in phloem sap of petioles, indicating that daily variations in the ability of stem tissue to donate N compounds to phloem were of lesser amplitude than, or were out of phase with, the translocation of N from leaves. Since amides dominated the spectrum of compounds exported to leaves from nodules via the xylem (Fig. 1), the expression [(Amide level in stem phloem sap - Amide level in petiole phloem sap) + Amide level in petiole phloem sap] was used to evaluate diurnal fluctuations in the relative rates at which leaves and stem tissue made recently fixed N available to the translocation streams serving root and apical regions of the shoot. The data (Fig. 5B) indicated that upper regions of the stem at night loaded almost three times (maximum value for the ratio 2.9) as much amide onto phloem as left the leaves in phloem translocate, whereas by day translocation of amide from leaves approximately equaled that loaded by the upper stem. A similar diurnal trend was observed for stem loading of amide onto the downward moving stream of translocate, but this activity at all times involved less amide than was currently being cycled through leaves.

Indications of the magnitudes and intensities of the net exchanges of individual amino compounds between stem tissues and the upward and downward translocation streams were obtained by comparing average concentration values for amino compounds

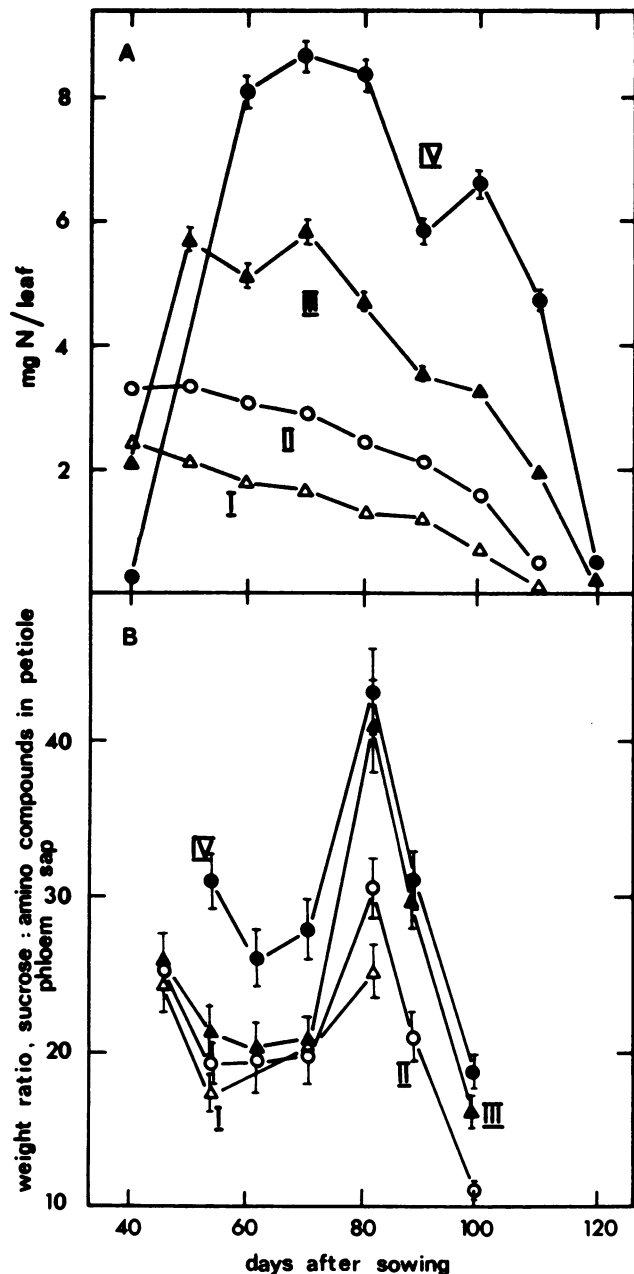


FIG. 3. Changes with age in N content (A) and the sugar to amino compounds weight ratio of petiole phloem sap (B) in four age groups of leaves from the primary shoot axis of nodulated *L. albus*. Standard deviations are shown for the data. Age group of leaves: I, leaves 1 to 4; II, leaves 5 to 8; III, leaves 9 to 12; IV, leaves 13 to 16 (age groups numbered from base of shoot, as shown in Fig. 2).

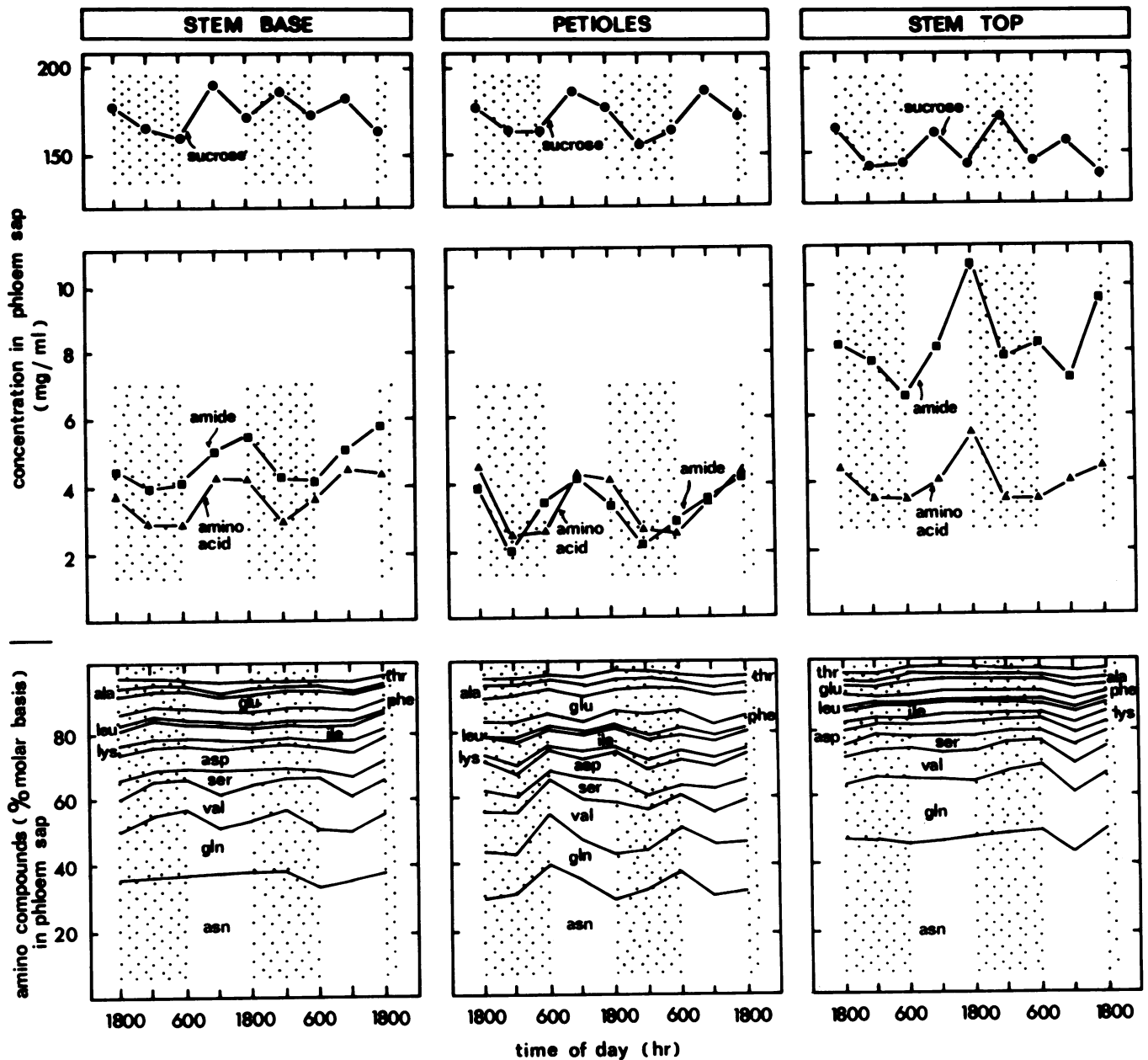


FIG. 4. Diurnal changes in concentrations of major solute fractions and in molar composition of the amino fraction of phloem sap collected from leaf petioles, stem base, and stem tops of the primary shoots of 56- to 58-day-nodulated, glasshouse-grown plants of *L. albus*. The 2 days of the experiment had 12.5 h of uninterrupted sunshine, the daytime maximum temperature was 27 C, minimum night temperature 8 C. Amide = Asn + Gln.

for the 48-h period in petiole phloem sap with comparable values for stem base or stem top phloem sap. Expressed as the ratio of stem phloem sap concentration to petiole phloem sap concentration (Fig. 5C), the data suggested effective loading (values significantly greater [$P > 0.05$] than 1) of His, Asn, Gln, Lys, Thr, Ser, Leu, and Val, the possibility of unloading (values significantly less than 1) of Glu and Gly, and little or no net flux in respect of the other amino compounds present in the translocate from leaves (Ala, Ile, Phe, Asp, Abu). Expressed in terms of absolute changes in concentration (Fig. 5D), the dominant part played by amides in stem loading of phloem became evident, with the two major xylem solutes, Asn and Gln, accounting for over 80% of the amino compounds loaded onto phloem by the stem. The third most important component of xylem, Asp (Fig. 2), did not appear to be transferred to phloem from stem tissue, a finding consistent with

recent autoradiographic evidence (3) showing little abstraction of this solute from the ascending transpiration stream by the stem. Indeed, in view of earlier labeling studies much of the Asp in phloem was likely to have originated from metabolism within the leaf, since the xylem supply of this compound from the root was shown not to be freely available for phloem loading in the leaf (3, 12).

DISCUSSION

The analyses of transport fluids presented here provided information on several key transport activities in *L. albus* L. The general finding of lower proportions of amides relative to amino acids in petiole phloem sap than in xylem sap substantiated our earlier conclusion that the N stream translocated from leaves

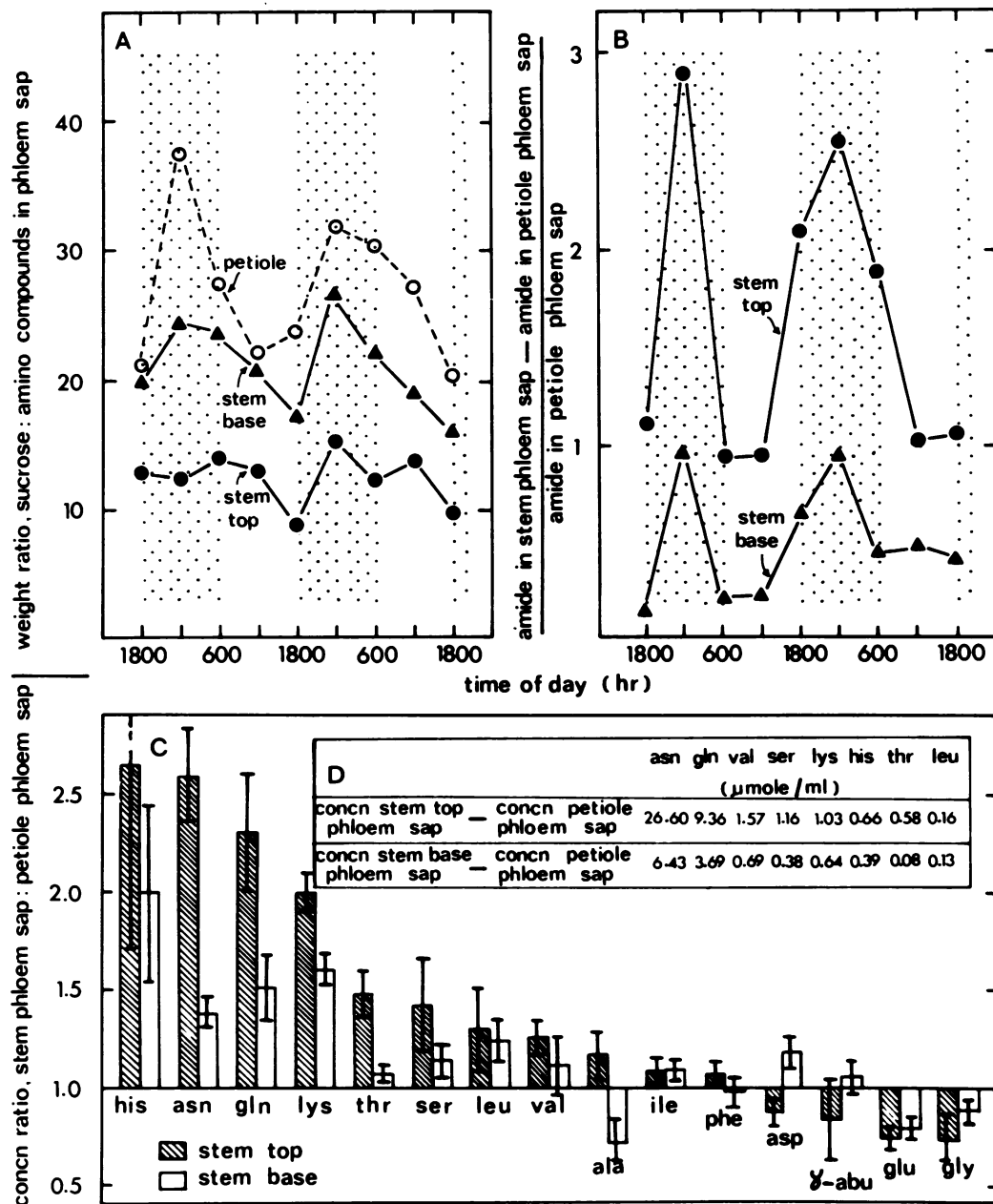


FIG. 5. Diurnal fluctuations in sucrose to amino compounds weight ratio of stem and petiole phloem sap (A) and in the relative capacities of leaf and stem tissue to load amides onto phloem (B) in 56- to 58-day plants of *L. albus*. Estimates are made of the relative effectiveness of leaf and stem to load specific compounds (C) and of the relative amounts of each compound loaded by the stem (D), using data for average phloem sap composition of petioles, stem top, and stem base for the 48 h of the study. See text and Figure 4 for further details of experiment. Standard errors are shown for the data of C.

consisted of amino acids synthesized in or mobilized from the leaf as well as N compounds cycling through the leaf in association with the xylem to phloem exchange of the vein retrieval system of the leaf (3, 4). Lower sucrose levels and higher amide levels in stem phloem exudates than in corresponding phloem sap of petioles suggested that sucrose was unloaded and amide loaded during the passage of the phloem stream through the stem. In the study of diurnal functioning of this system the upward stream of translocate was shown to be enriched to the extent of $41 \pm 8 \mu\text{mol}$ (SD) amino compounds per ml, the lower stream only to the extent of $12.4 \pm 4 \mu\text{mol}$ (SD) amino compounds per ml, enrichment values closely similar to the predictions made recently (9) for the C and N fluxes in a similarly aged plant of *L. albus*. On average,

1.5 mol sucrose was unloaded by the stem from the upward stream of translocate for each mol of amide loaded, implying that there would be a relatively small change in the osmotic value of the phloem contents during lateral exchange of these solutes with the stem, especially if counterions were to accompany the flux of amino compounds into the sieve elements. Inasmuch as earlier labeling studies (3, 13) demonstrated rapid and effective transfer of N from xylem to the phloem translocate of leaves, the relationships of the phloem-loading process for N in stem tissue to the pools of N stored in stem and to the abstraction of xylem-borne N by stems were not evaluated. Nevertheless, the recently constructed model for C and N flow in *L. albus* did indicate net transport of N between xylem and phloem even though the course

of this transfer appeared to be protracted and to involve long term exchanges with the reserve pools of N in stem segments (D. McNeil, J. S. Pate, and C. A. Atkins, unpublished).

It was concluded in an earlier study (9) that the principal effect of stem loading of N solutes onto phloem was to provide fruits and other growing parts with a phloem supply of N additional to that available from photosynthesizing leaves, and thus supply these sink regions with translocate of a C/N ratio close to their requirements for growth. Since upward streams of translocate were shown to be enriched with N relatively more than downward streams, the relevance of the stem-loading process to the high N demand of shoots compared with roots became apparent (9). The present study demonstrated that this principle applied throughout growth to both primary stem and secondary lateral shoots, and that the amides Asn and Gln were at all times the major compounds to be loaded onto phloem by the stem. Sink regions thus received translocate much richer in N than they would have done were leaves to have been their sole source of C and N.

Phloem sap exhibited remarkable constancy in composition of its amino fraction at each sampling site of the system, despite considerable variations in solute concentrations, particularly in the ratio of sugar to amino compounds. The amino acid spectrum of petiole phloem sap changed only slightly during daily functioning of the leaf, despite wide variations in accessibility of the loading system to N from the transpiration stream. During aging of the leaf the balance of exported amino acids and amides remained relatively constant as the relevant populations of leaves went through successive phases of accumulation, maintenance, and loss of N. It remains to be seen whether amino balance of phloem and xylem can be altered appreciably by physiological

manipulation of the plant, and, if so, how the transport systems adapt to gross changes in availability of specific solutes.

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