15th Annual Conference of the Metabolomics Society
METABOLOMICS 2019
JUNE 23–27 | THE HAGUE

Unveiling non-cyclic flux modes in the plant TCA cycle by GC-MS-based ^{13}C-positional labelling

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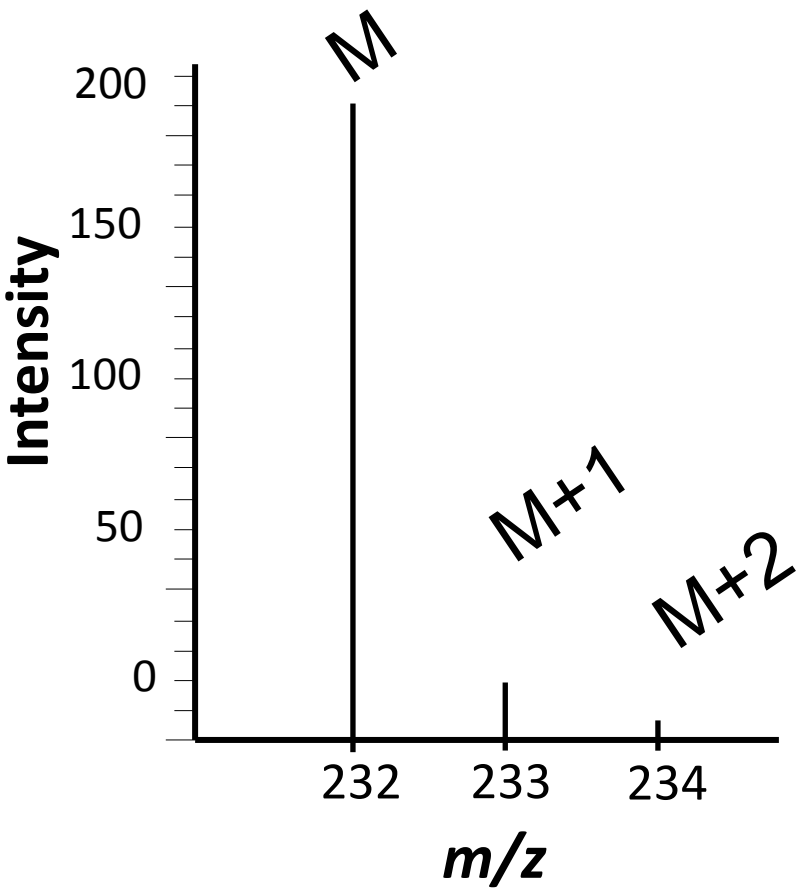
Federal University of Ceará, Fortaleza/CE, Brazil.

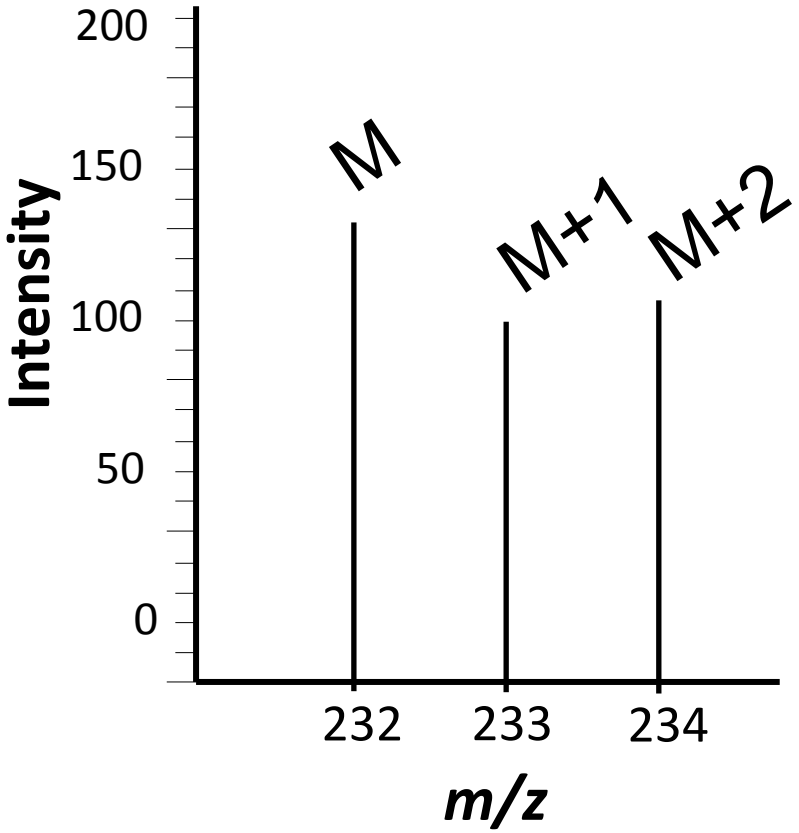
Contents

Metabolite profiling *vs* MFA

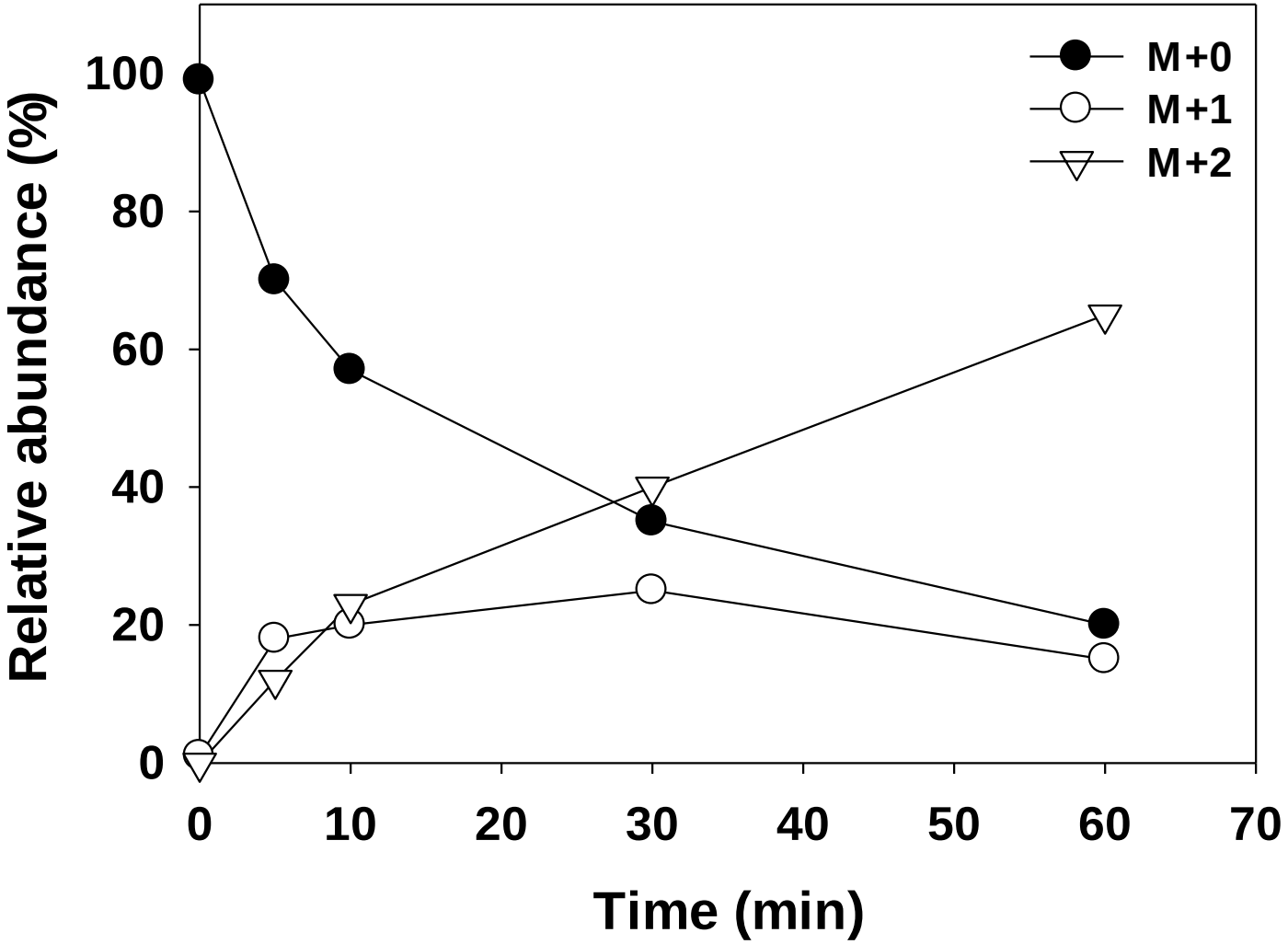
○ ^{12}C
● ^{13}C

M ○○ – monoisotopic peak
M+1 ●○ ○● – incorporation of one ^{13}C
M+2 ●● – incorporation of two ^{13}C





Mass spectral of a full labelled metabolite

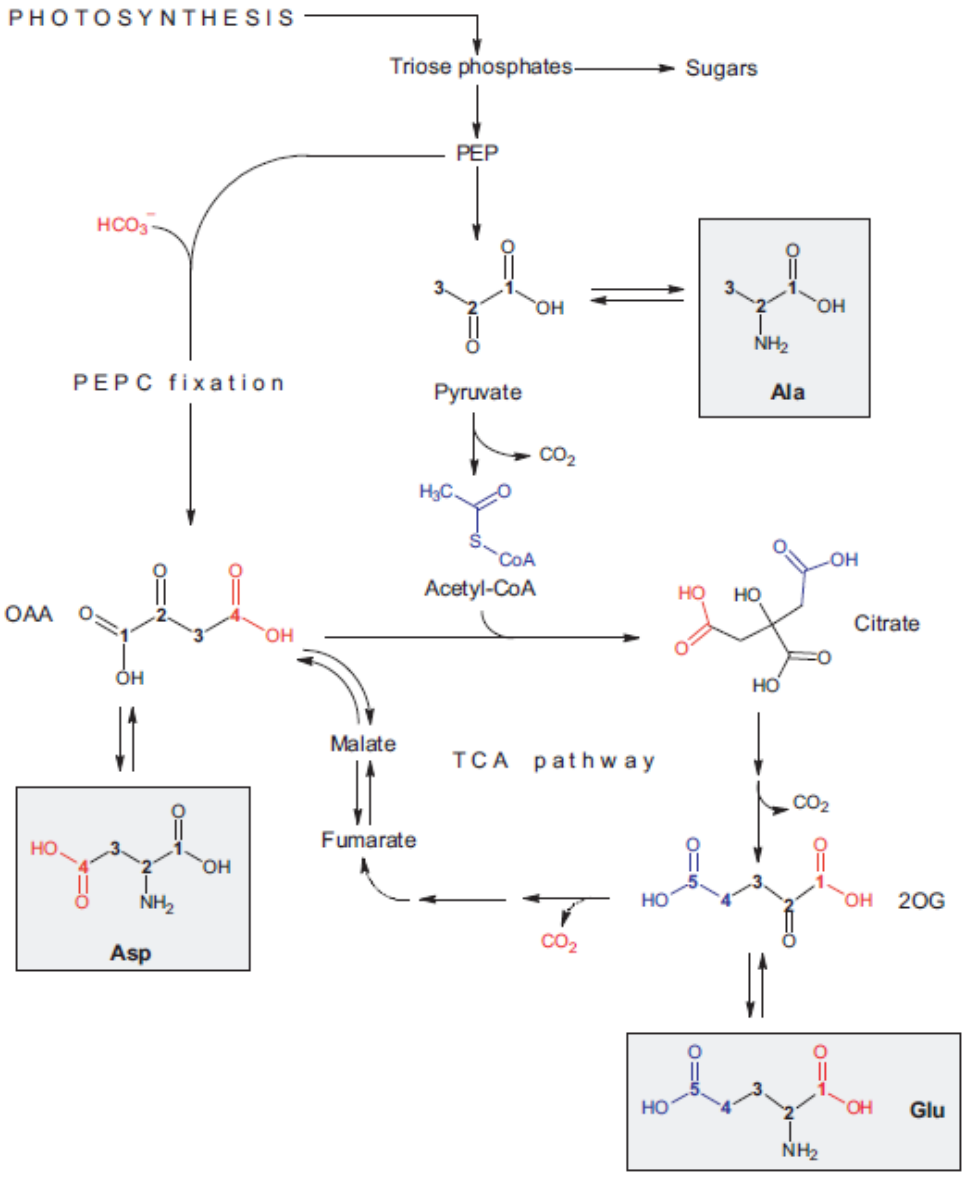


Lima et al. (2018) Plant metabolomics

Metabolite profiling *vs* MFA

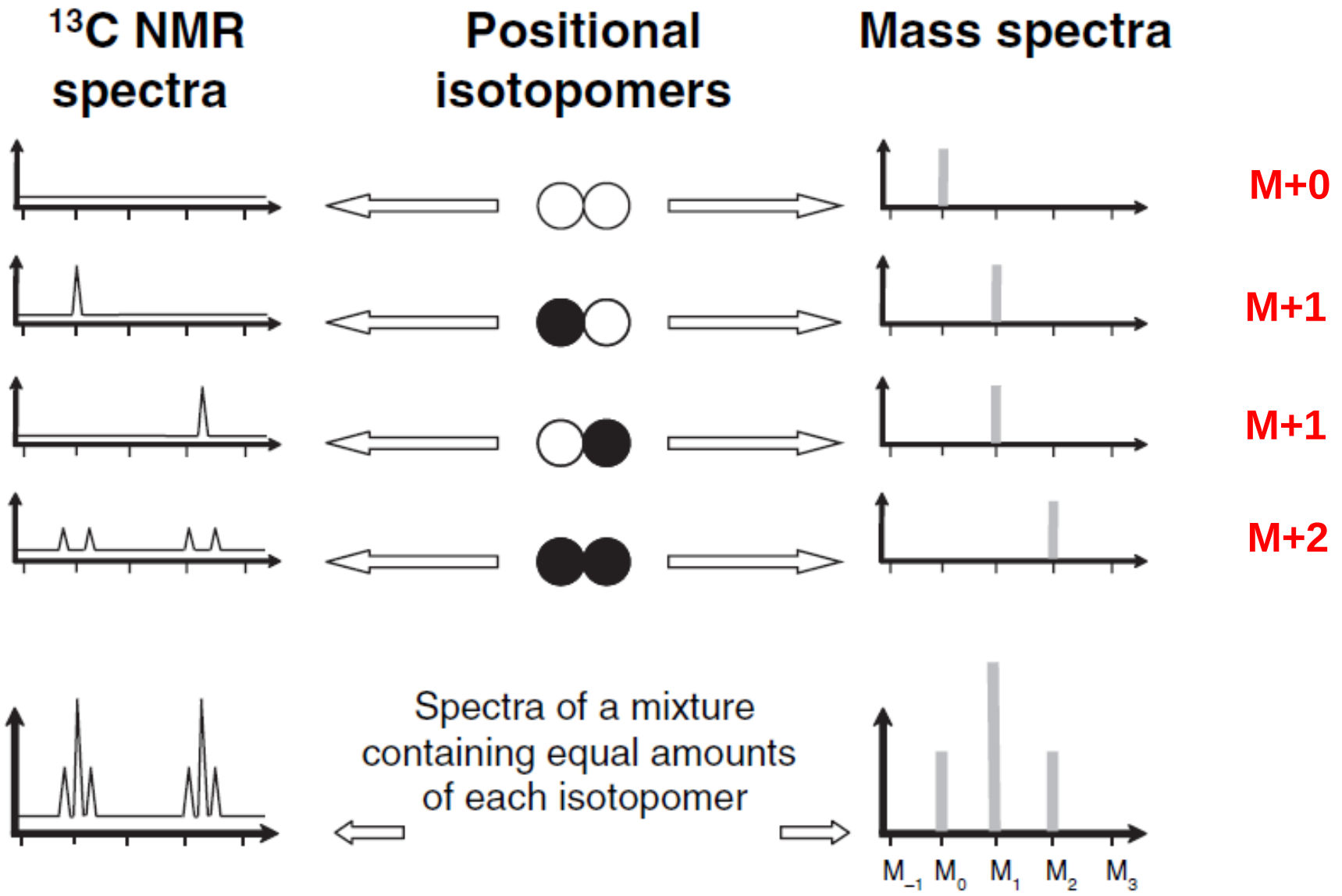
Ex: The level of Glu increase in plants transferred to light

Hypothesis:
Light
↳ **Photosynthesis**
↳ **Glutamate**



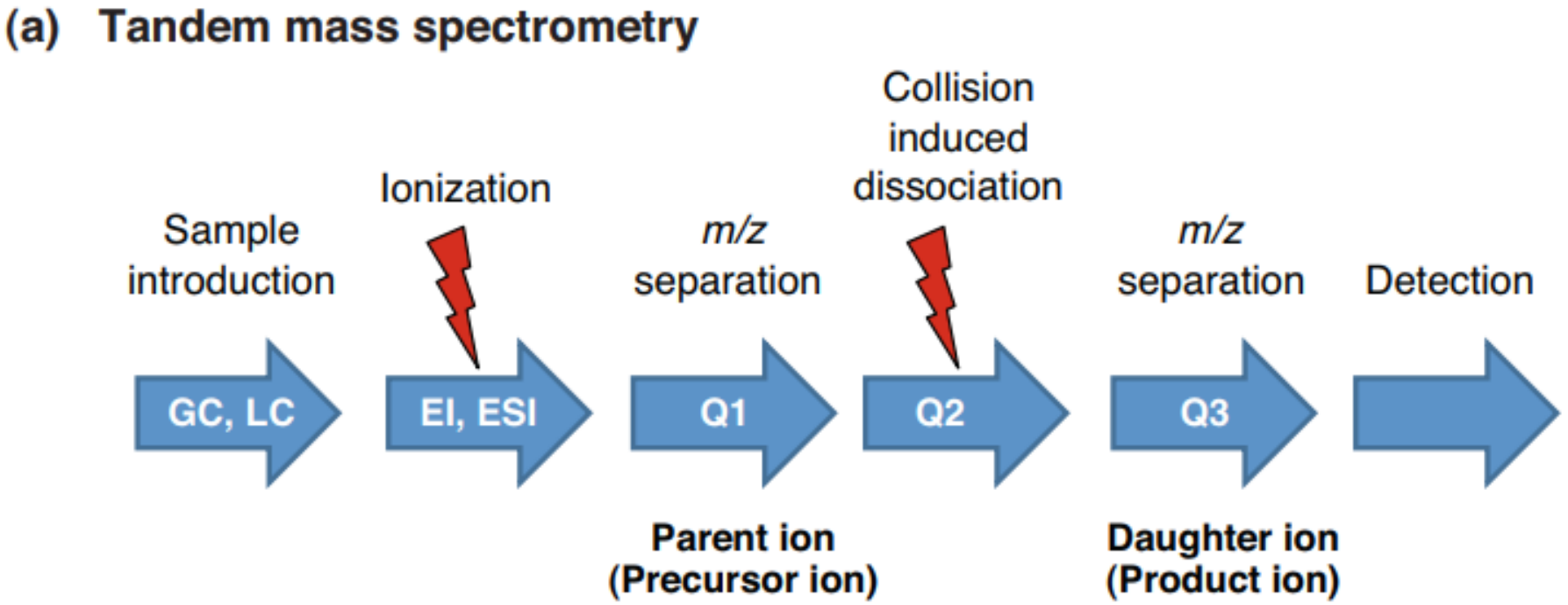
Using positional isotope labelling to investigate the source of C for Glu synthesis

Positional isotope labelling – NMR *vs* MS



Ratcliffe and Shachar-Hill (2006) Plant Journal

Positional isotope labelling via MS/MS

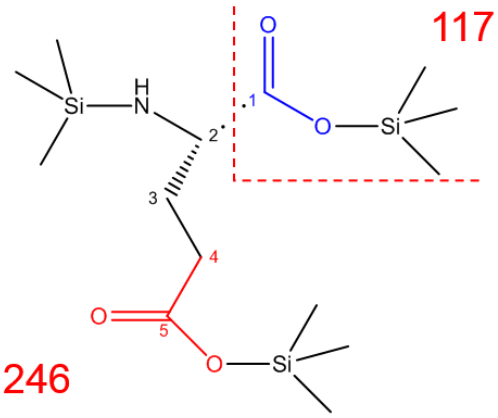


Antoniewicz et al. (2013) *Curr Op Biotech*

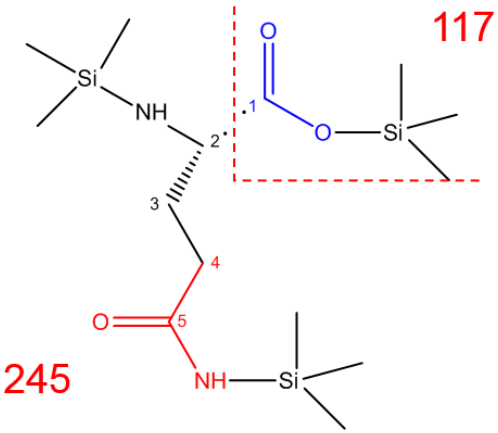
Positional isotope labelling via GC-EI-MS

Which fragment should I use?

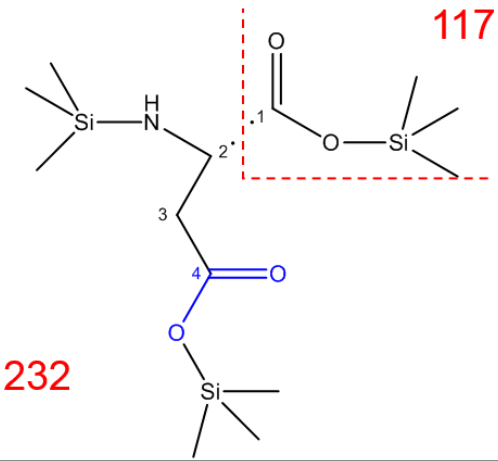
Glutamate (3TMS)



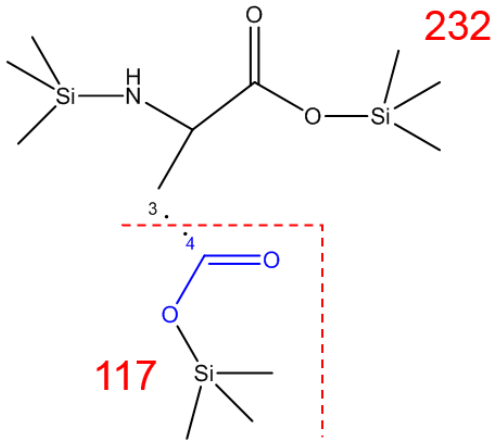
Glutamine (3TMS)



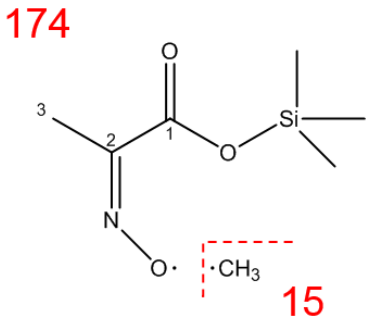
Aspartate (3TMS)



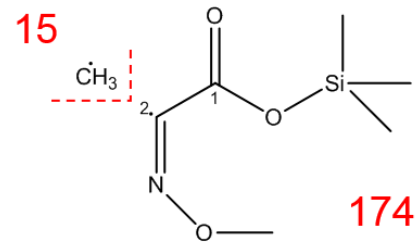
Aspartate (3TMS)



Pyruvate (1MEOX)(1TMS)



Pyruvate (1MEOX)(1TMS)



Applying the positional isotope labelling

New Phytologist

Research

Direct assessment of the metabolic origin of carbon atoms in glutamate from illuminated leaves using ^{13}C-NMR

Cyril Abadie[1], Jérémy Lothier[2], Edouard Boex-Fontvieille[3], Adam Carroll[1] and Guillaume Tcherkez[1]

New Phytologist

Research

In vivo phospho*enol*pyruvate carboxylase activity is controlled by CO_2 and O_2 mole fractions and represents a major flux at high photorespiration rates

Cyril Abadie and Guillaume Tcherkez

Abadie et al. (2017) New phytologist; Abadie & Tcherkez (2018) New phytologist

Origin of C for Glu synthesis

Labelling expected:

2 ^{13}C from glycolysis

1 ^{13}C from PEPc activity

2 ^{13}C from the TCA cycle

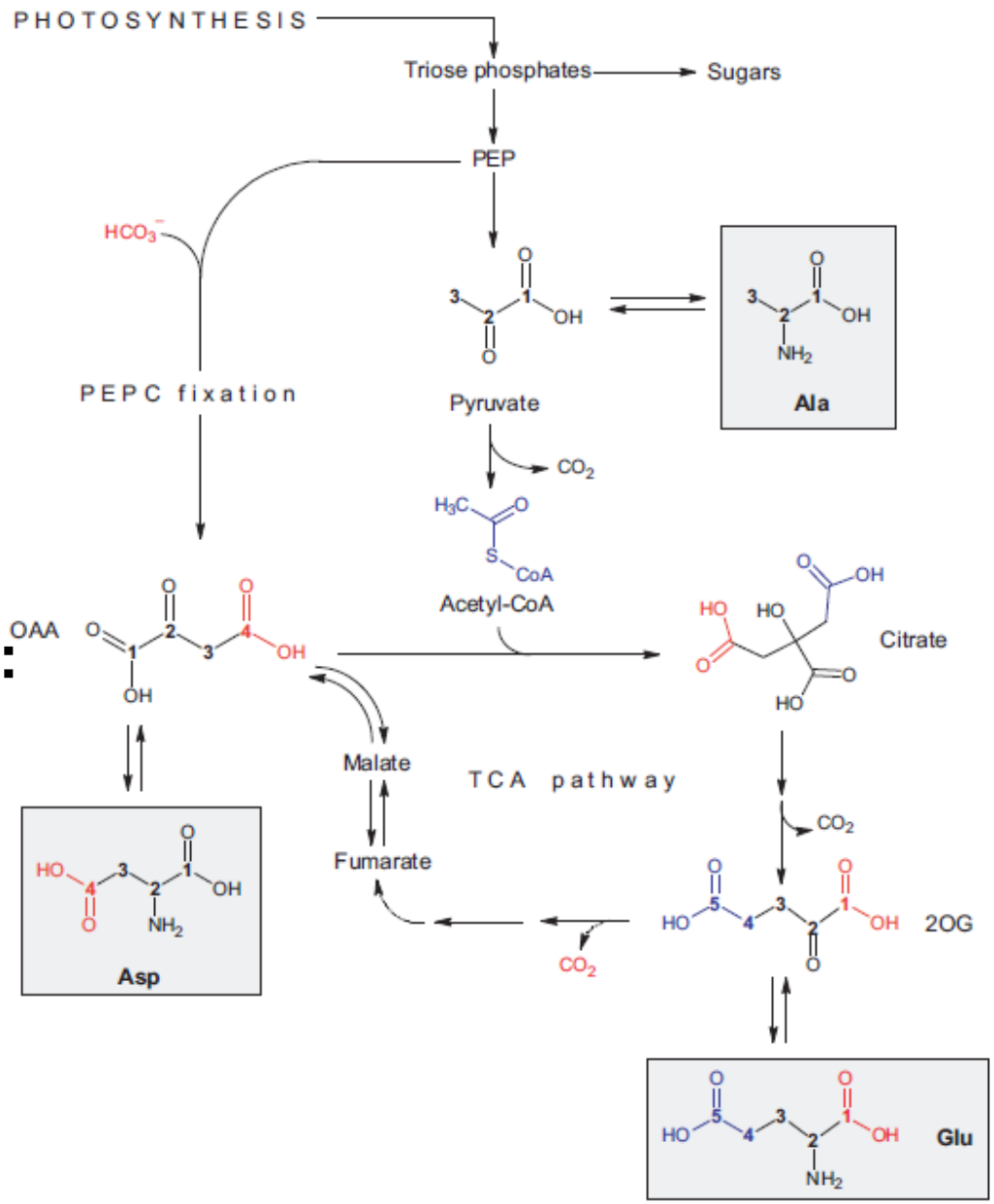
Labelling observed by NMR:

0 ^{13}C from glycolysis

1 ^{13}C from PEPc activity

0 ^{13}C from the TCA cycle

Presence of ^{12}C



Abadie et al. (2017) New phytologist

Non-cyclic flux modes of guard cell TCA cycle

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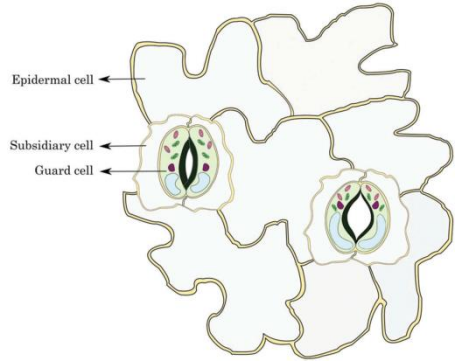

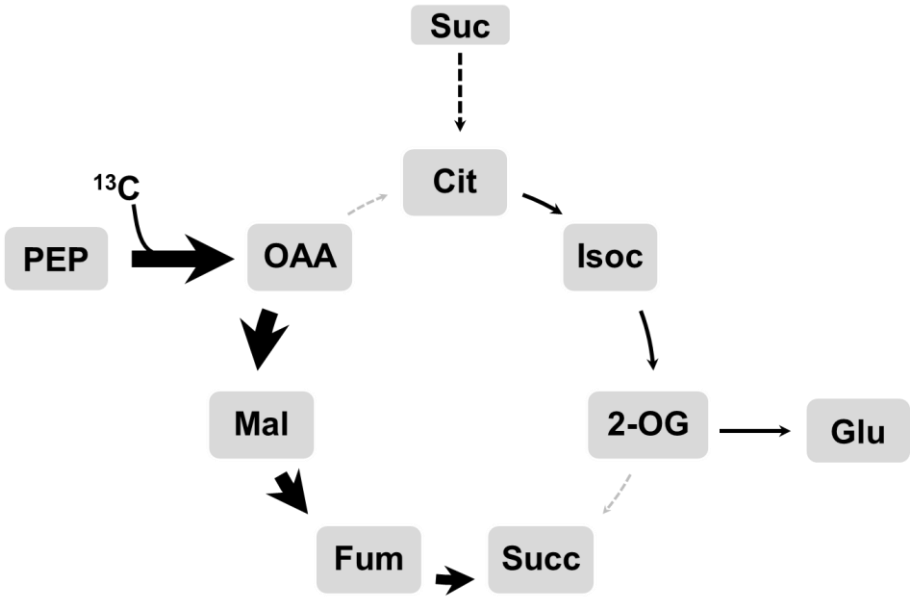

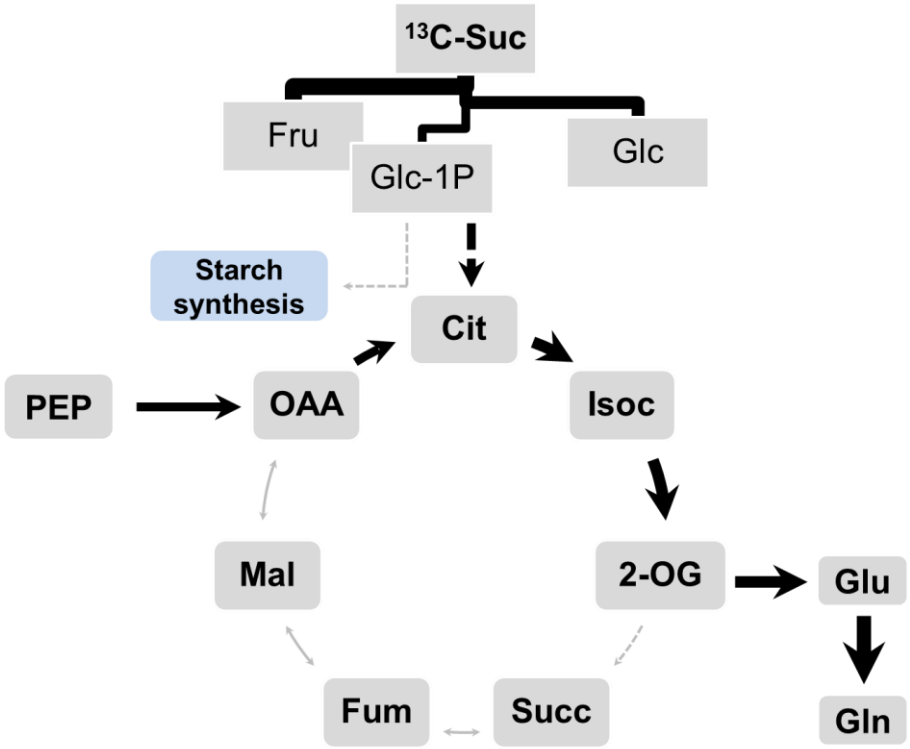

Daloso et al. (2016). New Phytologist 209

Daloso et al. (2015). Plant, Cell & Environment 38

Robaina-Estévez et al. (2017). Scientific Reports 7

Medeiros et al. (2018). Plant Journal 94

Positional isotope labelling in Glu *m/z* 246 - 146

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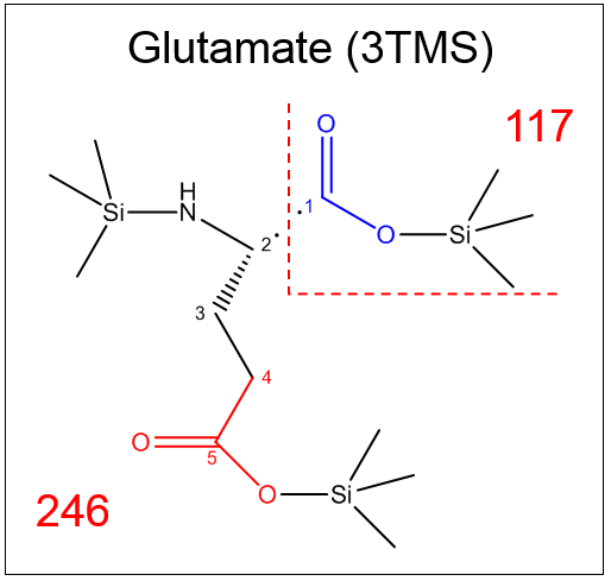
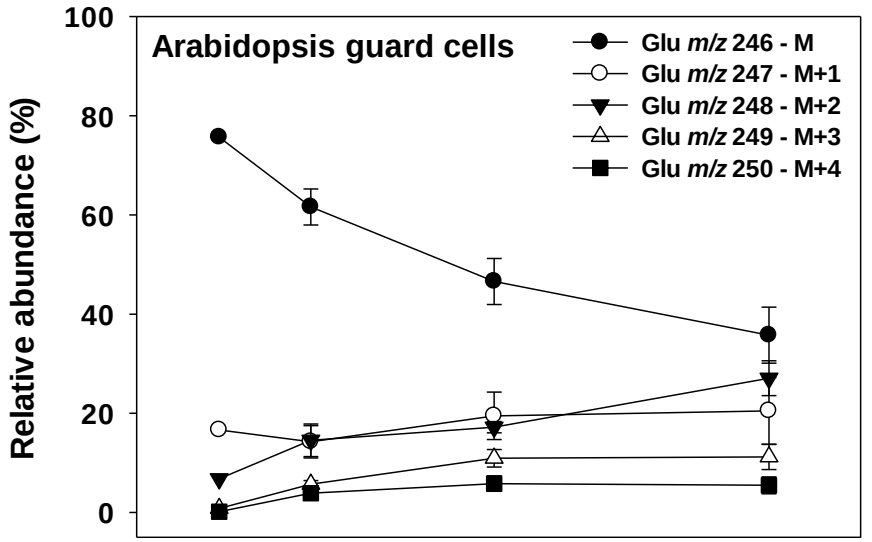
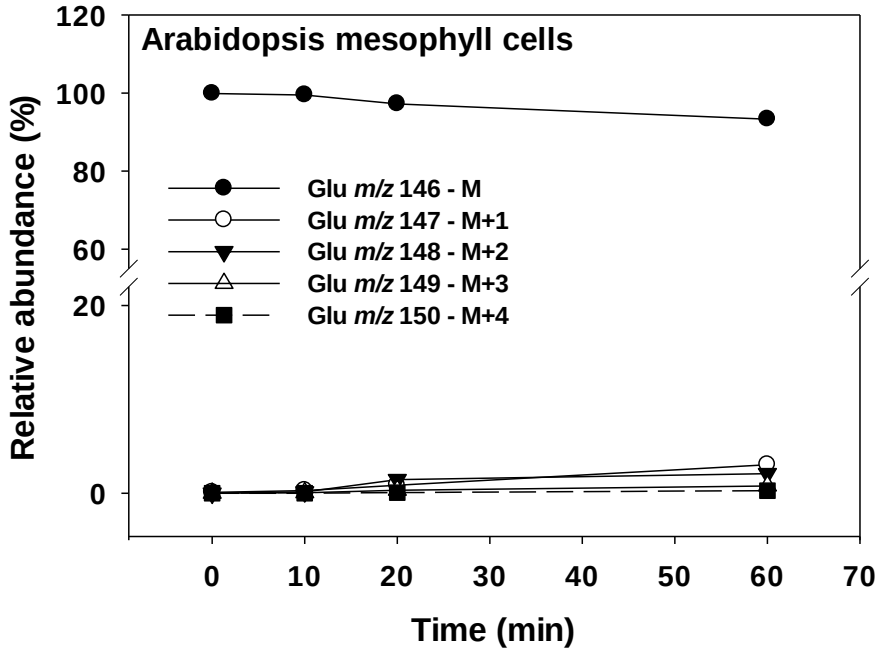


Labelled C:
***m/z* 146 - 2,3,4,5**
***m/z* 246 - 2,3,4,5**





Lima et al. (*in progress*)

Positional isotope labelling in Glu *m/z* 246 - 353

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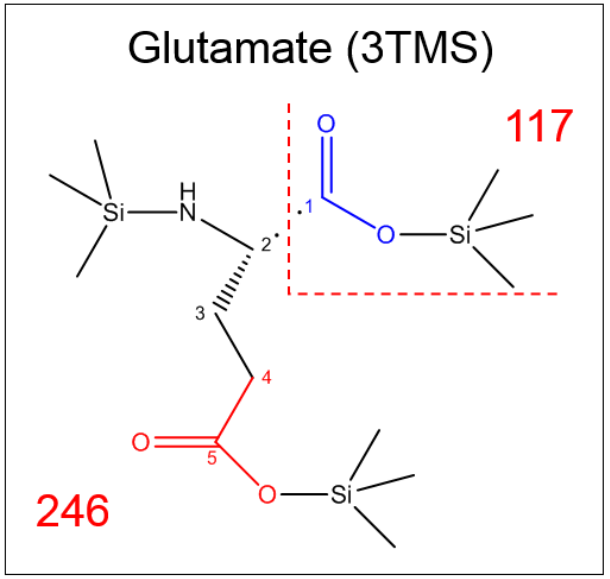
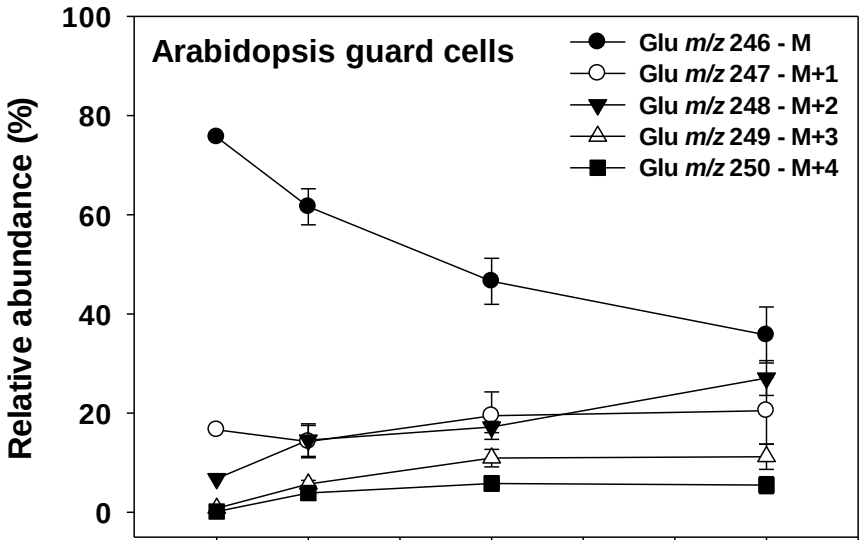
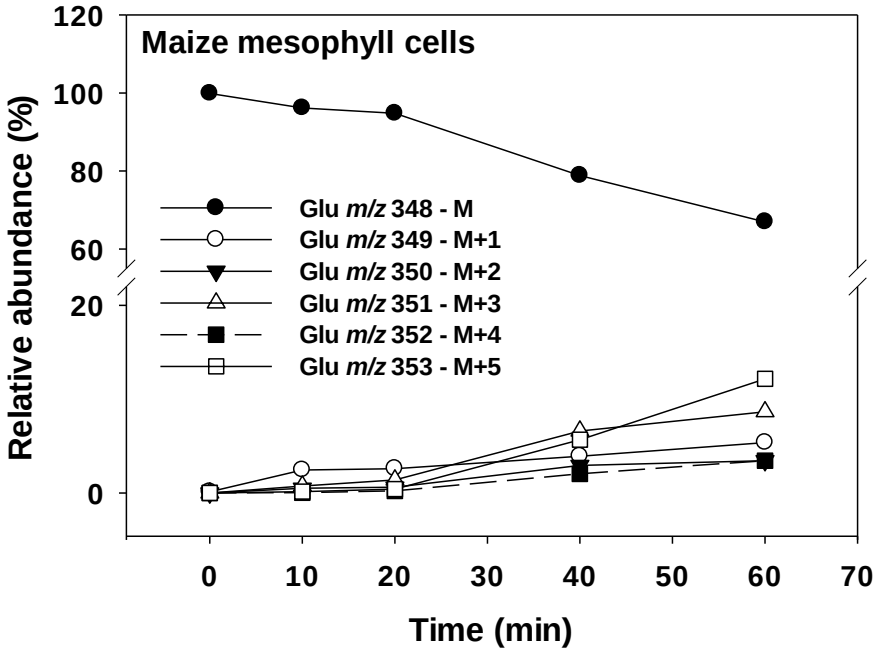


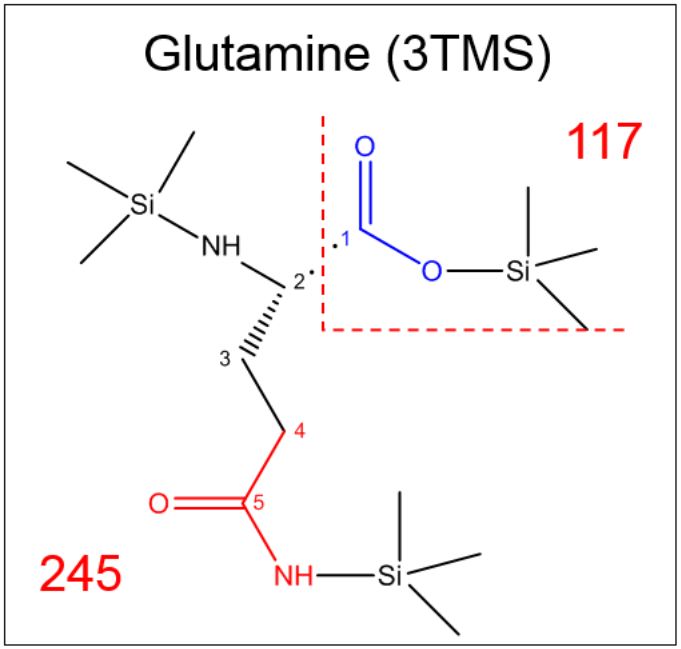
Labelled C:
***m/z* 246 - 2,3,4,5**
***m/z* 353 – 1,2,3,4,5**



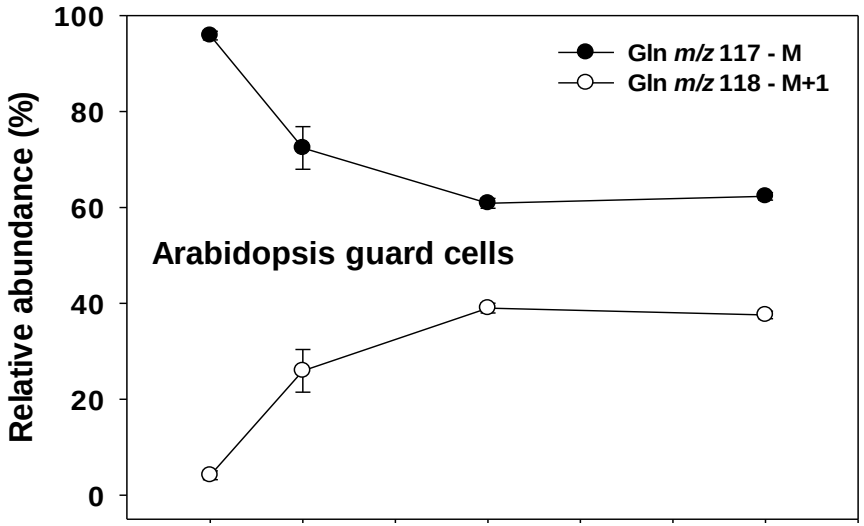


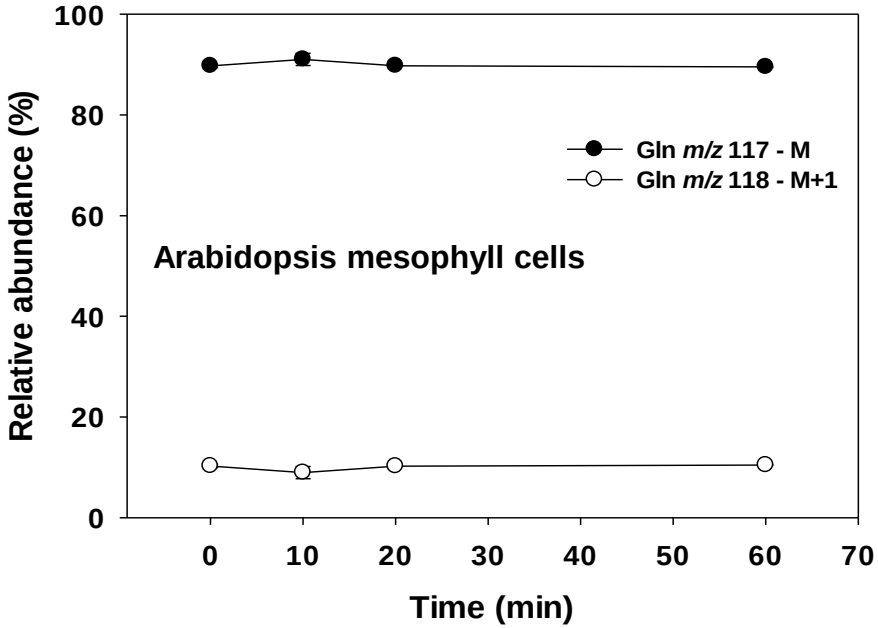
Lima et al. (*in progress*)

Positional isotope labelling in Gln *m/z* 117



Labelled C:
***m/z* 117 - 1**





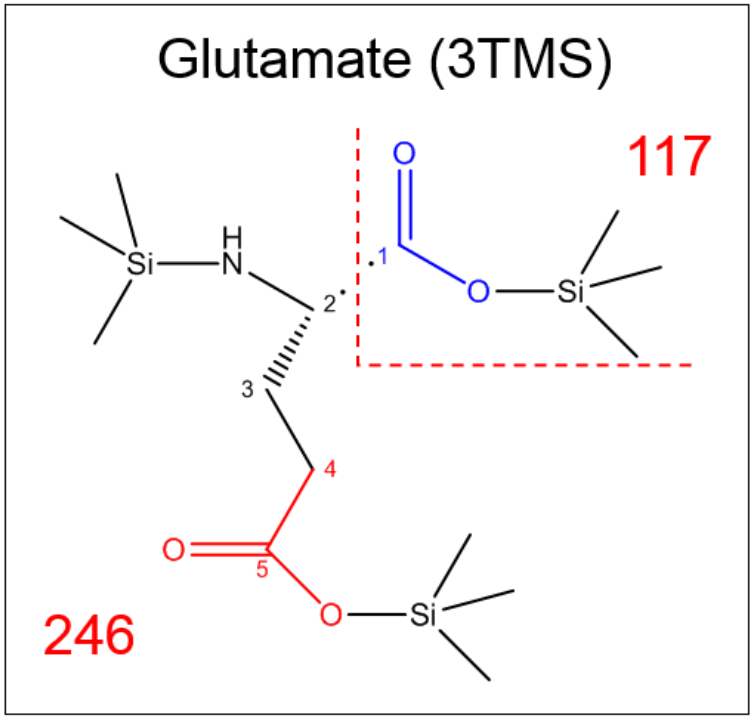
Lima et al. (*in progress*)

Take home messages

- **Do not use metabolite profiling data to discuss flux through the pathways**
- **The chosen labelled substrate will determine the fluxes that should be investigated**
- **Plant TCA cycle did not work as a cycle**
- **Positional isotope labelling is a useful approach to investigate ^{13}C redistribution throughout metabolic pathways**

Challenges and perspectives

- **To create a complete map of GC-EI-MS fragmentaion**



Glutamic acid 3TMS – GolmDB

m/z 117

m/z 128

m/z 156

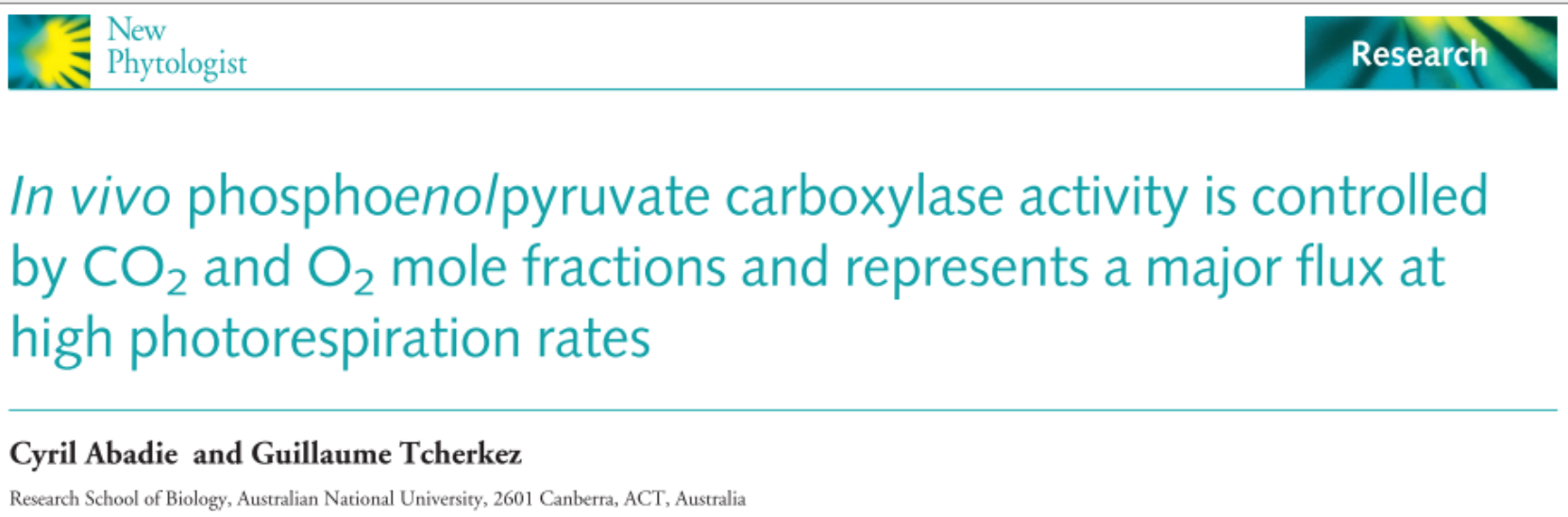
m/z 246

m/z 348

m/z 363

Challenges and perspectives

- **To discriminate small fragments from noise and TMS derivatives**
  - Dry different volumes before derivatization
  - SIM method?
- **Measure enzyme activity *in vivo* using MS data**



New Phytologist

Research

In vivo phospho*enol*pyruvate carboxylase activity is controlled by CO_2 and O_2 mole fractions and represents a major flux at high photorespiration rates

Cyril Abadie and Guillaume Tcherkez

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Acknowledgements

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LABPlant
Laboratory of Plant Metabolism

Financial support:

CNPq

Acknowledgements

Thanks for your attention