

# HETEROTROPHIC PRODUCTION OF PLANT LEAF LIPIDS IS HIGHLY ACTIVE THROUGHOUT SEASON: ISOTOPIC EVIDENCE ON AMINO ACIDS AND LIPIDS

Y. Takizawa<sup>1</sup>, and Y. Chikaraishi<sup>1</sup>

<sup>1</sup> Hokkaido University, Sapporo, Japan

## Introduction

Stable carbon ( $\delta^{13}\text{C}$ ) and hydrogen ( $\delta\text{D}$ ) isotopic compositions of lipid biomarkers have long been employed as powerful proxies in the study of paleo-environments and -ecosystems over a wide range of geological timescales. This is based on the fundamental mechanisms that the isotopic compositions of lipids produced by algae and plants primarily mirror those of carbon and hydrogen sources (e.g.,  $\text{CO}_2$  and  $\text{H}_2\text{O}$ ) in environments where/when they grew. On the other hand, a large variation in the  $\delta^{13}\text{C}$  and  $\delta\text{D}$  values are frequently found in leaf lipids within a plant between growth conditions (e.g., different seasons), which has been partially explained by phenological diversity in the use of isotopically distinct pools of carbon and hydrogen sources in the plant cell for lipid biosynthesis (e.g., Sessions 2006; Sachse et al., 2015). Although atmospheric  $\text{CO}_2$  and ground water are the primary sources for plant lipids, it is assumed that plants can produce not only lipids but also storage molecules (e.g., carbohydrates and proteins) during the period of active photosynthesis, and then that the storage molecules are used as alternative substrates for the lipid biosynthesis ‘heterotrophic production’ when plants grow under insufficient solar energy (e.g., night, winter, etc.). In particular, stone fruit plants (e.g., cherry, apple, almond, etc.) always bloom when their leaves are absent, generally 0-3 weeks before to the emergence of their first leaves. In this case, lipids in flowers indeed should be ‘heterotrophically’ biosynthesized from storage molecules without any photosynthetic activities.

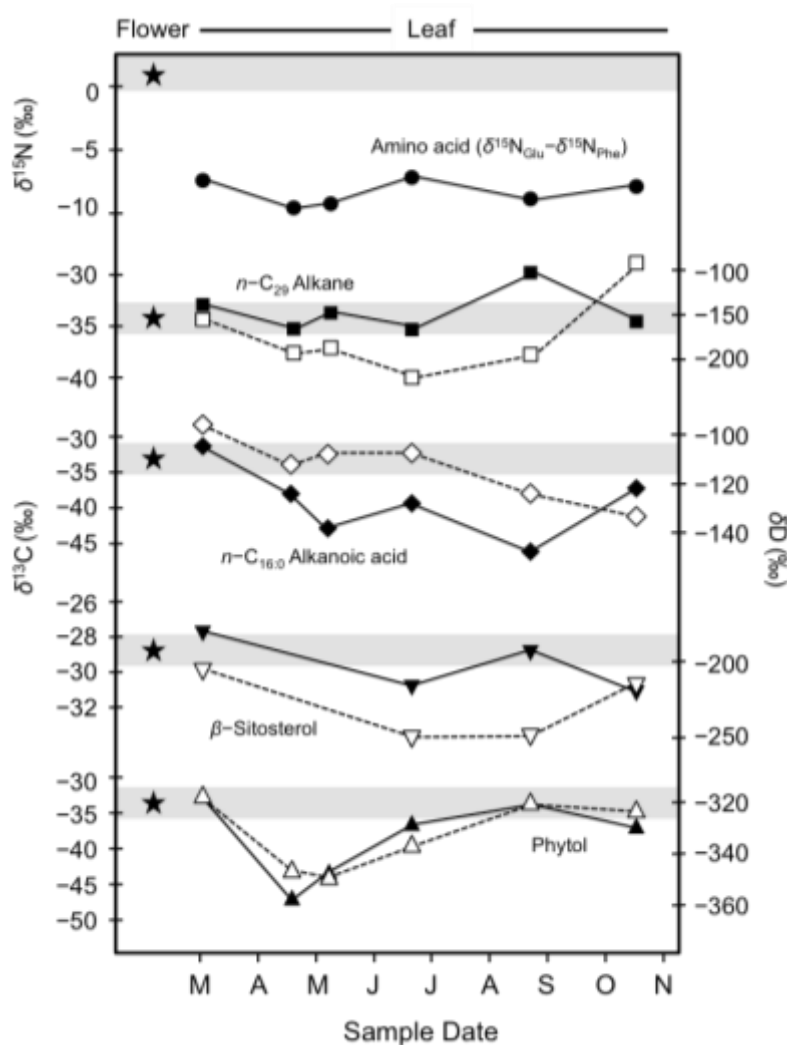
In order to characterize the isotopic compositions of storage-derived lipids and to illuminate the contribution of those to leaf lipids during the growing season, in this study we compared multi-element isotopic compositions ( $\delta\text{D}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) of amino acids and lipids (*n*-alkanes, *n*-alkanoic acids, sterols, and phytols) for flowers in early spring and leaves in spring to autumn for the stone fruit plant (cherry), *Cerasus lannesiana*.

## Results and Discussion

Difference in the  $\delta^{15}\text{N}$  value between glutamic acid and phenylalanine can account to the position of trophic hierarchies (TP) in the ecological, energetic food web, as  $-8.4\text{‰}$  and  $-0.8\text{‰}$  correspond to primary producers (TP=1.0) and primary consumers (TP=2.0), respectively. This difference is  $0.9\text{‰}$  (TP=2.2) and  $-8.3 \pm 1.0\text{‰}$  (TP=1.0 $\pm$ 0.1) for flowers in spring and leaves in March-November, respectively, for cherry in this study (Fig. 1), which well illustrate (1) the utilization of storage molecules (i.e., heterotrophic production) for blooming in early spring when no leaf operates photosynthesis, and (2) autotrophic production for leafing in March-November when mature leaves operate photosynthesis. Thus, heterotrophic production solely occurs for blooming and the isotopic compositions of flowers well record such ‘heterotrophic production’ in plant phenology.

The  $\delta^{13}\text{C}$  and  $\delta\text{D}$  values of lipids in leaves are not always clearly distinguishable from those in flowers (Fig. 1). Although the  $\delta^{13}\text{C}$  and  $\delta\text{D}$  values for plant leaves vary significantly ( $>10\%$  for  $\delta^{13}\text{C}$ ,  $>100\%$  for  $\delta\text{D}$ ) through season, these values are frequently close to those for plant flowers (i.e., heterotrophic production). For all lipids examined, there is a common trend that both  $\delta^{13}\text{C}$  and  $\delta\text{D}$  values of leaf lipids in spring are almost identical to those of the flower lipids in early spring. In contrast, leaves have *n*-alkanes, *n*-alkanoic acids, and sterols deviated from flower values in summer, but phytol deviated in spring. To interpret the significant temporal variation in the isotopic compositions of lipids, we suggest a two end member model, heterotrophic production vs. autotrophic production, within leaf lipids: (1) leaf lipids are produced with the storage molecules primarily at leafing in spring, which is characterized by less depletion in both  $^{13}\text{C}$  and D as similar to flower lipids, (2) such heterotrophic production is much active even in growing season, (3) further depletion in  $^{13}\text{C}$  and D of lipids is highly dependent on the contribution of autotrophic production, and (4) balance in autotrophic vs. heterotrophic productions allows much heterogeneity in the  $\delta^{13}\text{C}$  and  $\delta\text{D}$  values of leaf lipids throughout season even in summer.

Fig. 1. Seasonal changes in amino acid  $\delta^{15}\text{N}$  and lipid  $\delta^{13}\text{C}$  and  $\delta\text{D}$  values (‰) of flowers (star) and leaves (circle, square, diamond, and triangle) in the stone fruit plant *Cerasus lannesiana*. Filled and open symbols indicate the  $\delta^{13}\text{C}$  and  $\delta\text{D}$  values, respectively, in the leaf lipids. The gray mesh indicates the amino acid  $\delta^{15}\text{N}$  and lipid  $\delta^{13}\text{C}$  and  $\delta\text{D}$  in flower.



## References

- Sessions 2006, Seasonal changes in D/H fractionation accompanying lipid biosynthesis in *Spartina alterniflora*, *Geochimica et Cosmochimica Acta* **70**, 2153-2162.
- Sachse et al. 2015, Seasonal variation of leaf wax *n*-alkane production and  $\delta^2\text{H}$  values from the evergreen oak tree, *Quercus agrifolia*, *Isotopes in Environmental and Health Studies* **51**, 124-142.