

## Carbon partitioning: fructose 2,6-bisphosphate content as an indicator of specific changes in carbohydrate metabolism in needles from class II spruce trees

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### Introduction

It has been shown that very low doses of airborne pollutants (ozone, sulfite) can significantly change source-sink relationships. These shifts in allocation or transportation out of leaves can occur prior to reductions in photosynthesis (ozone; McLaughlin and McConathy, 1983) and can take place within minutes (Minchin and Gould, 1986).

In spite of intense research in this area, there is, however, only little information

available about metabolic acclimation of tissues to pollutants. It has thus been our aim to screen for biochemical indications of altered patterns of carbon allocation in needles of Norway spruce (*Picea abies*).

### Materials and Methods

The materials used for our investigations were needles from spruce trees from 2 locations in

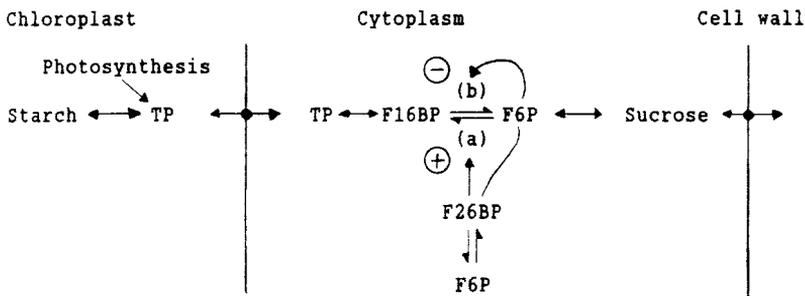
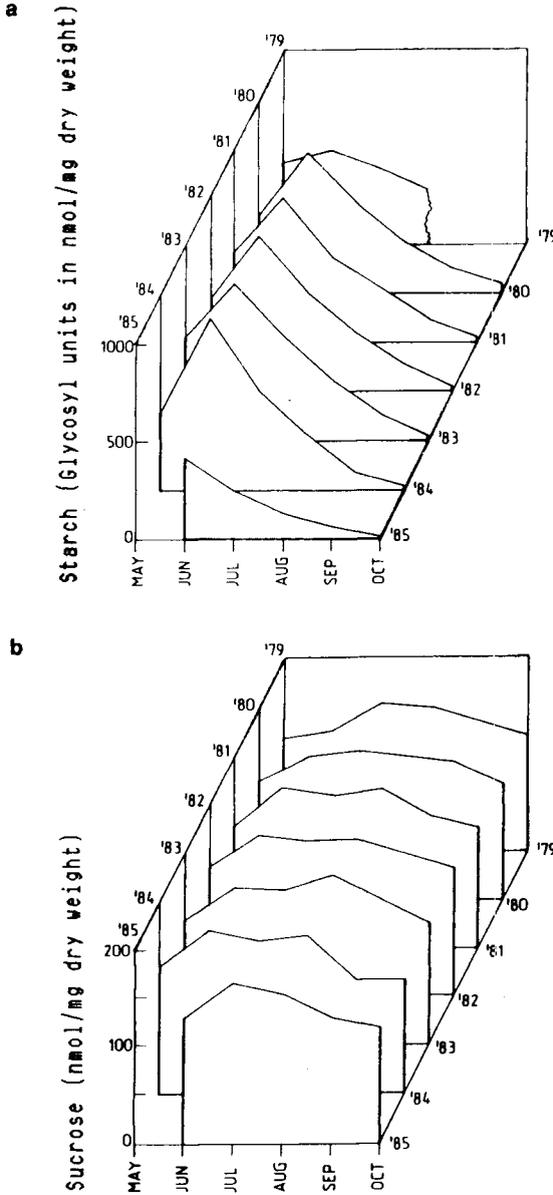


Fig. 1. Carbon partitioning between starch and sucrose. Intermediates and regulatory events. a: pyrophosphate-dependent fructose 6-phosphate phosphotransferase (PF6P); b: fructose 1,6-bisphosphatase; F16BP: fructose 1,6-bisphosphate; F26BP: fructose 2,6-bisphosphate; TP: triose phosphates.



**Fig. 2.** Content of starch (a) and sucrose (b) in Norway spruce needles in relation to needle age and vegetation period.

the southern part of the Black Forest (Kälbelescheuer and Haldenhof, near Freiburg, F.R.G.). Collection and freeze-drying of needle samples

as well as metabolite analyses were as described elsewhere (Einig and Hampp, 1988; Hampp *et al.*, 1989).

## Results and Discussion

### *Season- and age-dependent variations in pool sizes*

There is considerable evidence that the rate of starch synthesis is controlled by the rates of sucrose formation and transport.

Metabolites involved in the regulation of carbon partitioning between starch and sucrose are triose phosphates (TP; dihydroxyacetone phosphate, glyceraldehyde 3-phosphate), glyceric acid 3-phosphate (PGA), fructose 6-phosphate (F6P), orthophosphate ( $P_i$ ) and pyrophosphate ( $PP_i$ ). Levels of these metabolites control synthesis and degradation of the most important regulator, fructose 2,6-bisphosphate (F26BP). This compound affects cytosolic sucrose synthesis by inhibiting the fructose bisphosphatase (FBPase) reaction (gluconeogenesis) and activating a  $PP_i$ -dependent phosphofructokinase (PFK); active in both directions, glycolysis and gluconeogenesis (for a review see Stitt, 1987; compare also Fig. 1).

Sucrose and starch as 'endpoints' of this regulatory system show distinct differences in their pool sizes. Needles from

control trees have optimum starch levels in early summer (Fig. 2a). Independent of needle age, there is a continuous decline towards October. Sucrose, in contrast, is much more constant in its seasonal pool sizes (Fig. 2b).

There are, however, specific differences, when pool sizes of phosphorylated intermediates are compared. An intimate correlation between pool sizes of TP, F6P and F26BP is observed when the average contents of all needles (1980–1985) are plotted *versus* the sampling date (Fig. 3).

Under the assumption that the changes in pool sizes observed for F6P and TP also occur in the cytosol of our needle mesophyll cells, all these observations can easily be explained by the scheme shown in Fig. 1. In June samples, *e.g.*, starch, F6P and F26BP are high, while TP are low; high levels of F6P, possibly indicative of limited sucrose export (rates of synthesis exceed rates of export), activate F26BP synthesis. Increased levels of F26BP, however, favor glycolysis over gluconeogenesis and thus TP are diverted into starch synthesis. In July, in contrast, an opposite situation emerges with decreased amounts of F6P and F26BP and high levels of TP. This metabolic situation should thus be indicative of

**Table 1.** Intermediates of carbohydrate metabolism in spruce needles in relation to the degree of needle loss of the respective trees (class 0 and class II).

Intermediate	Needle loss (%)	
	0–10	30–40
Starch (hexose units)	814 ± 39	493 ± 45
Sucrose	168 ± 9	144 ± 5
Glucose	6 ± 1	6 ± 1
Fructose	5 ± 1	5 ± 1
Fructose 2,6-bisphosphate*	0.29 ± 0.05	1.09 ± 0.12
Triose phosphates	1.86 ± 0.23	2.97 ± 0.2
3-Phosphoglyceric acid	1.32 ± 0.29	1.61 ± 0.15
Fructose 6-phosphate*	98 ± 11	124 ± 13

Values are averages of individual samples (1980–1983), taken in June 1985. Values in nmol/mg dry weight; \* indicates pmol/mg dry weight.

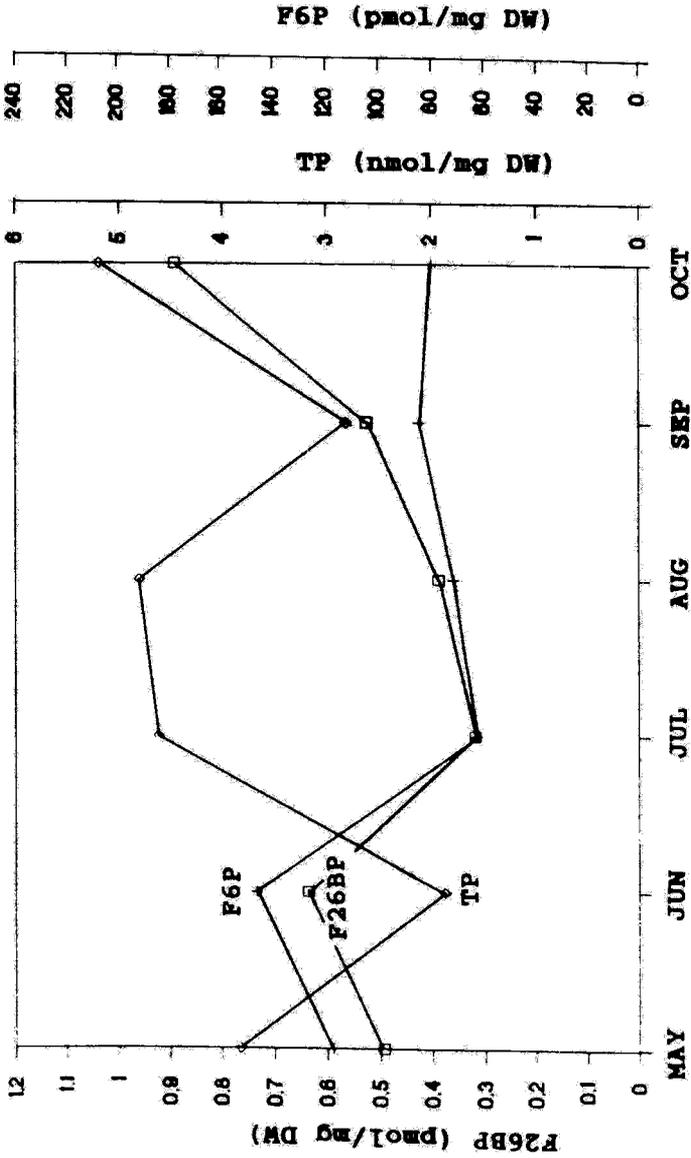


Fig. 3. Levels of fructose 2,6-bisphosphate (F26BP), fructose 6-phosphate (F6P) and triose phosphates (TP) in needles of Norway spruce trees. The values represent means of 1980-1985 samples.

increased partitioning of carbon into sucrose (starch decreases) and this situation is obviously continued during summer.

#### *Class II-specific changes in pool sizes*

There are also significant differences when the metabolite pools are compared with respect to needle loss (Table I). The average metabolite contents of NS needles from class 0 and class II trees (1980–1983; based on dry weight) differ significantly in the levels of starch, TP and F26BP, in that class II needles show a decrease in starch, compared to increased amounts of TP and F26BP. In contrast, sucrose, glucose, fructose, PGA and F6P only show minor differences. Compared to the observations reported for control needles above, the situation in class II needles is less straightforward to interpret.

The most interesting change in concentration is shown by F26BP. The significantly increased amount of this regulator will surely inhibit cytosolic FBPase and will thus largely reduce carbon flow towards sucrose (compare Fig. 1). The elevated level of F6P, if cytosolic, could be responsible for this increase in F26BP.

#### **Conclusion**

Needles from declining trees exhibit a significant increase of F26BP. This can be taken as evidence for impaired sucrose export. As such, a metabolic response towards altered carbon partitioning be-

tween starch and sucrose will precede any visible signs of damage. The determination of F26BP levels in needles could constitute an early indicator of affected carbon allocation.

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