



## **The effect of light intensity on the $^{13}\text{C}/^{12}\text{C}$ and D/H compositions of leaf wax n-alkanes in *Arabidopsis thaliana*: Implications for palaeoclimatic studies**

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Investigations of stable carbon and hydrogen isotopes in individual organic compounds from leaf waxes are increasingly being used as proxies to estimate various palaeoclimatic parameters. Several previous studies have suggested that a simultaneous analysis of the  $^{13}\text{C}/^{12}\text{C}$  and D/H compositions of n-alkyl biomarkers may help identify the type of palaeovegetation contributing to the sedimentary record and thus enhance the applicability of compound-specific methodology in studying past climate. The rationale is that in C3 plants the  $\delta^{13}\text{C}$  and  $\delta\text{D}$  values are linked because of their dependence on water use efficiency (WUE). In this study we show that the assumption about the link might not necessarily be correct and that during biosynthesis the D/H composition of n-alkyl lipids could also be influenced by other factors that are decoupled from those controlling the  $\delta^{13}\text{C}$  values.

We measured the  $\delta^{13}\text{C}$  and  $\delta\text{D}$  values of leaf wax n-alkanes from 4 lines of Thale cress (*Arabidopsis thaliana*) grown under controlled conditions in growth chambers. All 4 lines were subjected to 3 different light treatments (51, 175, 435 micromole  $\text{m}^{-2} \text{s}^{-1}$ ; LL, ML, and HL henceforth), while the rest of growth parameters (the duration of illumination, temperature, relative humidity, and water source) were constant. In addition, we determined transpiration rate, stomatal conductance to  $\text{CO}_2$ , and net photosynthetic rate. Both types of experimental data were used to develop a kinetic model to investigate D/H fractionation during n-alkane biosynthesis.

As expected, plants differed in their physiological response to various light treatments. Transpiration rates were higher but not significantly different between ML and HL (1.6-2.3 and 1.6-1.8  $\text{mmol m}^{-2} \text{s}^{-1}$ , respectively) than in LL (0.5-1.1). Stomatal conductance to  $\text{CO}_2$  was also higher in ML and HL (97-132 and 116-126  $\text{mmol m}^{-2} \text{s}^{-1}$ ) than in LL (24-66). However, the plants in HL had significantly higher rates of net photosynthesis (13.3-14.4  $\text{micromole m}^{-2} \text{s}^{-1}$ ) than the plants in ML (5.6-6.4) and LL (0.9-1.2). As a result, WUE was considerably greater in HL (7.2-8.8) than in ML (2.5-3.6) and LL (1.0-2.5). The  $^{13}\text{C}/^{12}\text{C}$  composition of n-alkanes is consistent with the differences in WUE among the plants at 3 light levels. The  $\delta^{13}\text{C}$  values of n-C31 alkane in HL are significantly more positive (-37.9 to -40.4 per mil) than those in ML (-42.6 to -43.5) and LL (-44.3 to -44.8).

Our measurements also showed that the  $\delta\text{D}$  values in HL are more negative (-172 to -182 per mil) than those in ML (-148 to -163) and LL (-152 to -163), which, unlike the  $\delta^{13}\text{C}$  values in ML and LL, almost totally overlap. Furthermore, the relationship among the  $\delta\text{D}$  values among 3 light levels is different from that shown by the transpiration data, which as indicated above, show a similarity between ML and HL. To investigate the nature of these discrepancies we used our plant physiological and stable hydrogen isotope data from one of the lines of Thale cress together with literature-based fractionation data to develop a kinetic model. The model allowed us to estimate the  $\delta\text{D}$  values of NADPH (the first product of  $\text{H}_2\text{O}$  splitting during biosynthesis) at 3 light levels. We found that the pattern among the  $\delta\text{D}$  values of NADPH is similar to the n-alkane dD data and dissimilar to the transpiration data.

The initial results of this work suggest that light intensity is an important environmental parameter that significantly influences both the  $\delta^{13}\text{C}$  and dD values of leaf wax n-alkanes. However, the D/H composition of leaf wax n-alkanes is decoupled from that of  $^{13}\text{C}/^{12}\text{C}$  because of the different biochemical mechanisms affecting hydrogen and carbon isotope fractionations during photosynthesis.