CO_2-fixation in poplar leaves treated with ozone

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Synopsis
Five weeks old cuttings of *Populus nigra* L. cv. Loenen were continuously exposed to 40 nL*L^{-1} (10.00–17.00 h) and 12 nL* L^{-1} ozone (17.00–10.00 h) for three weeks in a growth cabinet. After exposure the enzymes of CO_2-fixation i.e. the photosynthetic ribulose-1,5-bisphosphate carboxylase-/oxygenase (Rubisco) and the anapleurotic phosphoenolpyruvate carboxylase (PEPcase) were analysed. Older leaves, fully expanded prior exposure, exhibited a decrease in total activity (by 50 %) and quantity (by 70 %) of Rubisco; PEPcase activity was doubled. In younger leaves, which were developed during ozone treatment, activity of both enzymes was still unchanged. Rubisco content, by contrast, was decreased by 30 %. The observation made in older leaves indicates that a higher percentage of the newly fixed carbon is used to support catabolic metabolism in the ozone stressed poplar.

CO_2-fixation, ozone, Pepcase, Populus nigra L., Rubisco.

1. Introduction

In previous open-top-chamber-experiments the ozone-sensitive *Populus nigra* L. cv. Loenen exhibited a decrease in net photosynthesis (BALLACH & al. 1992) and alterations of carbohydrate levels (BÜCKER & BALLACH 1992) under the influence of ozone, especially in older leaves. These observations suggest an impact of ozone on the CO_2-fixation, and thus, plant productivity. Key enzymes in this process are Rubisco and /or PEPcase. Rubisco catalyses the first irreversible step of CO_2-fixation during photosynthesis leading to production of carbohydrates. In C3-plants, PEPcase is involved in anapleurotic reactions such as replenishment oxalacetate for tricarboxylic acid cycle (LATZKO & KELLY 1983). In this study the impact of ozone on both enzymes is investigated.

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2. Material and methods

2.1 Plant material and exposure

Cloned cuttings of *Populus nigra* L. cv. Loenen purchased from Algemene Keuringsdienst voor Boomkwekerijgewassenen (NAKB), The Hague/NL, were planted in 1 L pots filled with an unfertilized peat/sand mixture (17:3, v/v). Cultivation and following fumigation was performed in a phytotron (Häreus). The day/night-regime was 16h/8h with a light intensity of 420 µmol*m^{-2}*s^{-1}. Relative humidity was between 75 % and 45 %, temperature by 23°C and 16°C.

After five weeks of cultivation plants were exposed for three weeks. The ozone concentration was by 40 (±10) nL*L^{-1} from 10.00–17.00 h and 12 (±5) nL*L^{-1} from 17.00–10.00 h. The controls were supplied with charcoal (Purafil) filtered air.

Fertilizing was started three weeks after implanting of the cuttings by adding 100 mg of micronutrient (Hortrilon) to each plant, followed weekly by 0.5 g of N-P-K-fertilizer (15-10-15; Hakaphos blau).

For analyses samples were taken from leaves fully expanded prior fumigation, termed older leaves, and leaves developed during exposure, termed younger leaves.

2.2 Analyses

Extraction of enzymes was performed according to DANN & PELL (1989). Total activity of Rubisco was measured spectrophotometrically as described by LILLEY & WALKER (1974), the activity of PEPcase according to ASHTON (1990). The amount of Rubisco was determined by gel electrophoresis as described by KEYS & PARRY (1990) and chlorophyll content was measured according to ARNON (1949).

3. Results

After three weeks of exposure to low ozone concentrations, the older leaves exhibited a decrease in Rubisco activity and quantity by 50 % and 70 %, respectively (fig. 1). The activity of PEPcase was doubled (fig. 1).
The younger leaves did not achieve the Rubisco content observed in leaves of plants kept in filtered air.

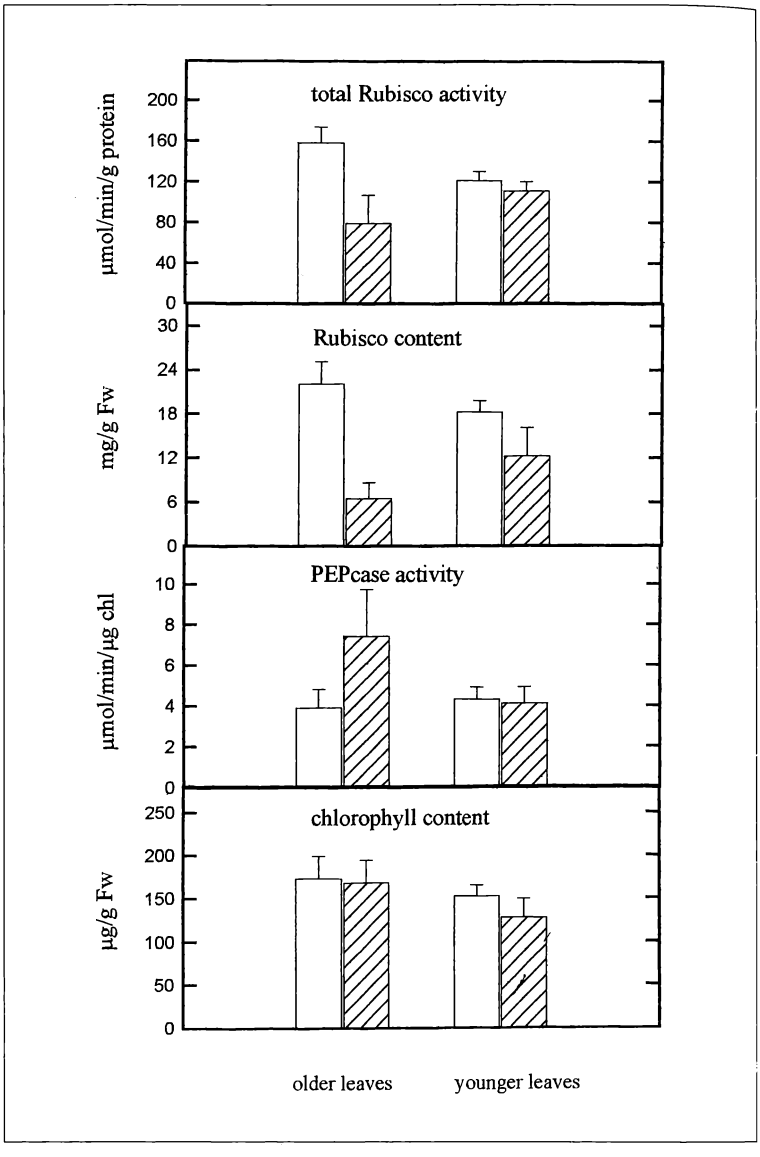

Fig. 1
Impact of ozone on total activity and quantity of Rubisco, on PEPcase activity and chlorophyll content in leaves of cv. Loenen. ☐ control ▨ ozone. Values are x (n=6); SD.

Rubisco- and PEPcase activity remained unchanged. All these alterations occurred before injuries were visible, only a slight decrease in chlorophyll content was detected (fig. 1).

4. Discussion

It can be concluded, that the alterations in photosynthesis and carbohydrate levels are also caused by the reduction in quantity and activity of Rubisco. The finding in younger leaves suggests a diminished synthesis of Rubisco protein under the influence of ozone. In protease assays with artificial substrate no increased activity was found (data not shown). Therefore an increased degradation of Rubisco is not very probable. Alterations in status of Rubisco after exposure to ozone has been found before in wheat (LEHNHERR & al. 1987), potato (DANN & PELL 1989), radish (PELL & al. 1992) and the hybrid *Populus maximowizii x trichocarpa* (PELL & al. 1992).

Behoynd this, increase of PEPcase-activity suggest that newly fixed CO_2 is used for feeding catabolic metabolism. Increase of PEPcase has been reported for spruce (TIETZ & WILD 1991).

Decrease in Rubisco quantity and activity is also assumed to contribute to normal leaf senescence (WITTENBACH & al. 1980) and premature senescence induced by air pollutants (LANDRY & PELL 1993). Premature foliar senescence due to air pollutants has also been described for cv. Loenen (BALLACH & al. 1988).

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