

Abstract

The aim of the present thesis was to choose and adjust a suitable methodology for counting particles in 3D space, which would be suitable for unbiased estimation of chloroplast number in needle mesophyll cells. The disector method was used for the first time to determine the number of chloroplasts. This method enables unbiased estimation of chloroplast number in needle volume from optical sections captured from fresh free-hand sections by confocal microscope. The sections did not need any pre-processing.

Another aim was to compare selected photosynthetic and anatomical characteristics of sun and shade Norway spruce needles, which were grown under different CO₂ concentration. The trees were grown for eight years in ambient (during the experiment increasing from 357 up to 370 $\mu\text{mol CO}_2 \cdot \text{mol}^{-1}$) CO₂ concentration or elevated (700 $\mu\text{mol} \cdot \text{mol}^{-1}$) CO₂ concentration in special glass domes on an experimental research site of the Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic at Bílý Kříž in Moravskoslezské Beskydy mountains. The sun needles were taken from the 3rd whorl (irradiance 1000–1200 $\mu\text{mol}(\text{photons}) \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) of the crown and the shade needles were taken from the 6th whorl (irradiance 300 $\mu\text{mol}(\text{photons}) \cdot \text{m}^{-2} \cdot \text{s}^{-1}$).

The elevated CO₂ concentration caused differences in the photosynthetic parameters, however only some of the anatomical parameters were significantly different from each other. The anatomical characteristics showed great variability even within the same variant of environment. However, it was not possible to extend the number of examined samples, because of limited time.

The effect of elevated CO₂ concentration mitigated the differences between sun and shade needle structure in EC. The average length of sun needles in AC was significantly longer than the average length of shade needles in EC. The needle volume and mesophyll volume estimations were significantly higher in sun AC needles than in shade needles in both CO₂ concentrations. The estimation of volume density of mesophyll, chloroplast density, mesophyll cell density and the average number of chloroplasts per cell didn't significantly differ among the variants of environment. The estimation of total number of chloroplasts per needle mesophyll was significantly higher in sun needles in AC in comparison with shade needles in AC and EC.