WAX-TURNOVER IN SUN-EXPOSED AND SHADED LEAVES OF MATURE BEECH TREE (*FAGUS SYLVATICA*)

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Plant cuticular waxes play an important role as they interact with the environment while protecting the leaf against water loss and other environmental stress. Furthermore, lipid biosynthesis and composition react to environmental stress such as drought. In general, plants exposed to increased water stress and solar radiation tend to form thicker wax layers than shaded plants and plants that are not exposed to water stress. To date, there is an understanding of variation in lipid composition and distribution within leaf waxes caused by environmental changes and stress, though little is known about variation in turnover rates of plant wax components within leaves. In this study, a $^{13}$CO$_2$ pulse-chase labelling experiment was conducted on a mature deciduous tree during late summer to identify the leaf wax-turnover in dependence of sun-exposure within the late growing season.

The $^{13}$CO$_2$ pulse-chase labelling experiment was conducted in August 2018 on a ca. 200 year old beech tree (*Fagus sylvatica*), located on the campus of the University of Zurich, Zurich, Switzerland. Six labelling chambers were placed on sun-exposed (n=3) and shaded (n=3) spots underneath the tree and wrapped around branches containing ca. 60 leaves. One additional branch was used as a control at each location. Selected branches were exposed to a $^{13}$C-enriched atmosphere for five hours. Afterwards, sun-exposed and shaded leaves were collected weekly (9 weeks) until October 2018. Variations in leaf chlorophyll content were monitored with a SPAD chlorophyll meter. Additional properties like leaf thickness, water content, carbon and nitrogen content as well as stable isotope composition ($\delta^{13}$C and $\delta^{15}$N) were measured. The composition of alkanes and fatty acids was assessed by GC-MS and GC-FID and their compound-specific stable carbon isotope ($\delta^{13}$C) composition was determined by GC-C-irmMS.

The results indicate a clear difference in leaf properties between sun-exposed and shaded leaves. Sun-exposed leaves have a larger leaf thickness (+20%) and a lower water content (-10%) compared to shaded leaves because of an increased irradiance and higher evapotranspiration (Lichtenthaler et al., 2007). In addition, sun-exposed leaves have a higher carbon content (+4%) compared to shaded leaves but a lower (-16%) nitrogen content. The
high C/N ratio observed in sun-exposed leaves can be explained by a higher lignin content (Sariyildiz and Anderson, 2003). Furthermore, compared to shaded leaves, sun-exposed leaves are characterized by a larger amount of epicuticular waxes (+42%). Since epicuticular waxes provide protection against environmental stress, sun-exposed leaves produce a larger amount of leaf waxes compared to shaded leaves to prevent water loss and to protect the aerial leaf parts against solar radiation. The maximum $^{13}$C-enrichment is largest in bulk leaves shortly after the isotopic pulse and is lower in fatty acids and alkanes by more than one order of magnitude. The maximum $^{13}$C-enrichment also occurred with a certain time lag in lipid fractions compared to bulk carbon, whereas the maximum enrichment is followed by a phase of decreasing $^{13}$C-abundance. This is due to the fact that the $^{13}$C, which is taken up during photosynthesis, is first used for the synthesis of primary biosynthesis products followed by fatty acids and n-alkanes that are synthesized as secondary biosynthesis products (Kunst and Samuels, 2003). As a consequence, a faster leaf wax-turnover is expected in sun-exposed compared to shaded leaves.

References