

## **Dissecting Crassulacean Acid Metabolism with compound-specific stable isotope techniques**

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Crassulacean acid metabolism (CAM) plants exhibit a complex interplay between CO<sub>2</sub> fixation by PEPC and Rubisco on the one hand, and carbon demand for growth and CAM maintenance on the other hand. This study investigated the contribution of carbon from PEPC and Rubisco fixation to metabolite pools over the diurnal cycle, as well as to phloem export. Therefore, amount and carbon isotope composition of carbohydrates and organic acids were determined over the diurnal cycle with HPLC-IRMS in leaves and phloem exudates of *Kalanchoe daigremontiana*. Three types of pools could be distinguished: starch and malate showed PEPC-like  $\delta^{13}\text{C}$  values and a pattern of reciprocal mobilization and accumulation indicating a high connection to PEPC fixation. Isotopic composition and concentration of hexoses were not affected by diurnal metabolism, suggesting a low turnover. Sucrose, in contrast, exhibited significant changes in  $\delta^{13}\text{C}$  values, which could not be explained by the net changes in concentration, indicating a high turnover, with carbon from nocturnal starch degradation and direct Rubisco fixation during the day feeding the pool.  $\delta^{13}\text{C}$  values of phloem and leaf sucrose were highly correlated. We conclude that the leaf sucrose pool was well equilibrated and the main source of phloem export.