

# Physiology of Ribulose Bisphosphate Carboxylase as Major Leaf Protein in Mulberry (*Morus alba* L.)

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The characteristics of the synthesis and degradation of ribulose 1,5-bisphosphate carboxylase (RuBPCase) have been extensively studied in annual plant leaves in relation to growth or senescence<sup>2,3,9</sup>. It has been confirmed that RuBPCase functions not only as the enzyme responsible for CO<sub>2</sub> fixation but also as a nitrogen source for the growth of sink organs through its own degradation. Very few studies have been conducted on the formation and loss of RuBPCase in the leaves of woody plants. For rearing of silkworms, RuBPCase which is the most abundant protein in the mulberry leaves must be the dominant nutrient. Hence, RuBPCase protein in mulberry leaves has to be kept at a level sufficient not only to sustain tree growth but also for satisfying the nutritional requirement of silkworms. To regulate the metabolism of this enzyme protein is one of fundamental problems for intensive culture of mulberry.

## Changes in content of RuBPCase during development and senescence of mulberry leaves<sup>7)</sup>

The formation and loss of RuBPCase were followed during two distinct phases of mulberry growth, i.e., from flushing to full development of leaves in spring and from the onset of the autumnal senescence to the falling of the leaves. In spring, the level of RuBPCase protein increased along with leaf development, and the increase was more rapid

in the leaves which opened later (Fig. 1). In the leaves that opened in early June, the level of RuBPCase protein was maximum within one month after the leaves opened. In the leaves which had completed their development, a gradual increase in the level of RuBPCase protein lasted for a while, and then the RuBPCase protein was mobilized during leaf senescence as a result of the sequential emergence and development of new leaves. Senescence-associated degradation of RuBPCase and redistribution of nitrogen within a plant have generally been confirmed in annuals. Ratio of RuBPCase N to total N increased with leaf development, and reached the maximum level of 0.43 in the upper leaves by late June. This value was higher than that in other plant species, being 0.28 for rice leaves<sup>4)</sup> and 0.11–0.28 for wheat leaves<sup>1)</sup>.

Changes in the content of total N and RuBPCase were followed throughout the autumnal senescence in mulberry leaves of varying ages (Fig. 2). Trees had shed nearly all their leaves, leaving a few on the shoot top, until the end of November. Total N content decreased toward the end of autumn. RuBPCase protein content in the lower and middle leaves declined in late autumn. The leaves, however, fell before this content had been reduced to a half. These results indicated that though a limited amount of RuBPCase protein could be mobilized into storage sink organs during autumnal senescence, a much greater loss in RuBPCase protein occurred during the senescence resulting from the sequential development of new

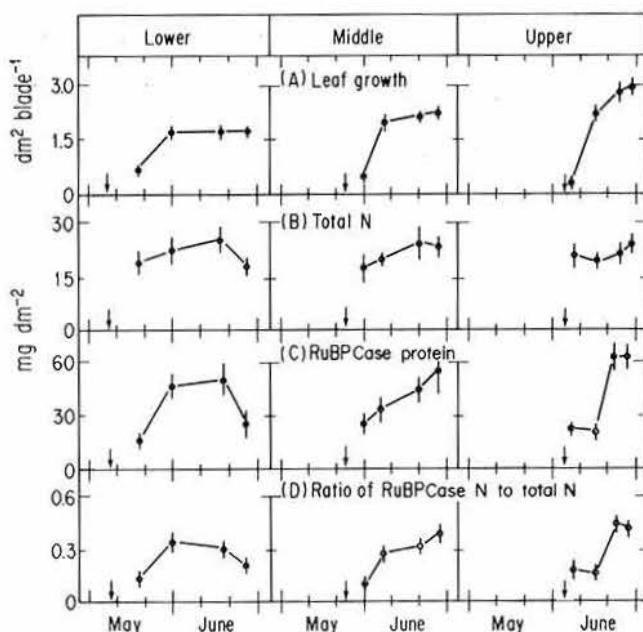


Fig. 1. (A) Leaf growth, and changes in contents of (B) total nitrogen and (C) RuBPCase protein, and (D) ratio of RuBPCase nitrogen to total nitrogen in leaves of *Morus alba* after leaf opening

Lower, middle, and upper refer to leaf position on an elongated shoot.

Vertical bars indicate s.e. of eight samples.

Arrows indicate the time of leaf opening.

leaves in the growing season. It appears that younger expanding leaves with a much greater requirement for nitrogen promote the mobilization of protein in older leaves, and RuBPCase may be degraded for the redistribution of reduced nitrogen.

### Changes in RuBPCase concentration due to external nitrogen supply in mulberry leaves<sup>5)</sup>

For intensive cultivation of mulberry trees a great deal of nitrogen fertilizer, annually more than  $300 \text{ kg N ha}^{-1}$ , is presently applied. Effects of external nitrogen supply on quantity of RuBPCase in the leaves were examined for mulberry saplings grown in a nutrient solution containing different amounts of N (10, 40 and  $120 \text{ mg l}^{-1}$ :  $N_{10}$ ,  $N_{40}$  and  $N_{120}$ ) together

with other nutrients (Fig. 3). The leaves supplied with the highest N ( $N_{120}$ ) showed twice the rate of  $\text{CO}_2$  fixation compared with those on lower N ( $N_{10}$ ,  $N_{40}$ ). Total N, total soluble protein and RuBPCase protein increased with the supply of N. The leaves of  $N_{120}$  plants contained double the RuBPCase protein found in  $N_{10}$  leaves. The ratio of RuBPCase protein to total soluble protein or to total N was also increased by increasing the N supply. RuBPCase protein functions as a storage of leaf-N and meets N requirement of newly developing organs by its remobilization from older leaves as described in the foregoing section of the present paper. Hence, enhancing the ratio of RuBPCase protein N to total N (or RuBPCase protein to total soluble protein) should be even more favorable for the economy of cellular N in the case of the deciduous tree.

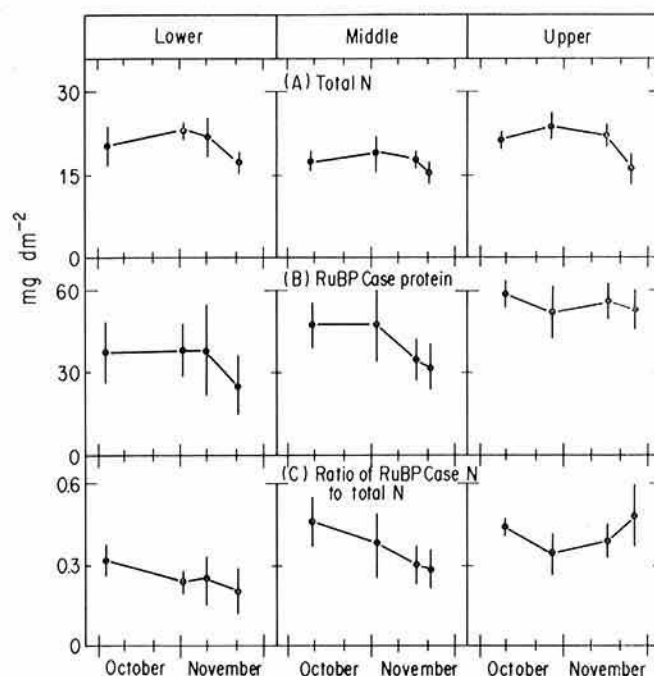


Fig. 2. Changes in contents of (A) total nitrogen and (B) RuBPCase protein, and (C) ratio of RuBPCase nitrogen to total nitrogen in leaves of *Morus alba* during autumnal senescence

Lower, middle, and upper refer to the leaf position on a shoot in which the elongation had stopped. Vertical bars indicate s.e. of eight samples.

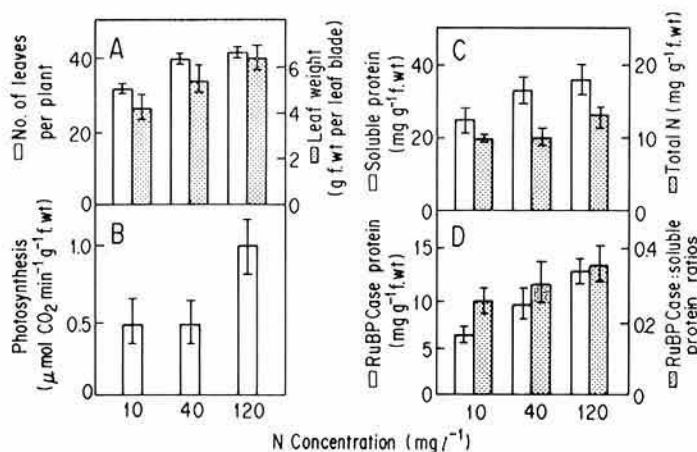


Fig. 3. (A) Leaf yield, (B) photosynthetic CO<sub>2</sub> fixation, (C) soluble protein and total nitrogen contents, and (D) RuBPCase protein content and its ratio to soluble protein in leaves of *Morus alba* grown in the nutrient solutions of different N concentrations

Vertical bars indicate s.e. of six samples.

## Effects of pruning of young and old shoots on amount of RuBPCase in leaves of the mulberry<sup>(1)</sup>

In intensive culture of mulberry, the foliated hardwood stems which have elongated in the previous year are cut off at their bases on the stumps in June–July. The new shoots then regenerated from the stumps are partially pruned (top half to top one-third) in August–September. As an alternative to this basic harvest method, shoots which have developed from the stumps since spring are sometimes partially pruned in August–September. Changes in the contents of RuBPCase and other cellular constituents were measured

in the leaves remaining on shoot after removal of the top one-third of shoots (old) which had developed since the spring and shoots (young) which developed the following shoot harvest on July 1.

### 1) Top-pruning of young shoots

After pruning on August 10, the changes in content of RuBPCase and other cellular constituents in the uppermost leaves on pruned shoots were followed (Fig. 4). Chlorophyll content increased in the leaves of pruned shoots by as much as 25% compared with the control leaves. Nitrogen content of the leaves of pruned shoots continued to increase after top-pruning, reaching 1.6 times that in the control leaves at day 30. Although

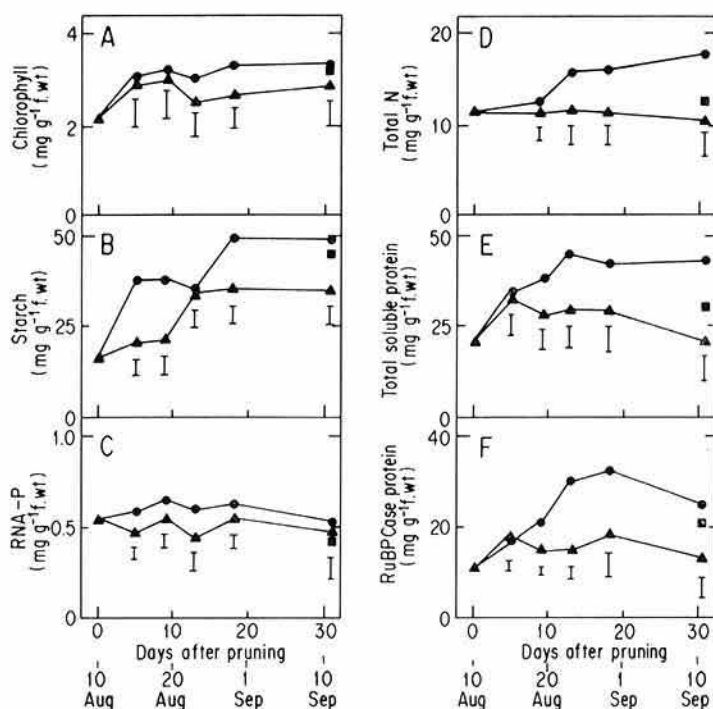


Fig. 4. Effects of top-pruning on August 10 on content of (A) chlorophyll, (B) starch, (C) RNA, (D) total nitrogen, (E) total soluble protein and (F) RuBPCase protein in leaves on new shoots regenerated from stumps of *Morus alba* left by stem harvest on July 1

● : When the axillary buds of the new shoots were removed,  
 ■ : When the axillary buds were allowed to grow,  
 ▲ : Comparable leaves on intact shoots.  
 Results are means for eight leaf samples.  
 Vertical bars represent l.s.d.,  $P=0.05$ .

sucrose content was not affected, the starch content was notably increased by top-pruning. Increase in RNA was also appreciable. By 9 day after top-pruning, notable increases in the contents of both total and RuBPCase protein had occurred in leaves from pruned shoots. Further increase continued up to day 18, and thereafter these raised levels remained steady (total protein) or slightly declined (RuBPCase protein). Top-pruning raised the contents of RuBPCase to maximum level of 2.0 times those in the control leaves. This was the most conspicuous change in the cellular constituents examined. An explanation for this change is non-execution of nitrogen redistribution resulting from removal of nitrogen demanding shoot tops.

## 2) Top-pruning of old shoots

The top one-third of the shoots which developed from mulberry stumps in the spring of the same year were pruned on August 24. The physiological events in the remaining leaves caused by top-pruning were examined throughout the autumn after pruning. No appreciable changes, except for RNA content, were caused by top-pruning. The content of RNA in the pruned shoot leaves exceeded that in the corresponding leaves on intact shoots throughout the experimental period. The elongation growth of old shoots was observed to slow down and the demand for nitrogen and photosynthate for source leaves in shoot tops decreased in late August when their tops were pruned. Therefore, in older shoots the removal of their tops may cause little change in the sink-source relationship.

## Modulated degradation of RuBPCase in leaves on top-pruned shoots of mulberry<sup>8)</sup>

One possible explanation for the increase in RuBPCase content in leaves on top-pruned shoots is the failure of nitrogen remobilization to occur in leaves, since RuBPCase degradation and remobilization are important means for providing nitrogen to the develop-

ing parts of a plant. The effects of top-pruning on the turnover of RuBPCase in the remaining leaves were investigated by examining the incorporation of L-<sup>14</sup>C(U)-leucine

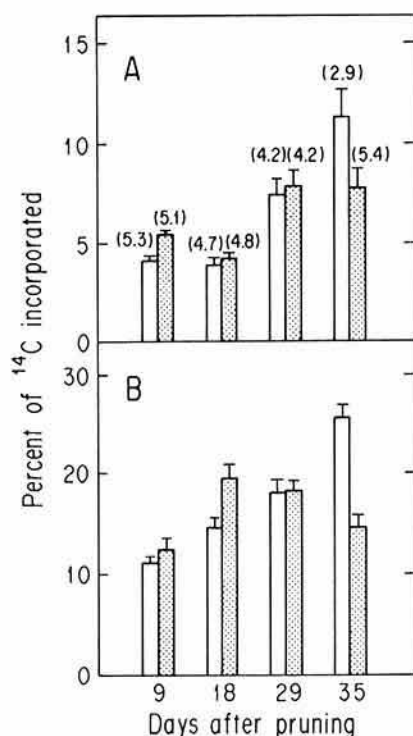


Fig. 5. Effects of top-pruning on <sup>14</sup>C incorporation into (A) RuBPCase and (B) total soluble protein from externally added L-<sup>14</sup>C(U)-leucine in leaf discs of *Morus alba*

▨ : Leaf discs obtained from leaves on top-pruned shoots,

□ : Leaf discs from comparable leaves on intact shoots.

Incorporation of <sup>14</sup>C is expressed as the proportion of <sup>14</sup>C incorporated into RuBPCase and total soluble protein to <sup>14</sup>C(U)-leucine absorbed by leaf discs.

The number in parenthesis is the radioactivity absorbed by the leaf discs (KBq per disc). Results are the means for six leaf samples.

Vertical bars indicate s. e.

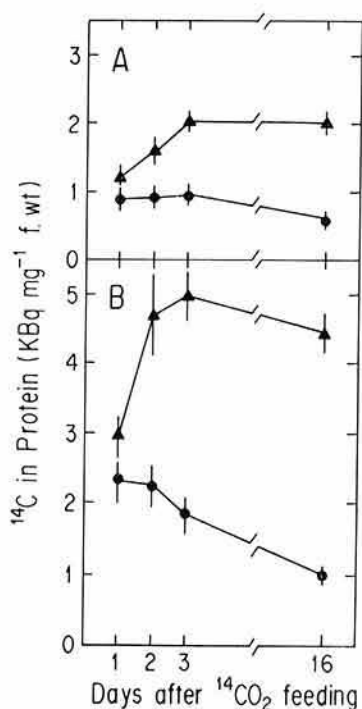


Fig. 6. Effects of top-pruning on changes in  $^{14}\text{C}$  in (A) RuBPCase and (B) total soluble protein in leaves of potted saplings of *Morus alba* previously fed  $^{14}\text{CO}_2$  for 2 hr

▲ : Leaves on top-pruned shoots,  
● : Comparable leaves on intact shoots.  
Results are the means of six leaf samples.  
Vertical bars indicate s. e.

into RuBPCase and following the loss of  $^{14}\text{C}$ -RuBPCase labelled photosynthetically. Leaf discs from field-grown mulberry trees were fed  $^{14}\text{C}$  labelled leucine for two hours and  $^{14}\text{C}$  incorporation into RuBPCase and total soluble protein was examined to compare the capacity of synthesizing them. The results in Fig. 5 are expressed as the proportions of  $^{14}\text{C}$  incorporated into RuBPCase and total soluble protein to  $^{14}\text{C}$ -leucine absorbed by leaf discs, respectively. Top-pruning occasionally raised slightly the proportions of  $^{14}\text{C}$  incorporation. The proportions of  $^{14}\text{C}$  in-

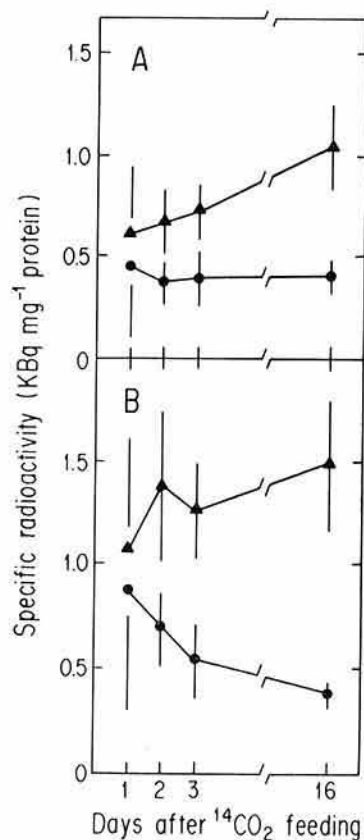


Fig. 7. Effects of top-pruning on specific radioactivity in (A) RuBPCase and (B) total soluble protein in leaves of potted saplings of *Morus alba* previously fed  $^{14}\text{CO}_2$  for 2 hr

▲ : Leaves on top-pruned shoots,  
● : Comparable leaves on intact shoots.  
Results are the means for six leaf samples.  
Vertical bars indicate s. e.

corporated into RuBPCase and total soluble protein were significantly higher ( $P < 0.01$ ) in the leaves on top pruned shoots at days 9 and 18, respectively. In contrast, at day 35, the proportions of  $^{14}\text{C}$  incorporation into both of them were considerably lower in the leaves compared to those in the control leaves, falling 58% (RuBPCase) and 68% (total soluble protein) the latter values, respectively. In the control leaves the proportions of  $^{14}\text{C}$



incorporation into RuBPCase and total soluble protein both increased progressively throughout the experimental period, but no appreciable changes in their concentrations could be detected. In the leaves on the top-pruned shoots, the extent of increase in the proportions of  $^{14}\text{C}$  incorporation into RuBPCase and total soluble protein was less than that in the control leaves (it decreased in total soluble protein after day 18), yet their contents rose progressively beyond those in the control leaves. Hence, the rate of the breakdown of RuBPCase and total soluble protein, which is slower than that of their simultaneous synthesis, showed decline by top-pruning. In the control leaves, the synthetic capacity for RuBPCase rose at the later period in the experiment, but no net increase in its amount was observed. This suggests that the degradation of RuBPCase occurring simultaneously with its synthesis parallels variation in the capacity for RuBPCase synthesis. This was also noted for total soluble protein in control leaves.

Changes in radioactivity in RuBPCase and total soluble protein in the leaves of mulberry saplings previously fed  $^{14}\text{CO}_2$  were followed (Fig. 6). Six days after top-pruning, the plants were fed  $^{14}\text{CO}_2$  and subsequently cultured in non-radioactive air. In the control leaves,  $^{14}\text{C}$  in RuBPCase became nearly maximum the day after  $^{14}\text{CO}_2$  feeding, and thereafter remained steady for a while. By day 16, about 40% of it was lost. In the leaves on top-pruned shoots,  $^{14}\text{C}$  in RuBPCase continued to increase up to day 3, and the maximal level persisted until day 16. Trends similar to these were also noted for total soluble protein. Specific radioactivity (Fig. 7) in RuBPCase continued to increase in leaves on top-pruned shoots even after attaining a maximum level in the control leaves. It in total soluble protein declined steadily in the control leaves, and increased in the leaves of top-pruned shoots.

Though there are factors that complicate the situation, distinct altering of trends in change in  $^{14}\text{C}$  in RuBPCase and in specific radioactivity by top-pruning as mentioned

above supports the assumption that the increase in RuBPCase by top-pruning results from a cessation of its degradation for the remobilization of nitrogen for newly developing leaves on shoot tops.

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