Carbon, Nitrogen and Energy Source Requirements during Shooting in Mulberry Plants

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One characteristic of the mulberry plant is its ready ability to regenerate new shoots from a stem or stump. The stem plays an important role in the regeneration of this perennial plant by supplying substances and energy needed for new growth. At the time of budding or rooting, the amounts of reserve carbohydrates in hardwood cuttings of the mulberry have been shown to decrease rapidly as new organs develop.[3] Decreases in the concentration of free amino acids such as proline, alanine, γ-aminobutyric acid and arginine have also been recognized in the parent stems after cutting.[11] Thus, the stem supplies carbon and nitrogen required for growth during bud opening. Depletion of ATP levels due to its use in synthetic reactions is also conceivable. Concomitantly, such depletion in ATP levels immediately affects amino acid and carbohydrate metabolism.

Thus, the development of new shoots and leaf yield in the mulberry are greatly affected by the amount of food material accumulated in the harwood stems or stumps of the mulberry during the autumn of the previous year. Regulation of the distribution of photosynthetic products during the growing season is considered to be closely related to improvement of leaf yield in the following year.

Adenine nucleotides, carbohydrates and ribulose bisphosphate carboxylase activity during shooting[17]

Budding and rooting is an energy-requiring process and thus it is supposed that a large amount of ATP is required and supplied. When leaves unfold and the activity of ribulose bisphosphate carboxylase (RuBPCase), which is responsible for photosynthetic carbon dioxide fixation, increases, photosynthate will be supplied to new growing organs to serve as a carbon and

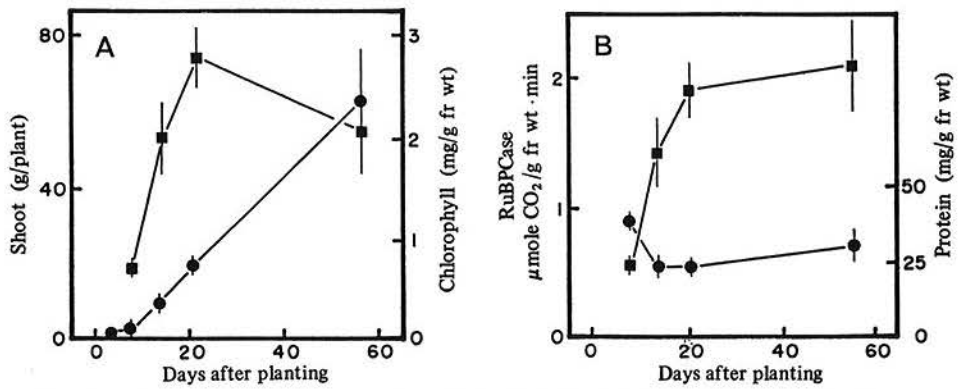

Fig. 1. (A) Growth of shoots (●) and changes in chlorophyll content (■) in the leaves
(B) Changes in the activity of RuBPCase (■) and soluble protein (●) in the leaves

energy source there. Results obtained from mulberry saplings following budding are given in Figs. 1 to 3 (means for six samples and bars indicate s.d.).

Budding appeared the day after planting at a temperature of 25°C. The first leaf opened on the 7th day, after which the young shoot grew steadily and the chlorophyll content increased rapidly (Fig. 1A). RuBPCase activity began when the first leaf opened and reached near

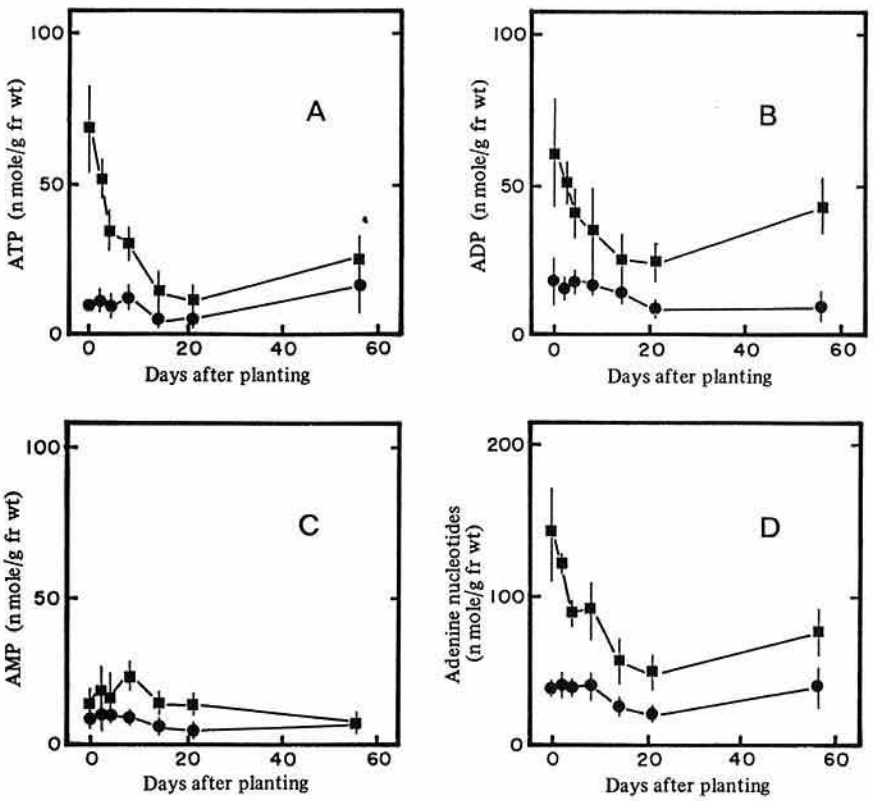

Fig. 2. Changes in the contents of ATP(A), ADP(B), AMP(C) and total adenine nucleotides(D) in the bark (■) and wood (●) of the hardwood stem

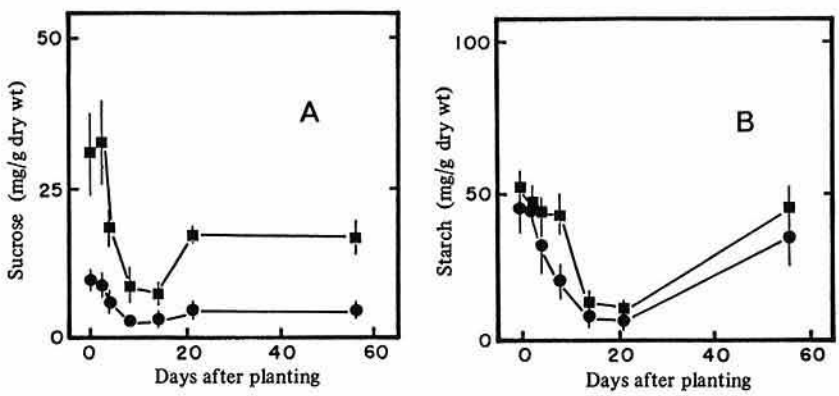

Fig. 3. (A) Changes in the sucrose content in the bark (■) and wood (●) of the hardwood stem
(B) Changes in the starch content in the bark (■) and wood (●) of the hardwood stem

maximum two weeks later (Fig. 1B).

Both the ATP and ADP concentration in the bark decreased sharply after planting and continued to decline until RuBPCase had nearly reached its maximam activity in the leaves (Fig. 2). Similar, but not so rapid, patterns of decrease in ATP and ADP content took place in the wood, but the beginning of these decreases lagged behind the decreases in the bark. The decrease in the total of adenine nucleotides could not be explained by energy consumption alone. Assuming that ATP is consumed as energy, no change should take place in the total amount of adenine nucleotides because of the formation of AMP. Thus, the stored ATP may be consumed as a substrate in the synthesis of RNA.

Sucrose and starch are the major metabolizable carbohydrates stored in the stem of the mulberry plant.[3] Changes in the initial sucrose and starch contents in the bark and the wood were slight during the first two days, after which a sharp decrease took place that continued until the 14th day (Fig. 3). As the leaves developed and their RuBPCase activity increased, a significant increase in the amount of surcose present was noted prior to the accumulation of starch. However, by day 56 the depleted carbohydrates had not been restored to their initial values. The value for the adenylate energy charge,[1] ATP+1/2 ADP/ATP+ADP+AMP, also remained appreciably low (0.62 in the bark, 0.58 in the wood). This indicates that the mulberry plants were still in an unfavorable condition for supplying carbon and energy for regrowth after the spring harvest.

Free amino acids and protein following budding in the hardwood stem[18]

The prominent existence of arginine and proline in the stems of a mulberry tree in mid-winter has been recognized.[7] At the time of planting of mulberry stem cuttings in the spring, the most predominant amino acid has been shown to be proline, followed by asparagine.[11] It is also known that, in mulberry stems, the arginine content is relatively stable during the early stage of growth, but decreases sharply during the season of active new shoot growth.[4] Also, a rapid decrease in the content of proline takes place in the early stage of growth. The role of proline as a storage compound of nitrogen has been recognized in the mulberry.[7]

Quantitative changes in protein and various amino acids in the hardwood stem of mulberry saplings after planting at a temperature of 25°C are given in Fig. 4 and Table 1, respectively. Budding appeared the day after planting and the protein content in the bark decreased gradually (Fig. 4). In the wood, however, protein levels hardly changed and were less than one-seventh that in the bark. Proline and asparagine content decreased continually following budding in the bark with comparable trends. Though a similar pattern of decrease in proline was noted in the wood, the decrease in asparagine was preceded by a temporary increase. Arginine content in the bark steadily increased up to day 8, and then declined to its initial value at the end of the experimental period. This trend of arginine content in the bark, i.e. an initial increase followed by a rapid decrease, was also observed for the γ-aminobutyric acid content of both the bark and wood.

Asparagine was found to be the most abundant organic nitrogen compound in mulberry xylem

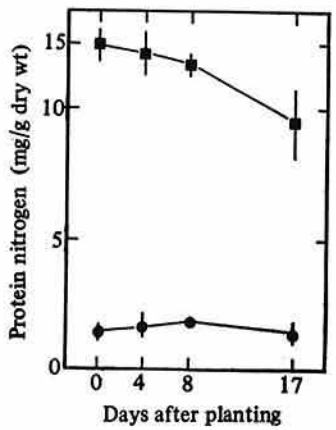

Fig. 4. Changes in protein nitrogen content in the bark (■) and wood (●) of the hardwood stem
The values are the means from four samples.
Bars indicate s.d.

Table 1. Changes in amino acid contents in (a) the bark and (b) the xylem of hardwood stems after planting

	Days after planting (μ mol g^{-1} d. wt)			
	0	4	8	17
(*a*) *Bark*				
Aspartic acid	8.1±2.4	8.5±1.9	5.8±1.1	3.3±0.6
Threonine	2.6±0.4	3.7±0.6	3.6±0.9	1.4±0.4
Serine	5.8±2.6	3.2±0.6	3.7±0.4	1.7±0.2
Asparagine	63.3±11.7	52.5±9.3	44.8±7.4	27.1±7.5
Glutamic acid	13.1±3.9	14.7±2.1	16.8±5.9	3.3±0.8
Glutamine	4.9±1.7	5.1±1.6	6.5±1.6	2.4±0.5
Proline	53.8±10.6	33.3±1.5	38.0±6.2	20.3±13.8
Glycine	0.6±0.1	0.8±0.1	1.0±0.3	0.7±0.3
Alanine	3.3±0.8	4.9±1.6	4.2±1.6	1.3±0.2
Valine	2.9±0.6	2.7±0.4	3.5±1.2	1.2±0.4
Leucine	0.2±0.1	0.6±0.2	0.4±0.1	0.3±0.1
Phenylalanine	2.9±0.4	1.8±0.3	1.2±0.3	0.4±0.1
γ-Aminobutyric acid	10.5±3.0	14.0±1.6	18.2±5.7	9.0±2.7
Histidine	1.1±0.2	2.2±0.8	3.8±0.7	1.7±0.7
Lysine	1.9±0.5	3.1±0.5	3.5±0.8	1.7±0.2
Arginine	30.4±9.1	38.4±6.5	48.8±17.4	32.5±10.9
Total	195.4	189.5	203.8	108.3
(*b*) *Xylem*				
Aspartic acid	2.6±0.6	2.3±0.4	1.9±0.3	1.4±0.2
Threonine	0.5±0.1	0.8±0.1	0.7±0.1	0.4±0.1
Serine	1.8±0.3	0.9±0.2	0.6±0.1	0.4±0.1
Asparagine	16.3±3.4	25.2±0.7	15.0±2.3	11.5±2.0
Glutamic acid	3.7±1.0	4.7±0.1	3.6±0.8	1.1±0.4
Glutamine	1.3±0.6	0.9±0.3	1.1±0.2	0.5±0.1
Proline	12.5±2.4	9.9±0.4	8.6±1.5	2.0±0.4
Glycine	0.1±0.0	0.2±0.0	0.2±0.0	0.2±0.0
Alanine	0.5±0.1	1.4±0.4	1.0±0.2	0.3±0.1
Valine	0.7±0.2	0.9±0.2	0.8±0.0	0.4±0.1
Leucine	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
Phenylalanine	0.6±0.2	0.4±0.1	0.2±0.0	0.1±0.0
γ-Aminobutyric acid	1.7±0.2	3.3±0.4	3.7±0.3	2.7±0.7
Histidine	0.3±0.1	0.6±0.2	0.4±0.0	0.5±0.1
Lysine	0.9±0.2	1.2±0.3	1.0±0.0	0.8±0.1
Arginine	7.4±0.9	7.5±0.8	5.6±2.4	5.9±1.5
Total	51.0	60.3	44.5	28.3

Means of four samples±s.d.

saps throughout the growing season of the plant.[12] It has also been reported that asparagine is present in high concentrations in young developing mulberry leaves where ^{14}C-asparagine is hardly formed from ^{14}C-precursors.[15] Thus, asparagine is considered to be not only a storage compound of nitrogen but also a nitrogen transport compound in mulberry trees.

Top pruning and stem carbohydrate reserves during autumn[19]

As in other deciduous species, the carbohydrate content in the mulberry is greatest in the autumn as the growth rate decreases, and lessens sharply as new growth begins in the spring.[3,8] The stems and stumps of mulberry trees function as major sinks for photosynthate[9,16] and may compete with young expanding leaves and growing shoots. The removal of organs competing with other sinks has a direct influence on assimilate partitioning. Source leaves also respond in various ways at the time of removal of any sink, but the results that have been reported in a number of studies are incompatible.[14]

For the autumnal rearing of silkworms, only

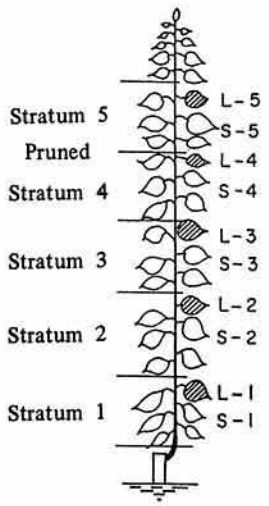

Fig. 5. Diagram of the sampling plan used and sample identification
Stems were divided into five strata, each with six leaves.
The uppermost leaves of each stratum were used for experiments.
L-1 to L-5 : the uppermost leaves of strata 1 to 5.
S-1 to S-5: stem samples of strata1 to 5.

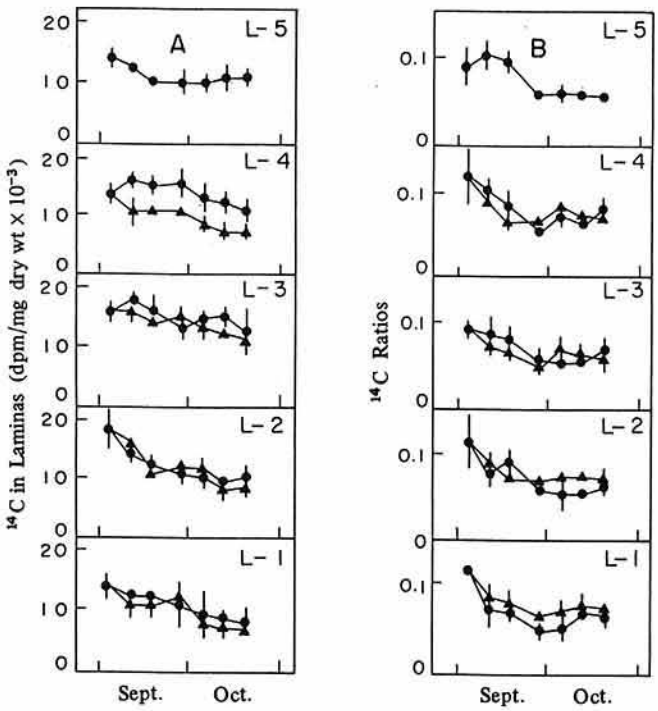

Fig. 6. Changes in the ^{14}C of laminas (A) and changes in the ratios of ^{14}C (dpm/g dry wt) in the midrib plus the first order veins to the ^{14}C in the laminas (B)
Leaves of control plant (●) and top-pruned plants (▲).
Values are the means of two leaf samples.
Bars indicate deviations.

the upper parts of the shoots of the mulberry are harvested in order to accumulate the reserve materials needed for growth of the tree the following spring.

Changes in the carbohydrate content of the stem and in the rate of photosynthetic $^{14}CO_2$ fixation in the remaining leaves were followed after top pruning had been done from early September through late October. Shoot tops, including shoot stratum 5, were pruned as shown in Fig. 5 and growth that appeared after this treatment was removed. The uppermost leaves of each stratum were fed $^{14}CO_2$ for 10 min and then transferred to a $^{14}CO_2$-free chamber and kept there for 30 min for the translocation of ^{14}C-assimilates.

Changes in radioactivity in the laminas and ratios of radioactivity (dpm/mg dry wt) in the midrib plus that in the first order veins to radioactivity in the lamina are shown in Fig. 6. The ^{14}C fixed in the uppermost leaves of top-pruned stems was reduced markedly compared with that in the corresponding leaves of control plants. Similar cases have been reported for the photosynthetic rates of other plant species, such as potato, pine and barley.[5,6,13] The reverse also has been reported for the leaves of the mulberry[10] and Italian ryegrass.[2]

The gradual decrease in the amount of ^{14}C-assimilates associated with autumnal sensecence began in leaves at all positions on the stems from early or mid-September. The ratio of radioactivity (dpm/mg dry wt) in the midribs plus the first order veins to radioactivity in the laminas, which may represent the intensity of export of ^{14}C-assimilates, also continued to decrease until late September. No perceptible difference could be detected in the ratios for leaves of top-pruned and intact plants. It is widely recognized that deciduous trees have a maximum concentration of reserve carbohydrate in the autumn. The results shown in Fig. 7 also

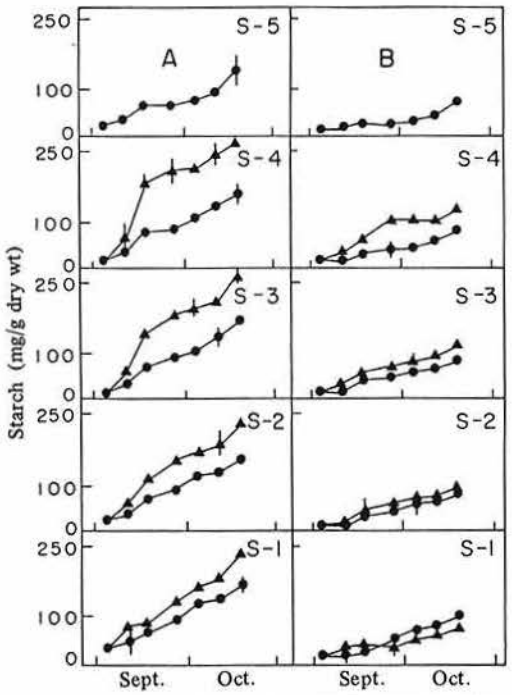

Fig. 7. Changes in the content of starch in the bark (A) and wood (B) of mulberry stems
Stems of control (●) and top-pruned trees (▲).
Values are the means of two stem samples.
Bars indicate deviations.

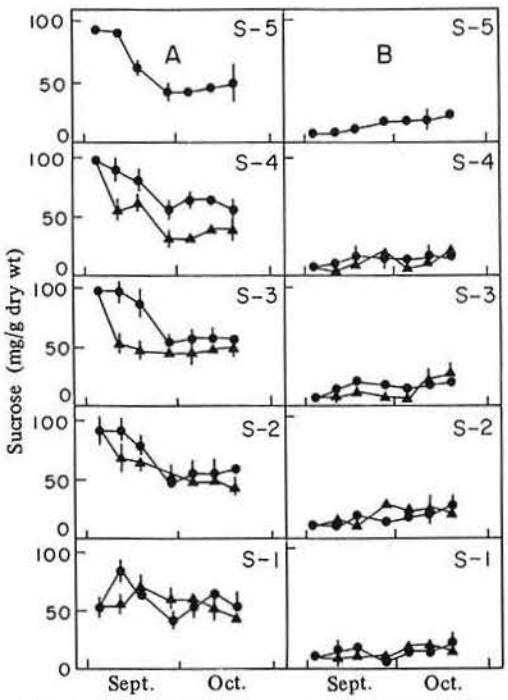

Fig. 8. Changes in the sucrose content of the bark (A) and wood (B) of mulberry stems
Symbols and values are the same as in Fig. 7.

demonstrate the steady accumulation of starch in the stems of both top-pruned and control mulberry trees towards the end of autumn. This accumulation was accelerated by top pruning, and was larger in the upper parts of the stems. In the bark, except for the lowest parts of the stem (S-1), the sucrose content (Fig. 8) decreased until late September. The glucose and fructose contents did not exceed 20 mg/g dry wt in the bark and 10 mg/g dry wt in the wood. The total amount of carbohydrate reserves (starch plus sugars) in the stem was larger in the pruned trees than in the controls throughout the plant. The sucrose content in the stems of the mulberry has been reported to reach its lowest value by September and then to increase steadily to a maximum by early December.[8] The effect of top pruning on the contents of protein, starch and sucrose in mulberry leaves was imperceptible throughout the experimental period.

In conclusion, romoval of the shoot tops accelerated the accumulation of starch in mulberry stems during autumn senescence even though photosynthesis in the remaining uppermost leaves was suppressed. The amount of assimilate loaded into the veins and the contents of carbohydrate and protein in the leaves were not affected by top-pruning treatment.

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