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Komarov Botanical Institute
Founded in 1714

LECO Simply GC×GC®

Gregory Pozhvanov[1], Alexey Shavarda[2,3]

Enhancing the Analysis of Plant Tissue using Pegasus 4D Comprehensive Gas Chromatography – Time of Flight Mass Spectrometry

LECO workshop: metabolomics
Berlin, 27–28.11

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GC×GC-TOFMS
Leco Pegasus 4D

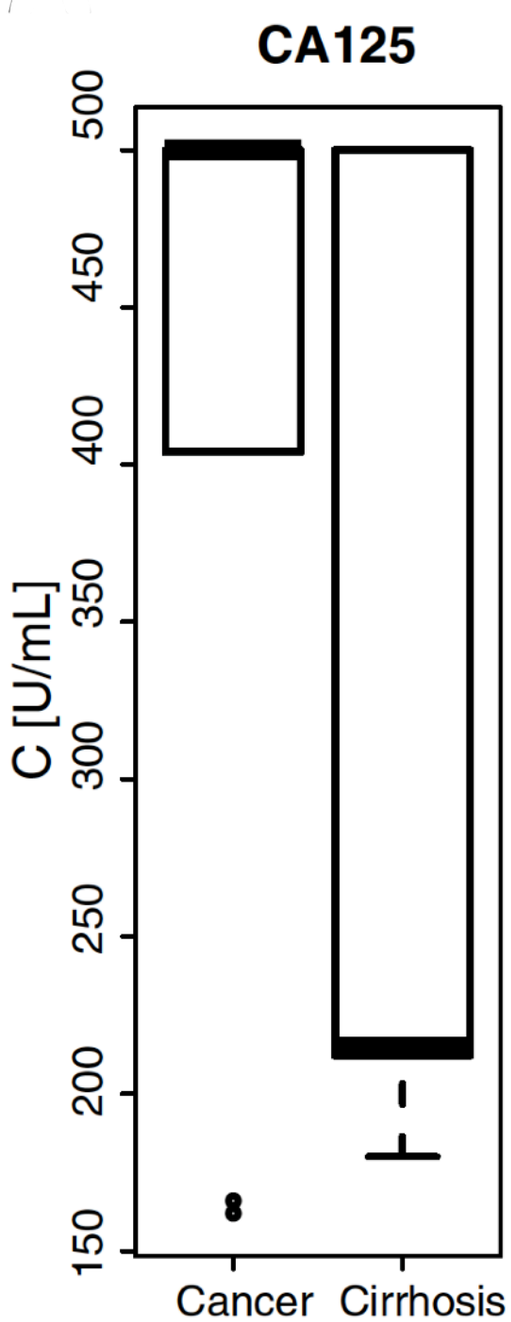
LC-MS
Agilent 6538

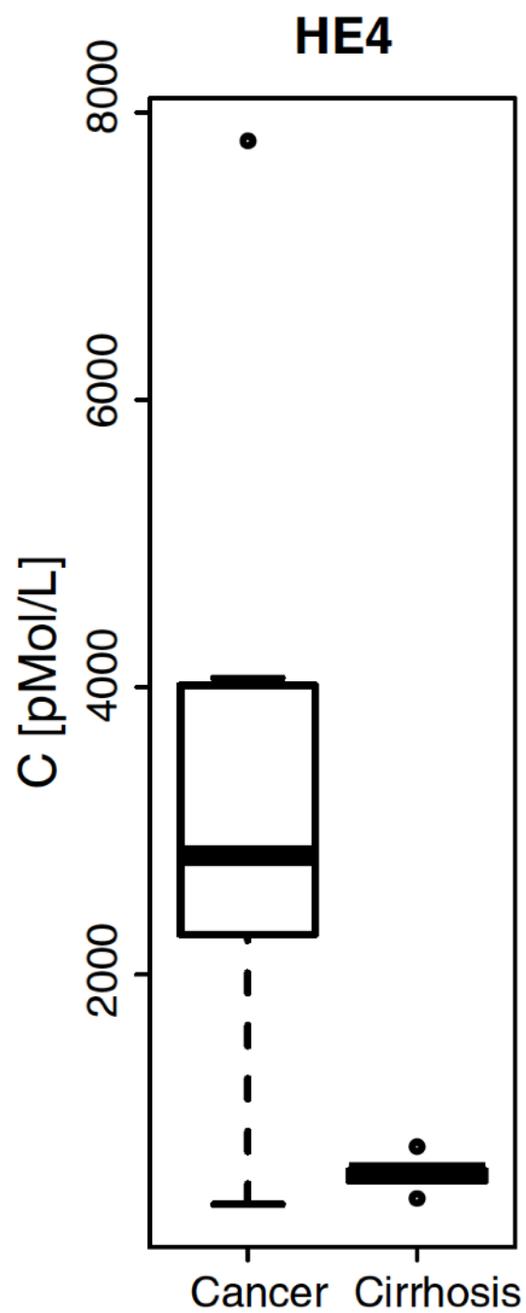
MALDI
Bruker ultraflextreme

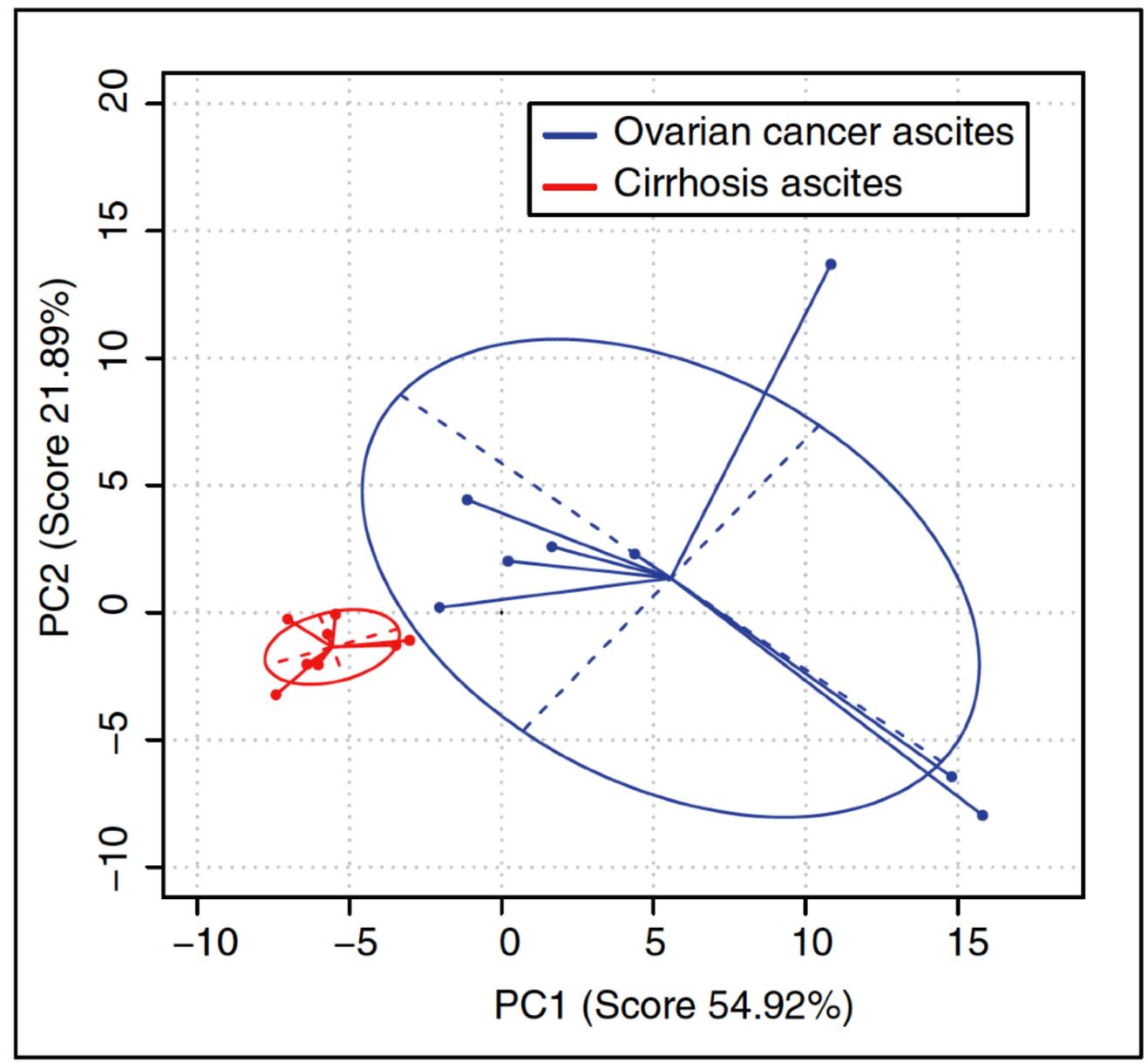
Confocal
Leica TCS SP5 MP

EM
Jeol JEM-1400

Metabolome Profiling of Ovarian Cancer Ascites







Shender et al., Molecular and Cellular Proteomics, 2014

Metabolic activities of *Bacteroides fragilis* OMVs

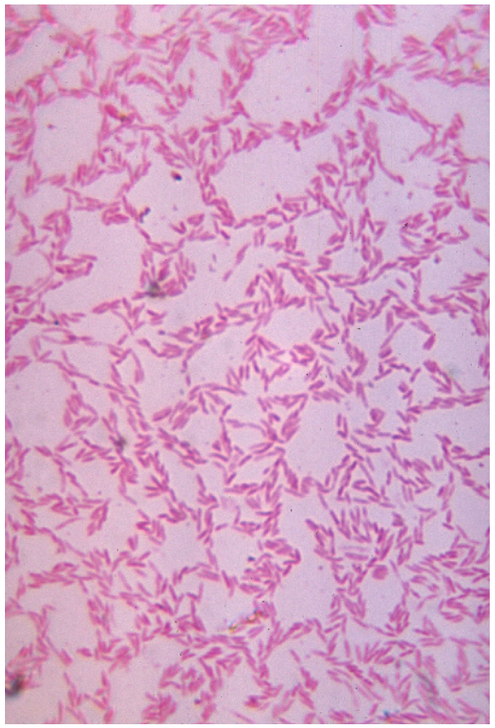
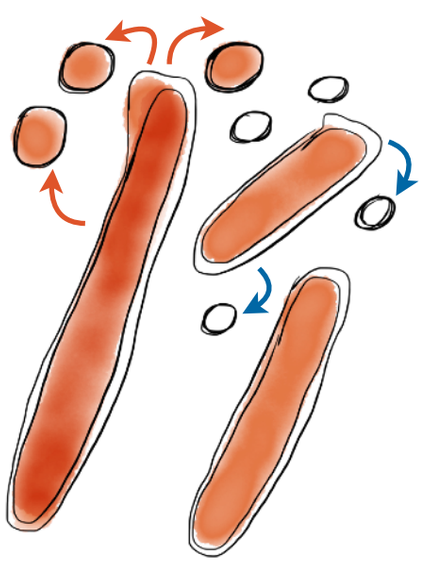
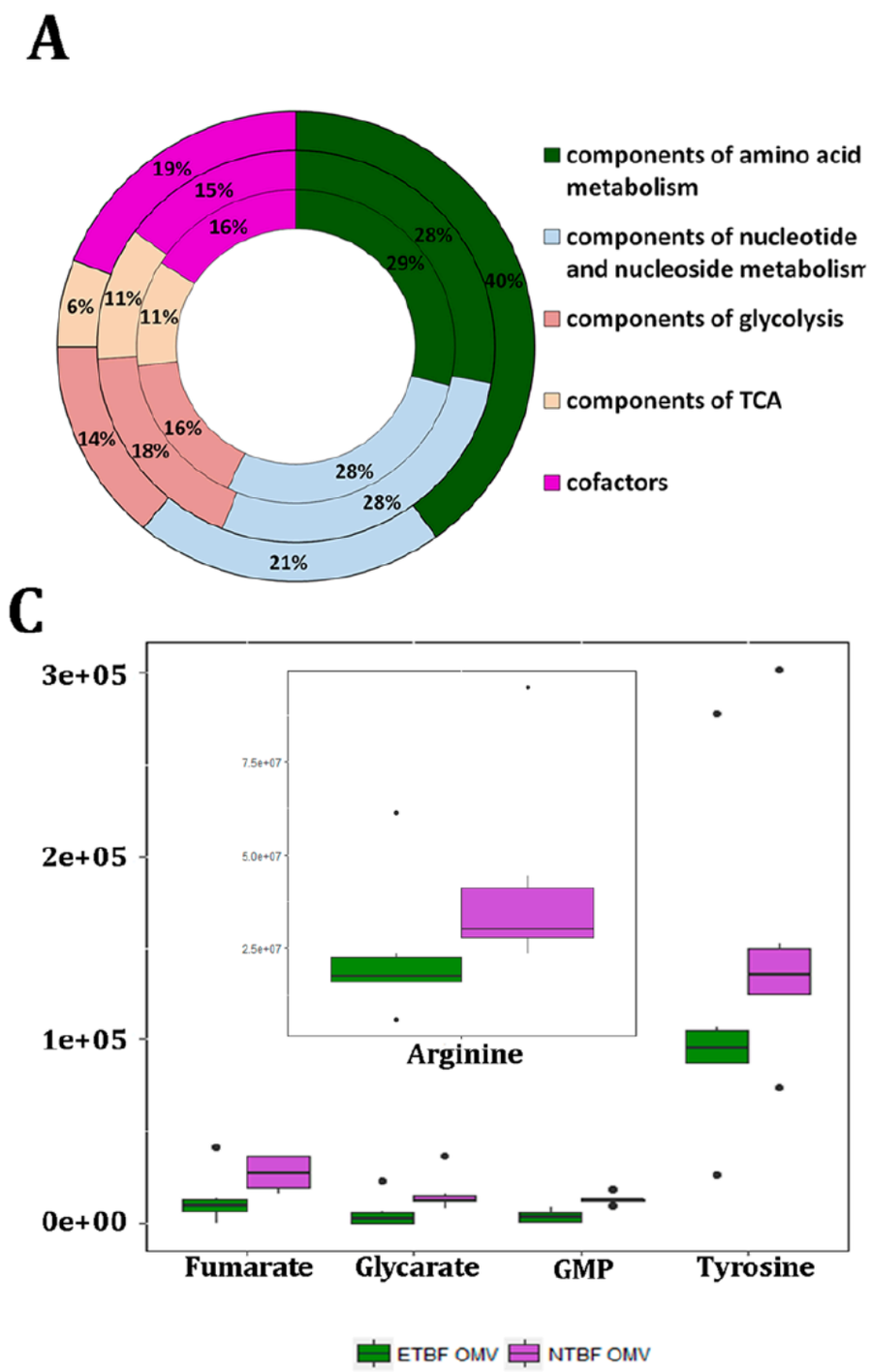


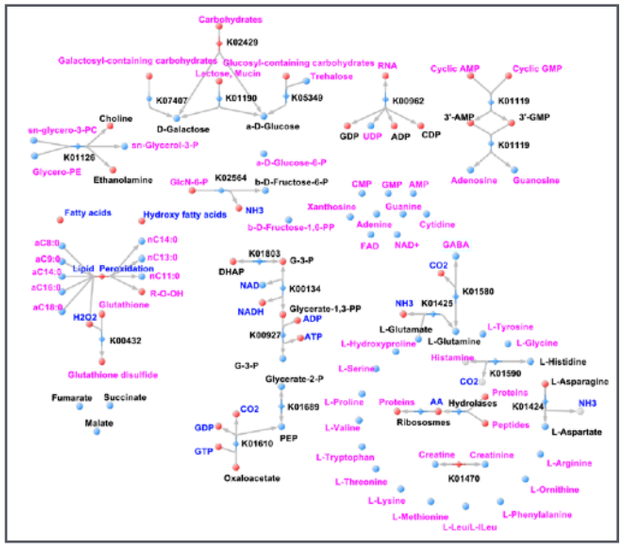
Image: CDC/Dr. V.R. Dowell, Jr. (PHIL #3087), 1972.





Zakharzhevskaya et al., Scientific Reports, 2017

Metabolic activities of *Bacteroides fragilis* OMVs



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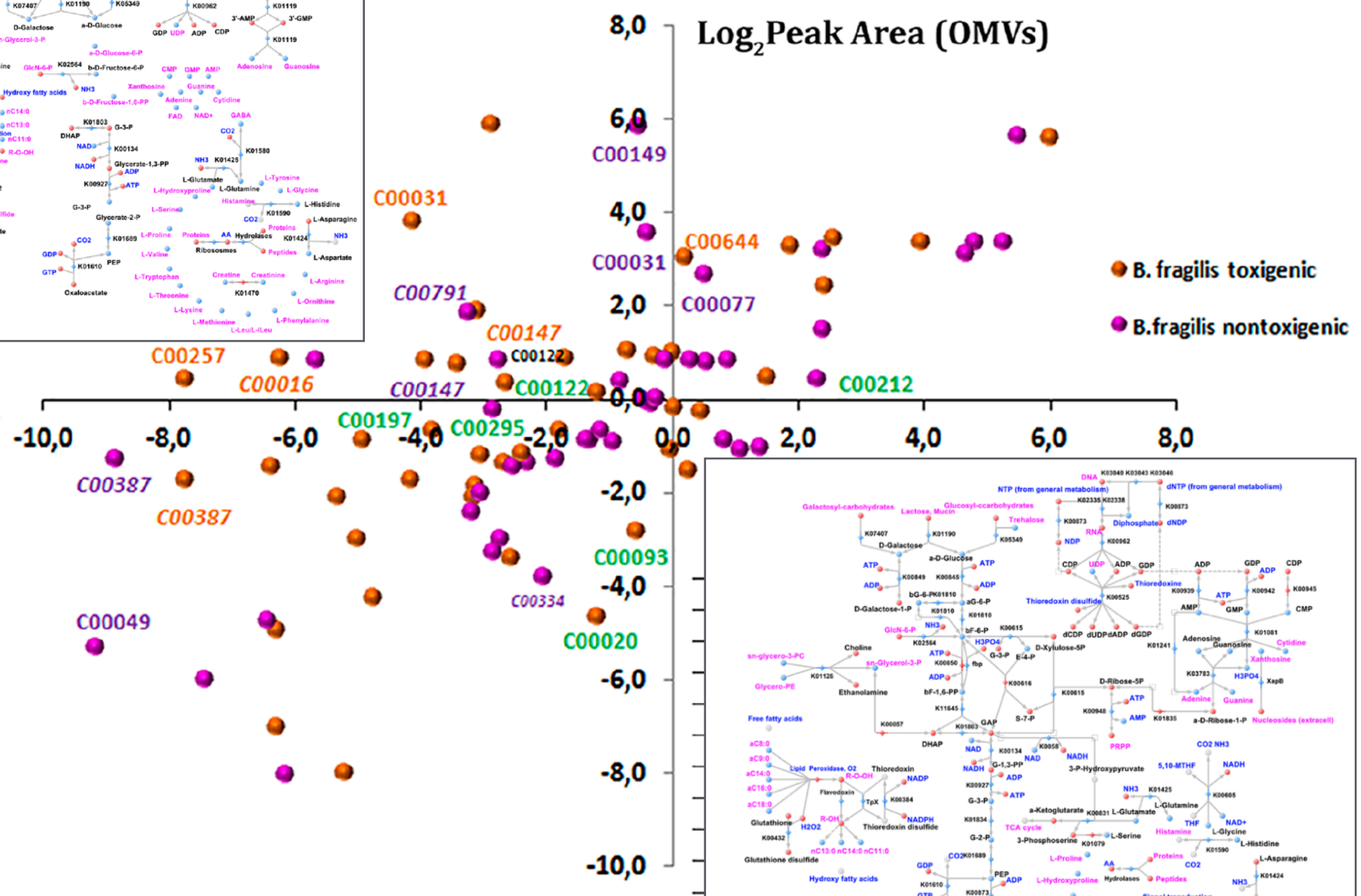

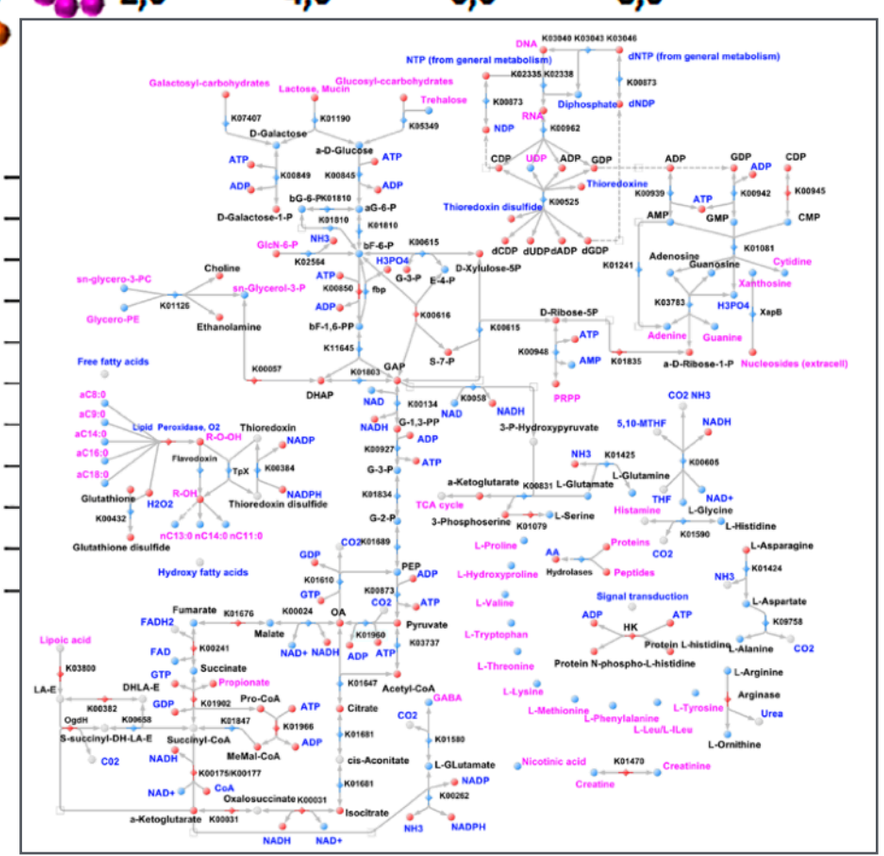

ETBF

Zakharzhevskaya et al., Scientific Reports, 2017

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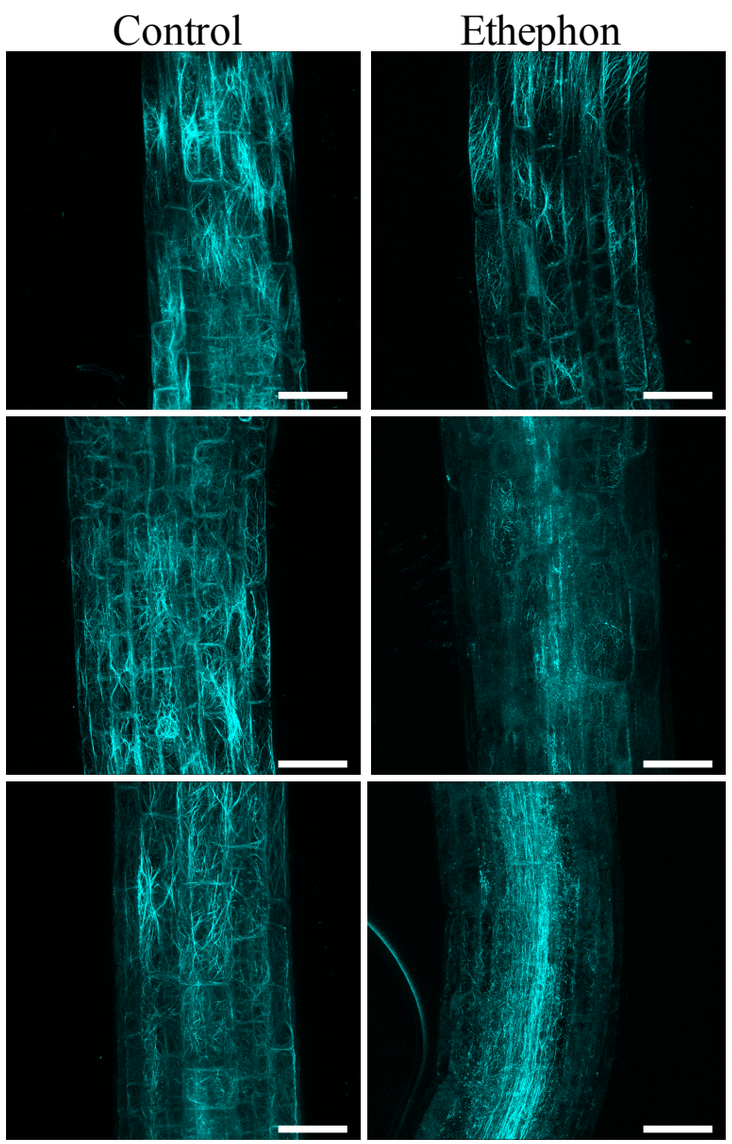
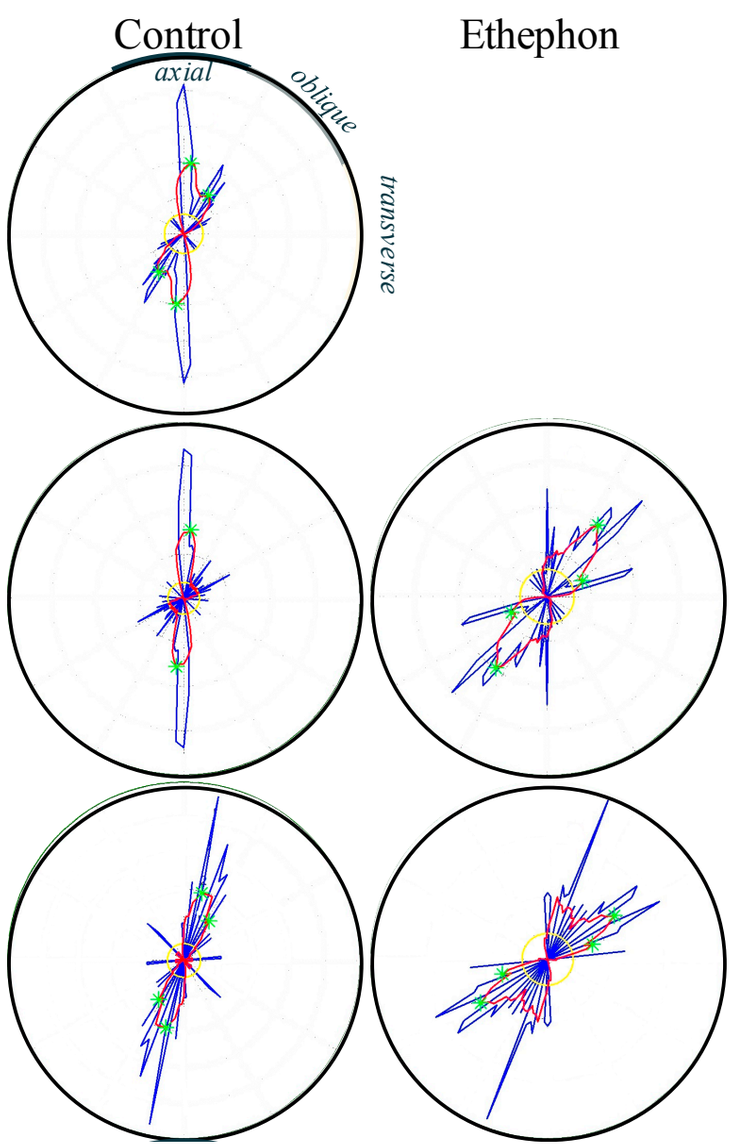
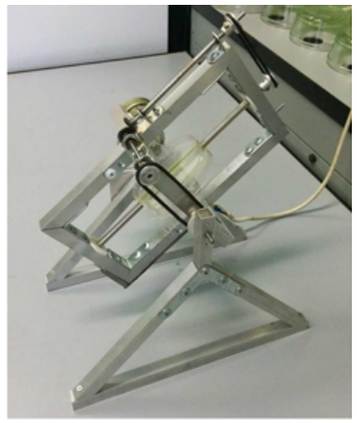
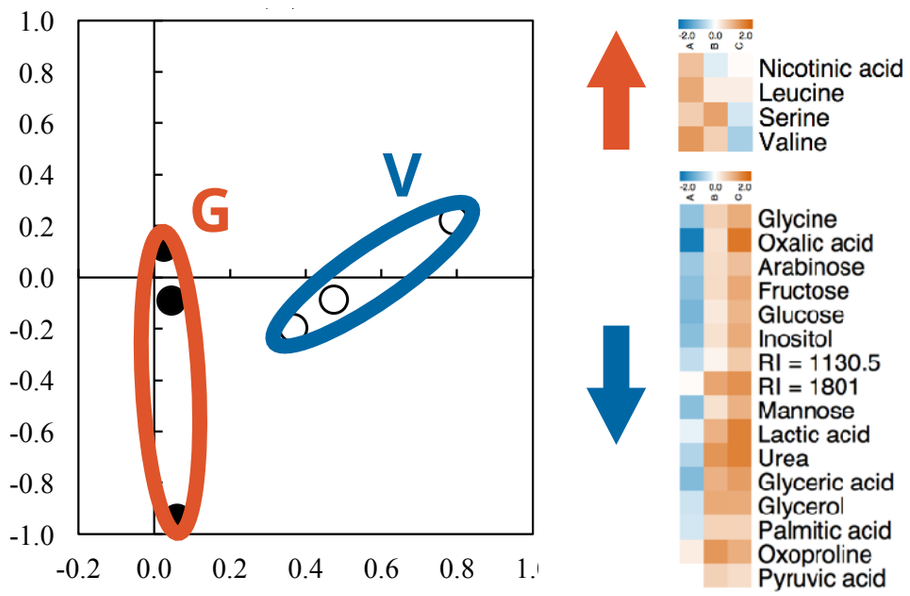
Plant cell polarity and gravitational biology group

Prof. **Sergei Medvedev**
St. Petersburg
State University









Pozhvanov et al., 2016; Pozhvanov et al., 2017.

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Microgravity modelling and gravitropism

$\vec{g}$

Earth

μg

Space

clinorotation

Earth

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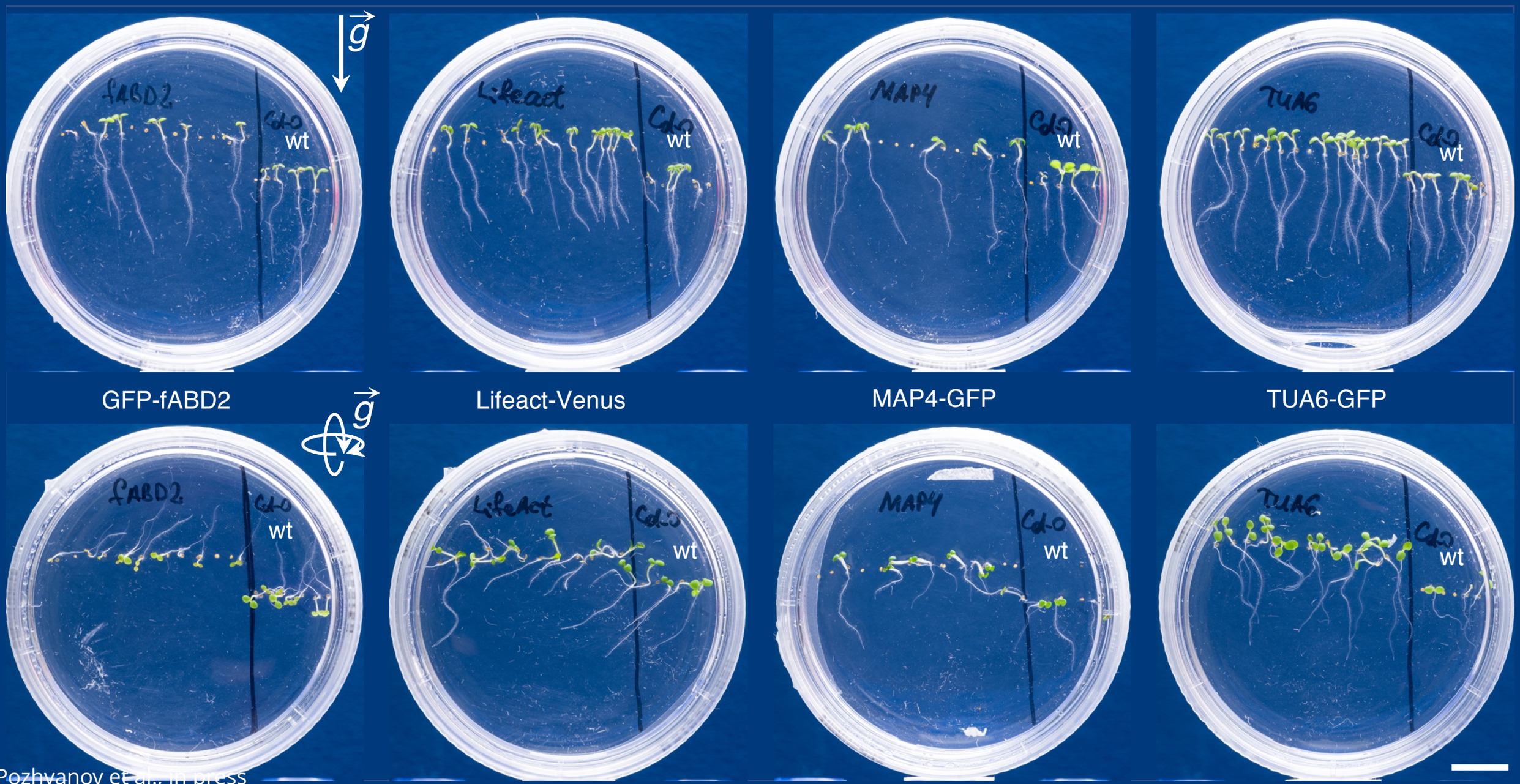
Microgravity modelling by 3D-clinorotation affects plant growth and development



Pozhvanov et al., in press

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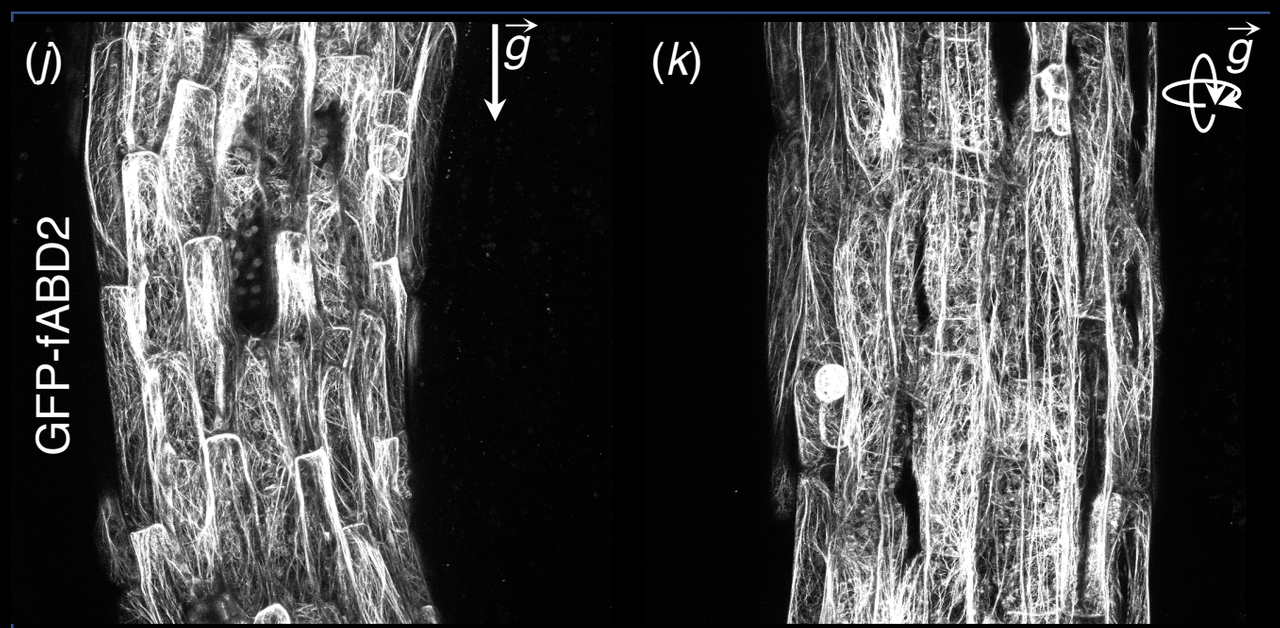
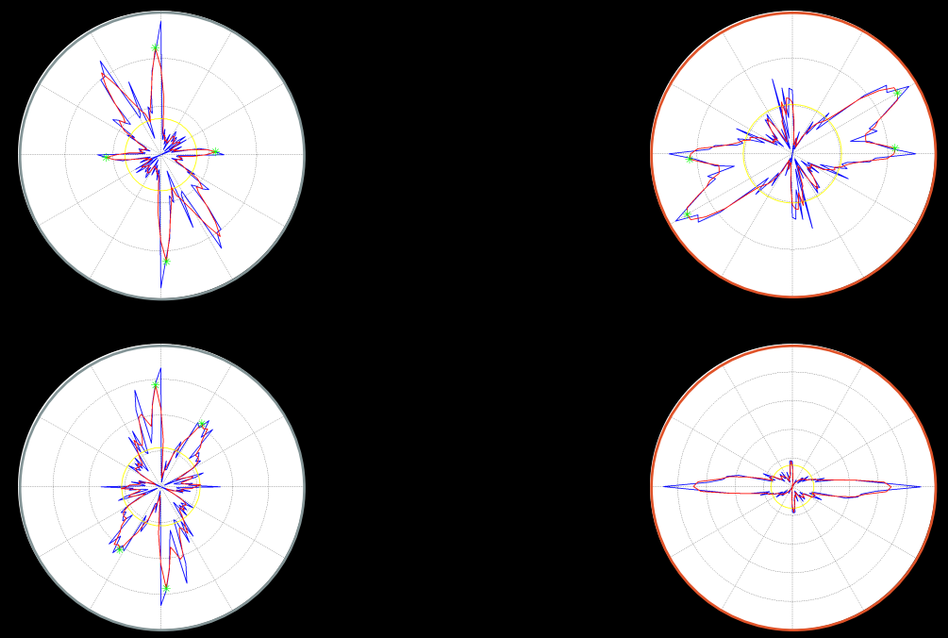
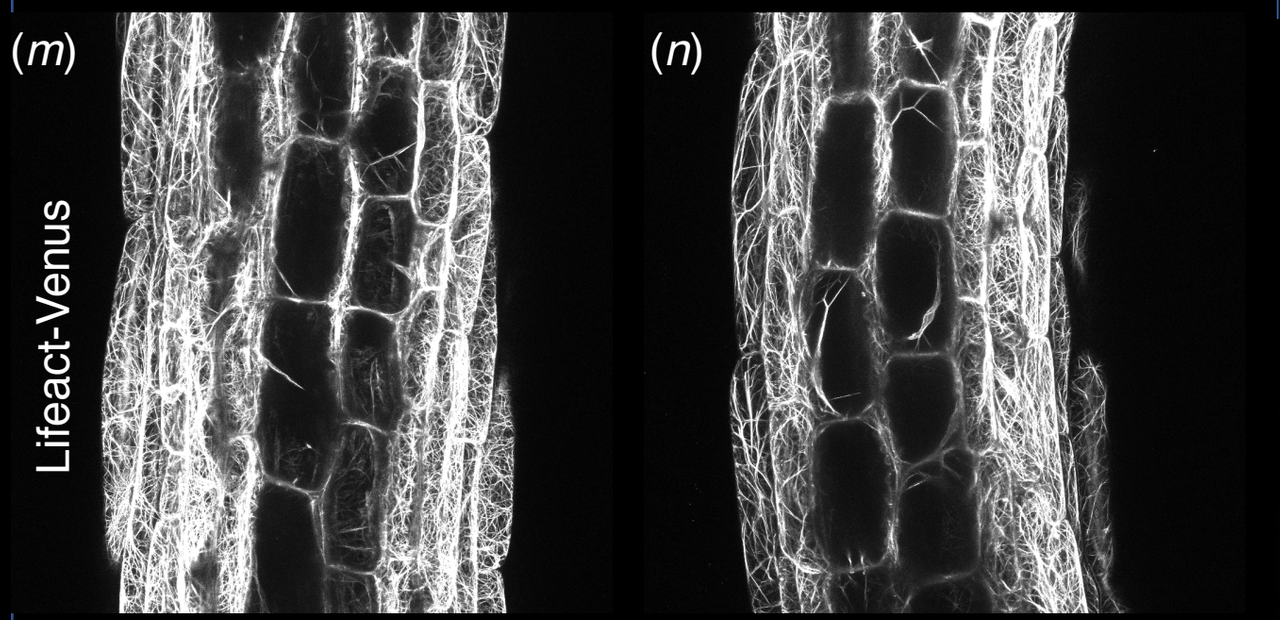
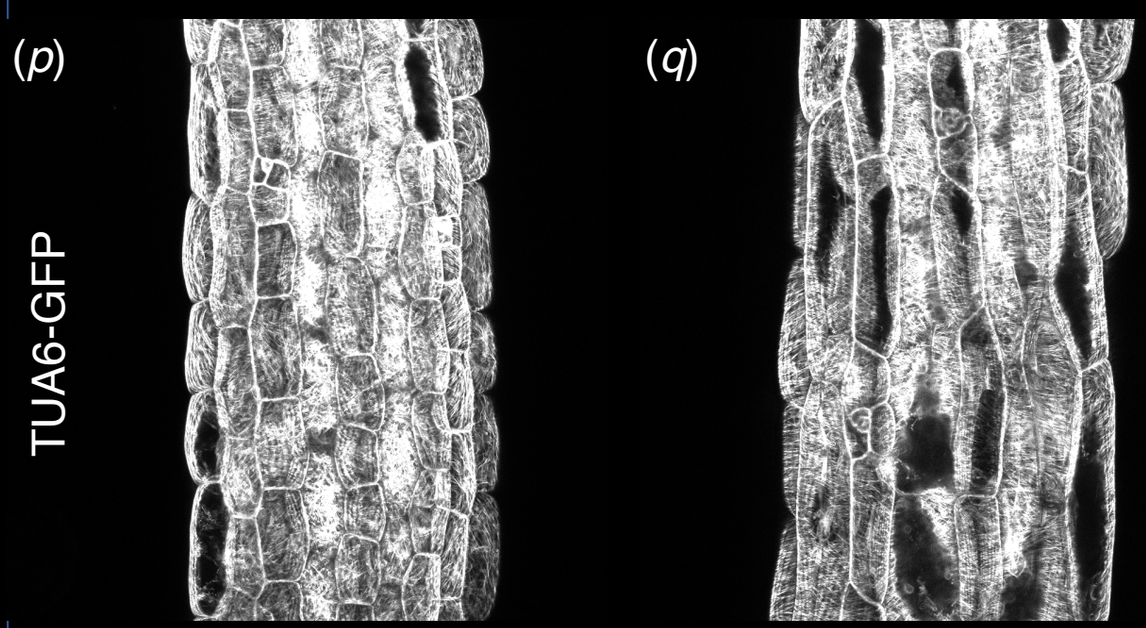
Cytoskeleton rearrangement in hypocotyls under 3D-clinorotation







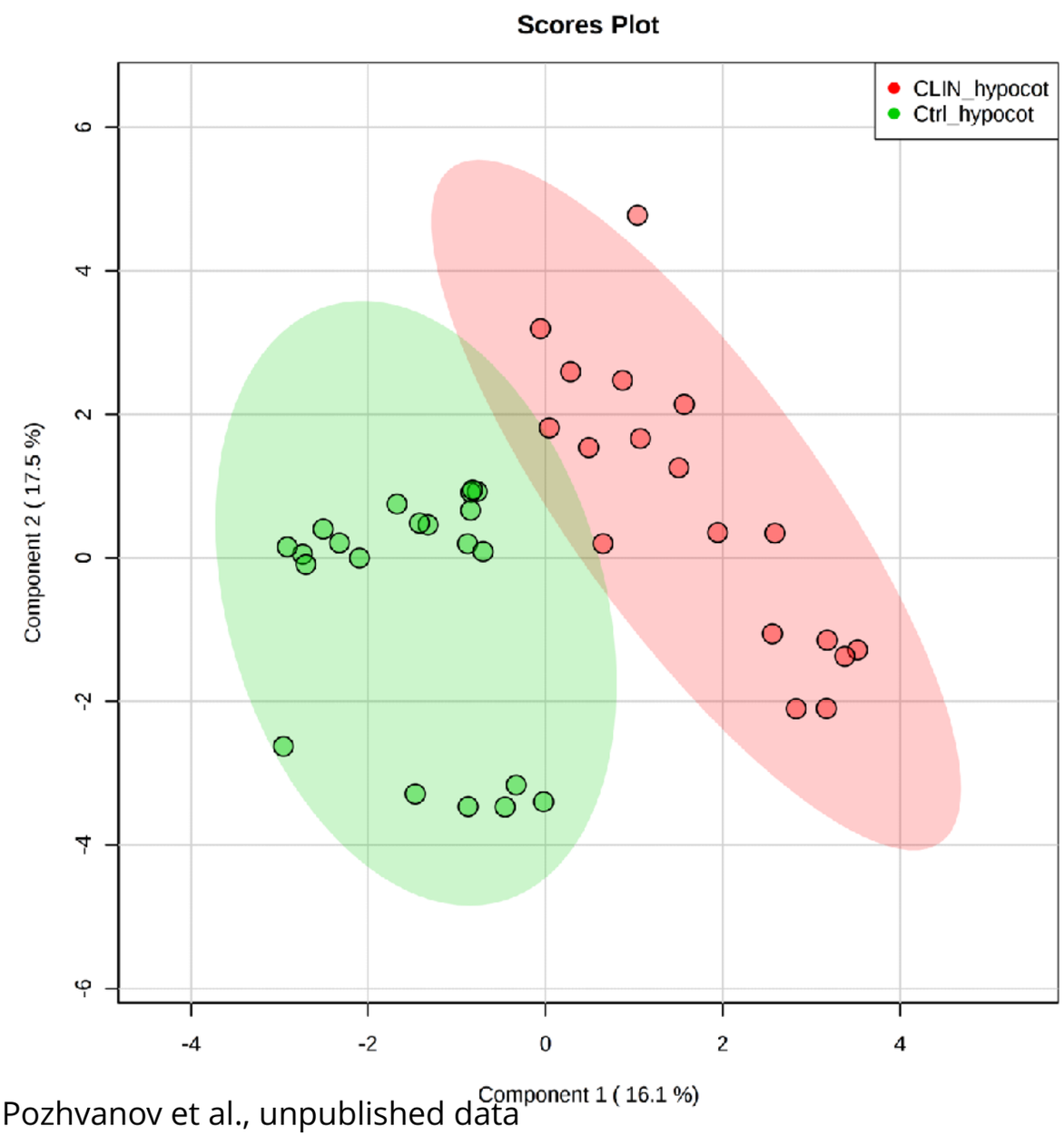


Actin microfilaments

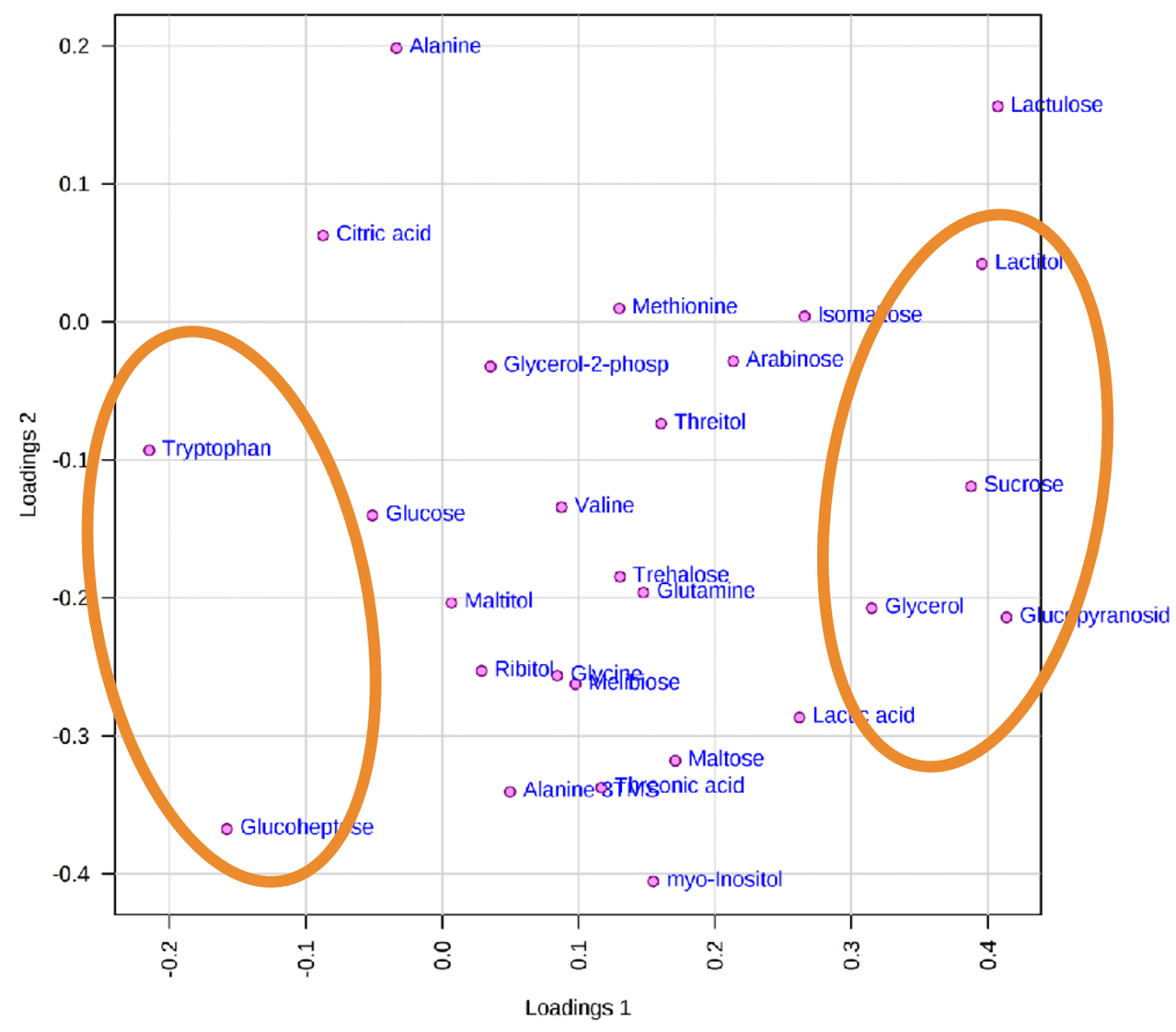
Microtubules

Pozhvanov et al., in press

Metabolite profiles in seedlings are adjusted by microgravity modelling

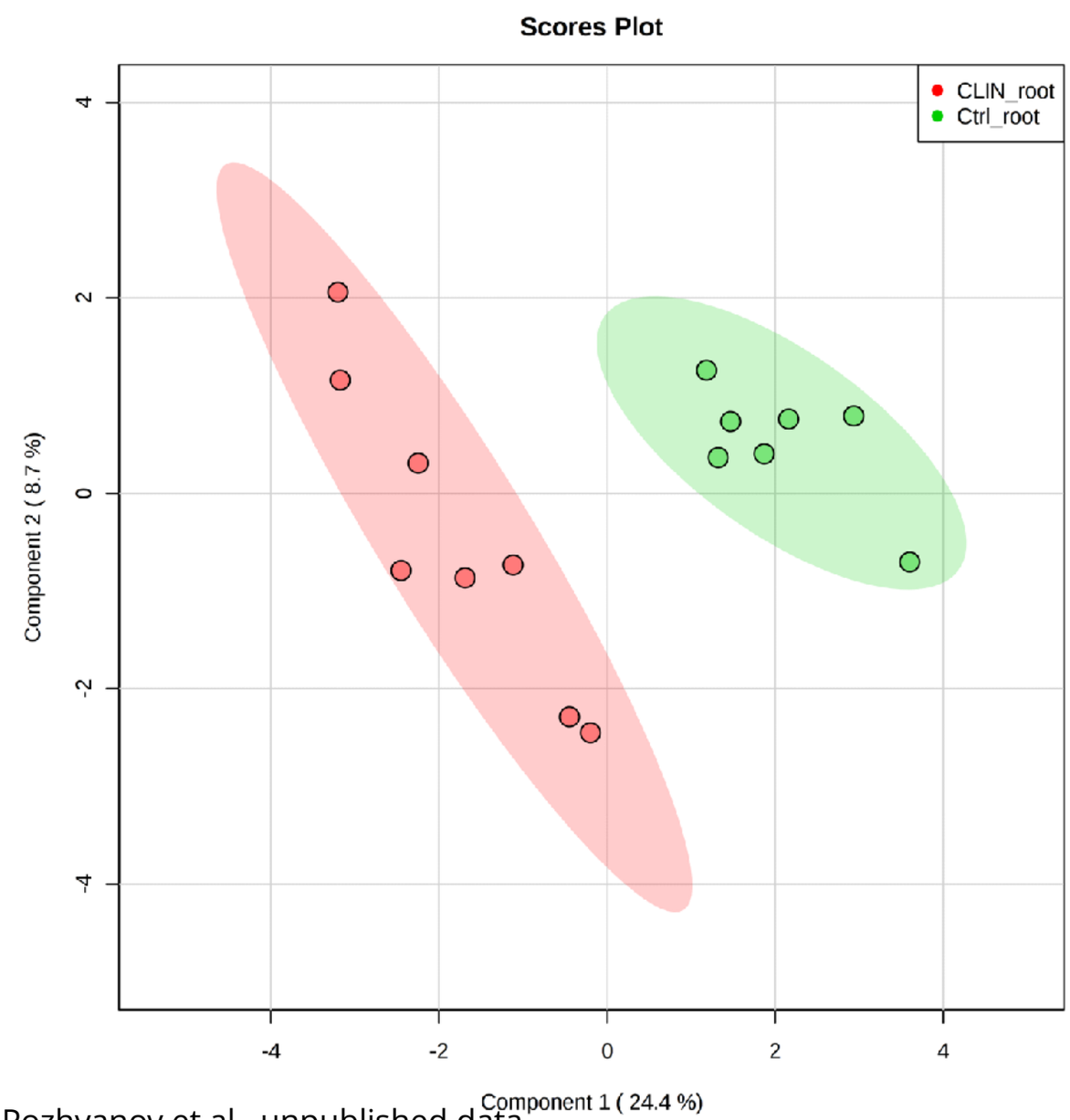


hypocotyls

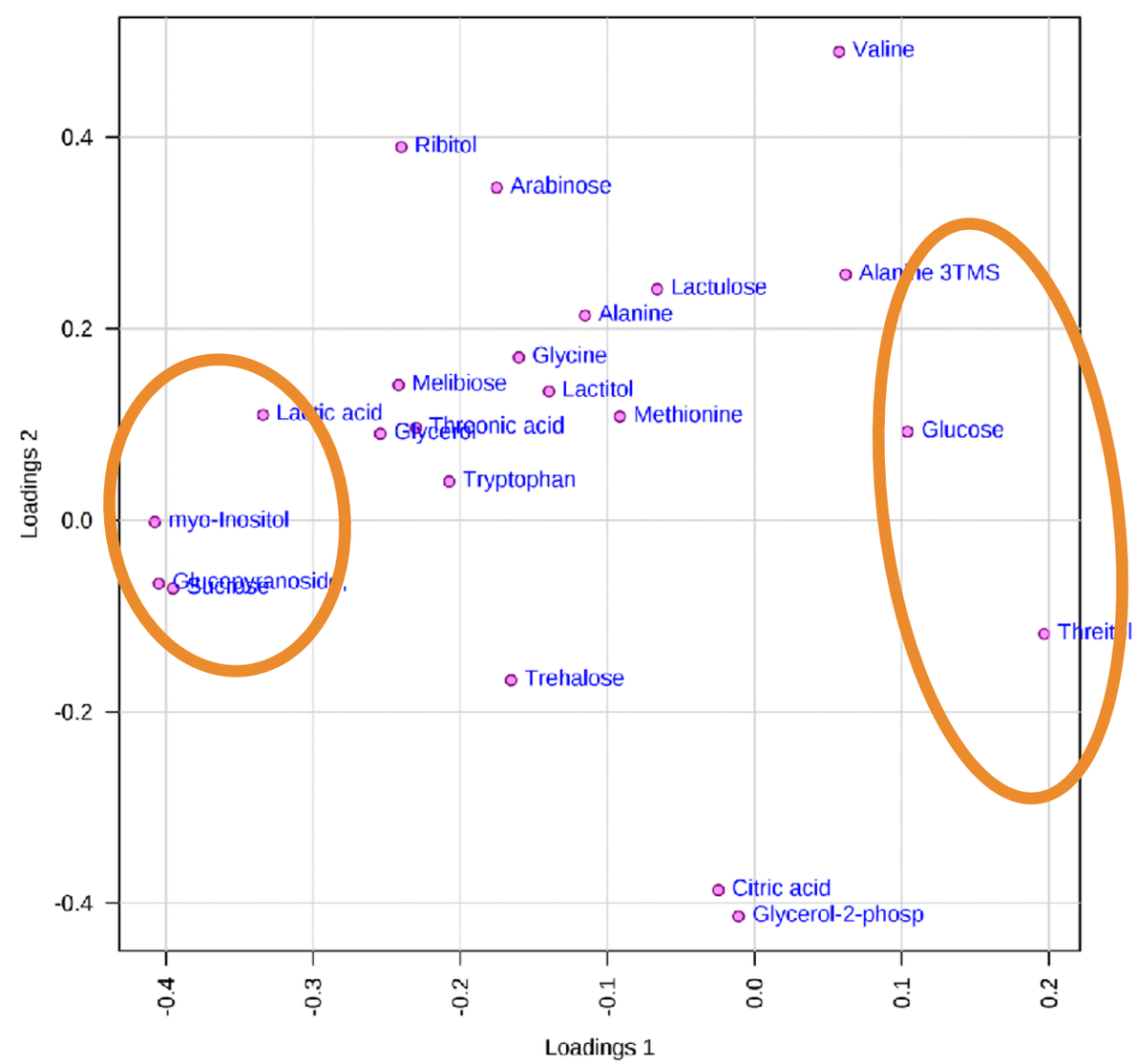


Pozhvanov et al., unpublished data

Metabolite profiles in seedlings are adjusted by microgravity modelling

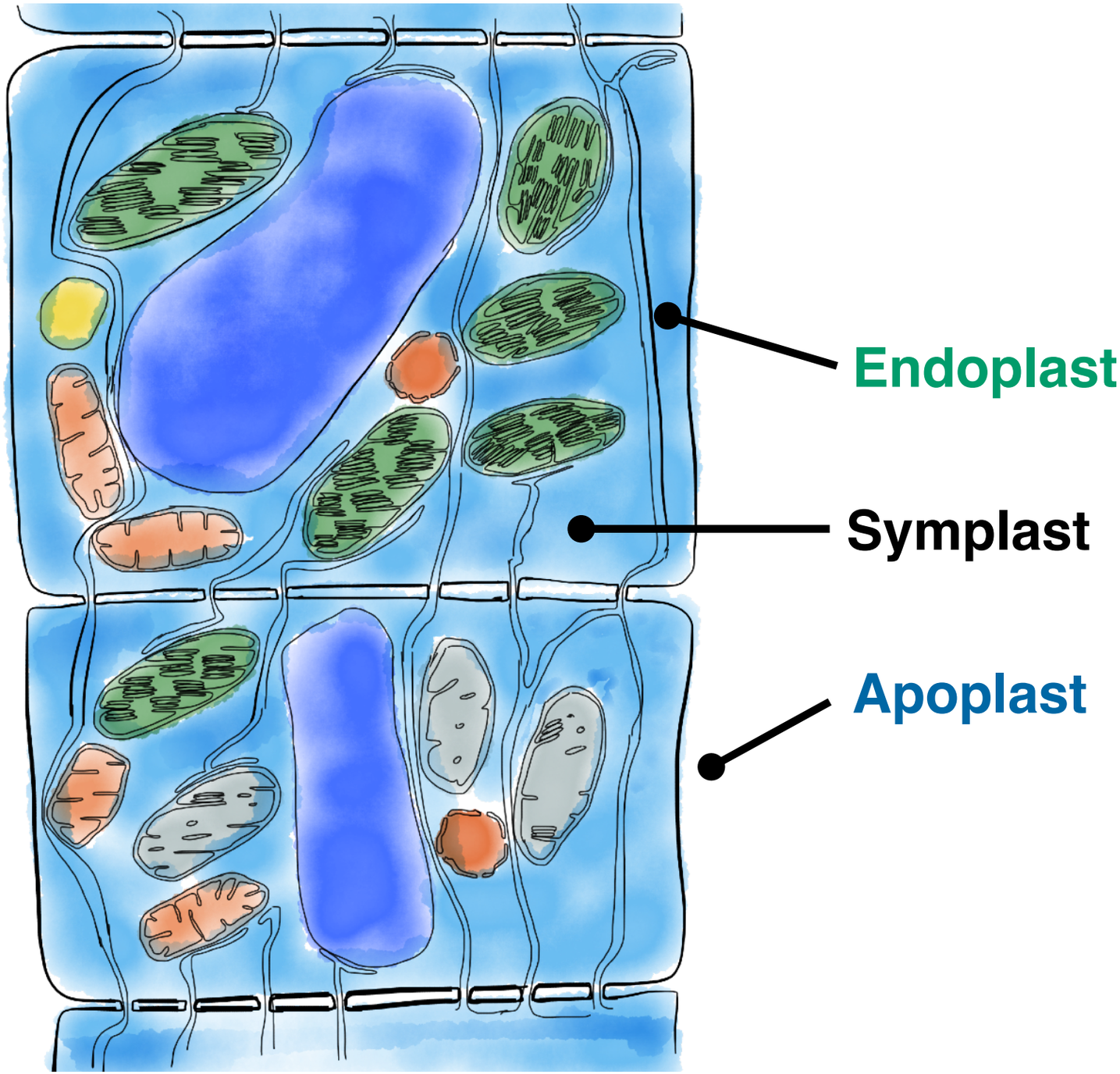


roots



Pozhvanov et al., unpublished data

Plant organisms consist of three enclosed domains



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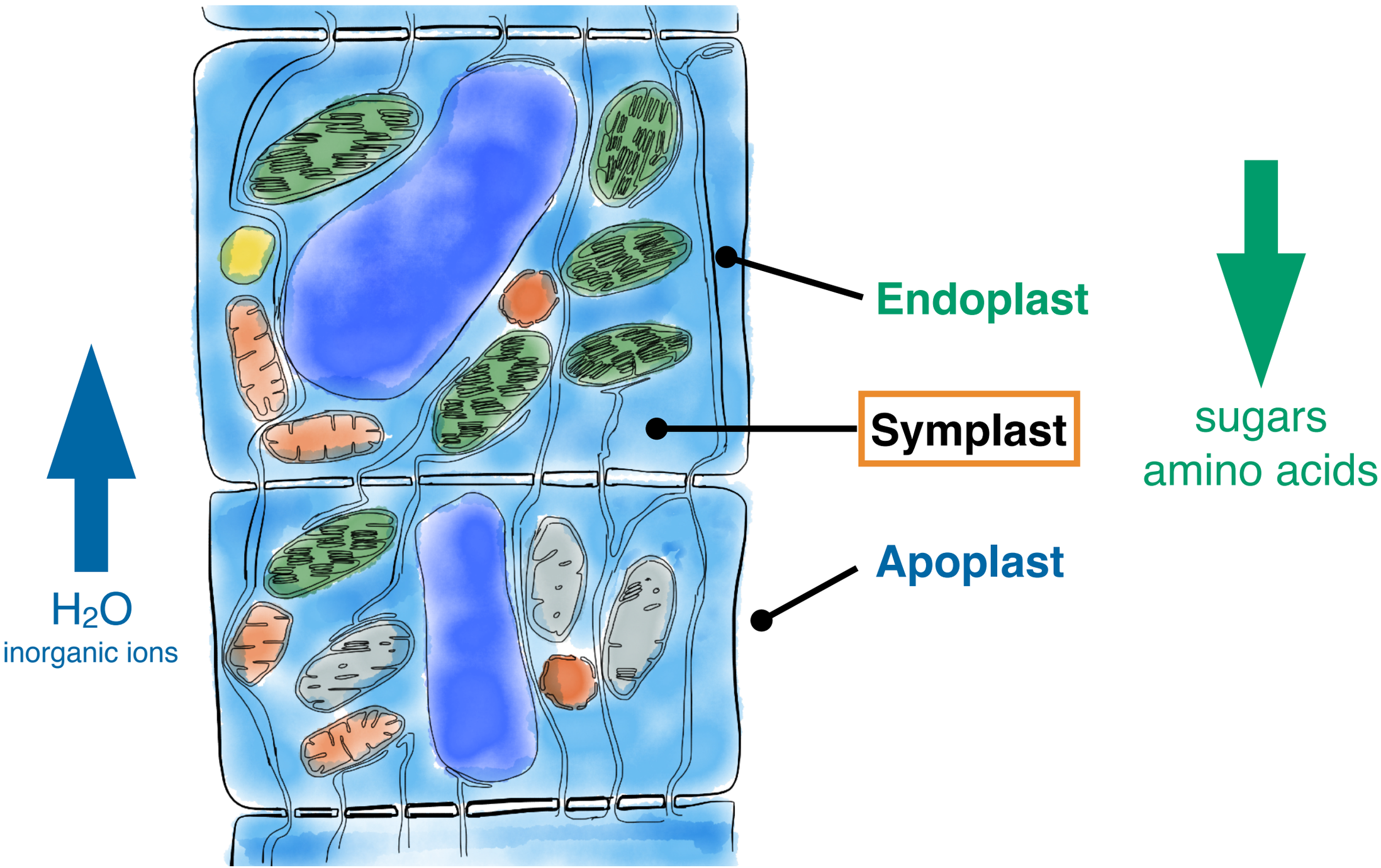
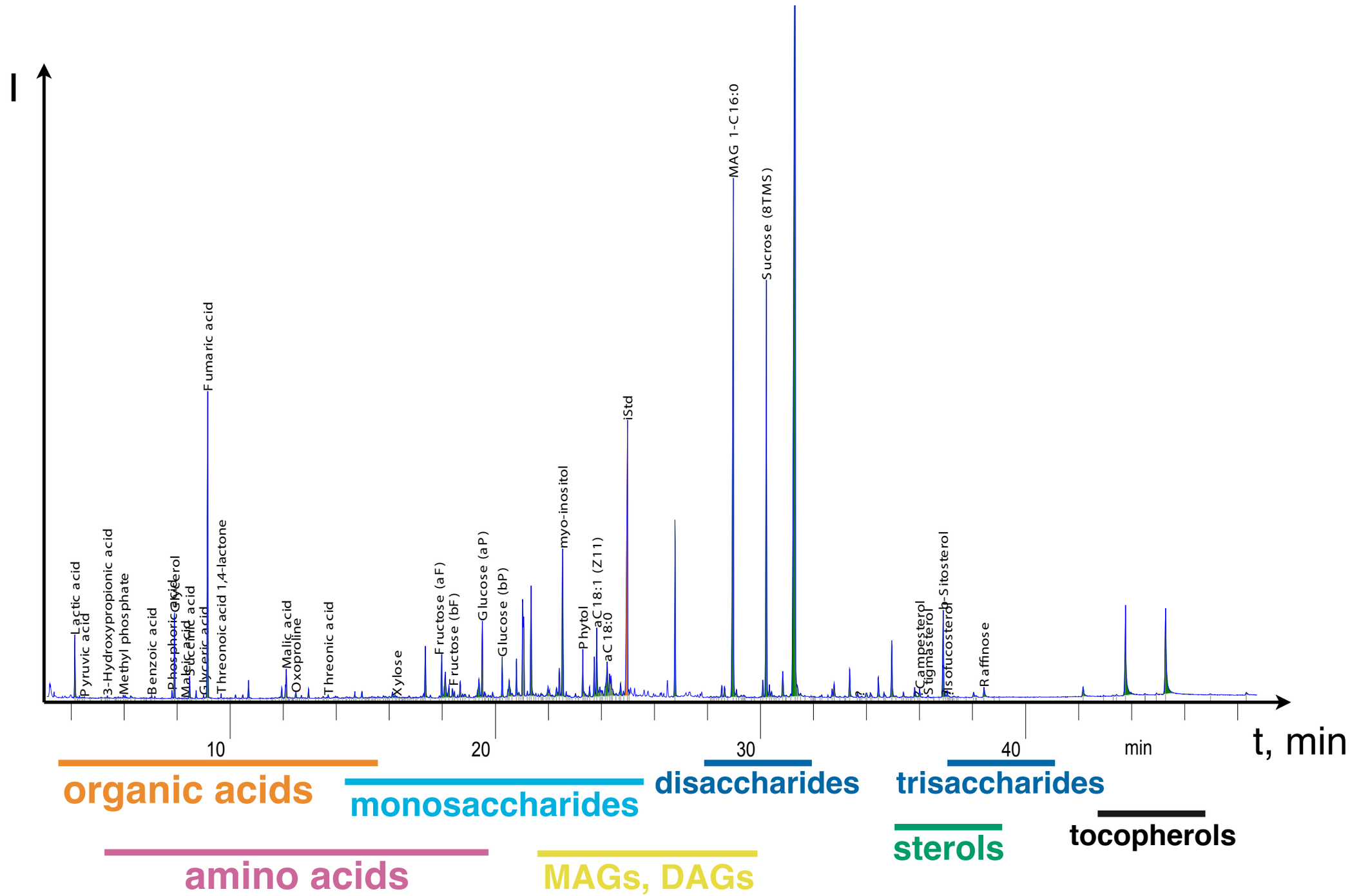


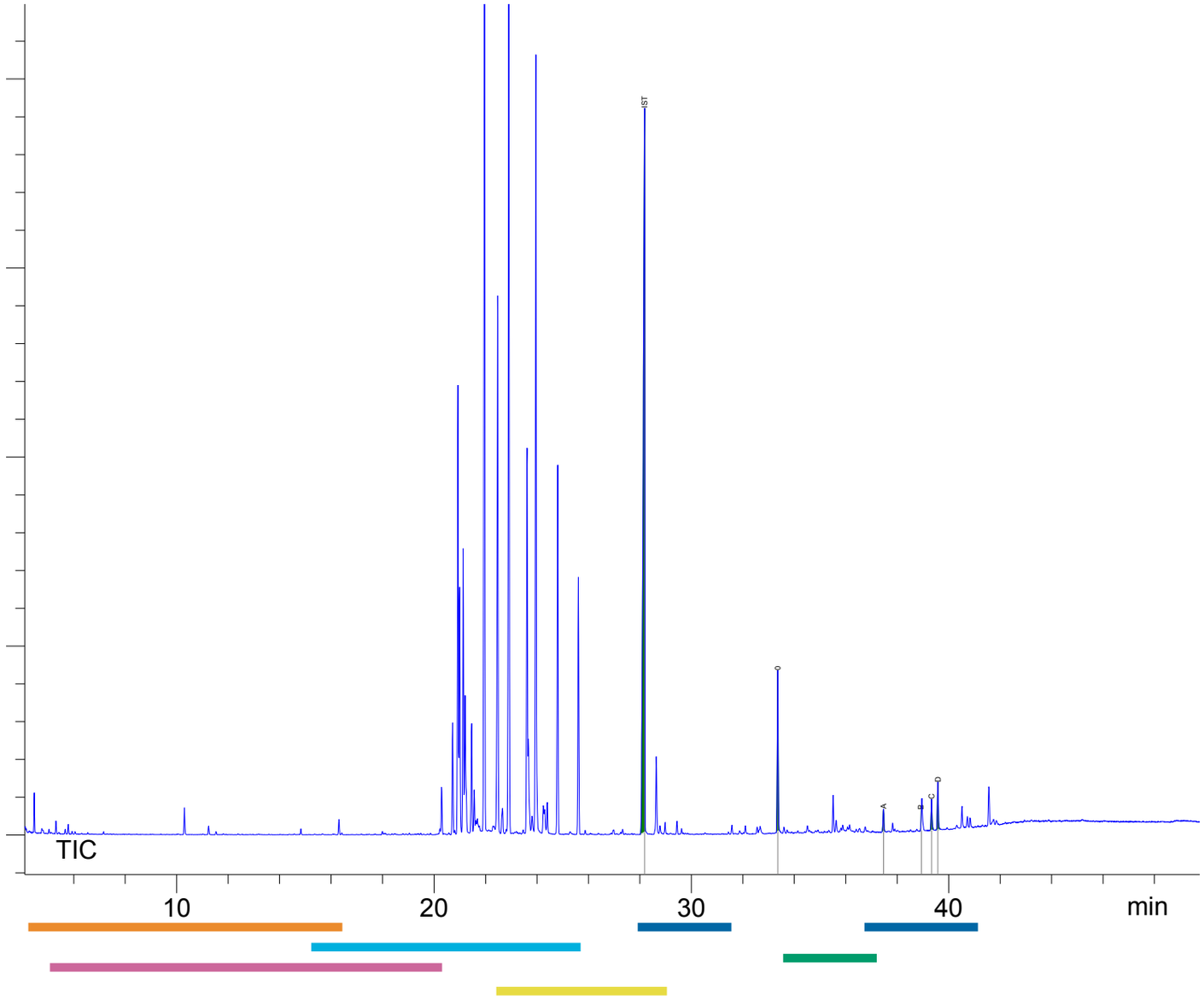
Typical plant metabolite profile, GC-MS



Plant metabolite profile is stable

Image credit: Roland Tsandekidis, plantarium.ru

Metasequoia glyptostroboides
Park of Botanical Institute RAS



Shavarda, Ozerov, unpublished data

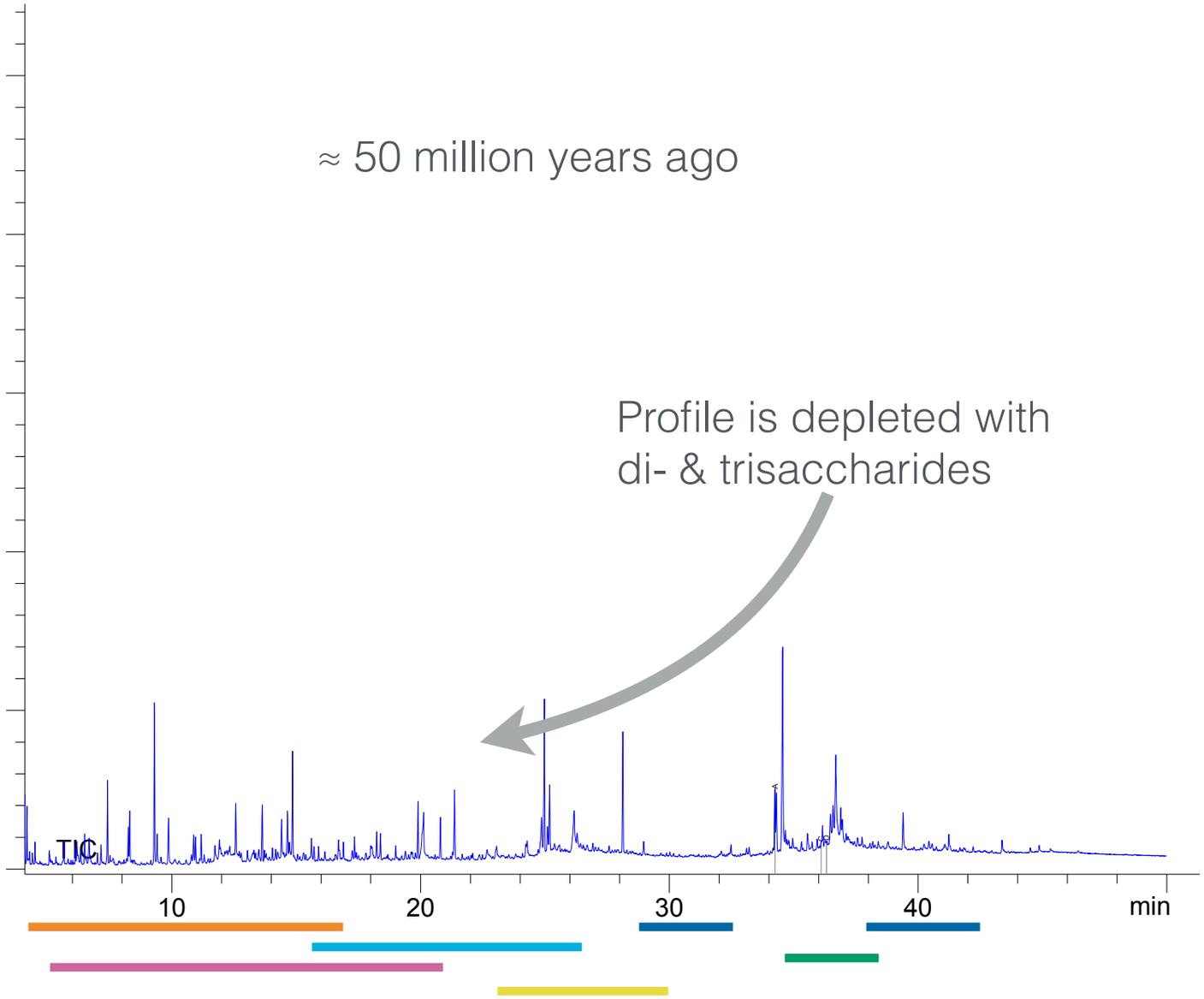
Plant metabolite profile is stable

Image credit: Roland Tsandekidis, plantarium.ru

Metasequoia nathorstii
Lower Eocene epoch

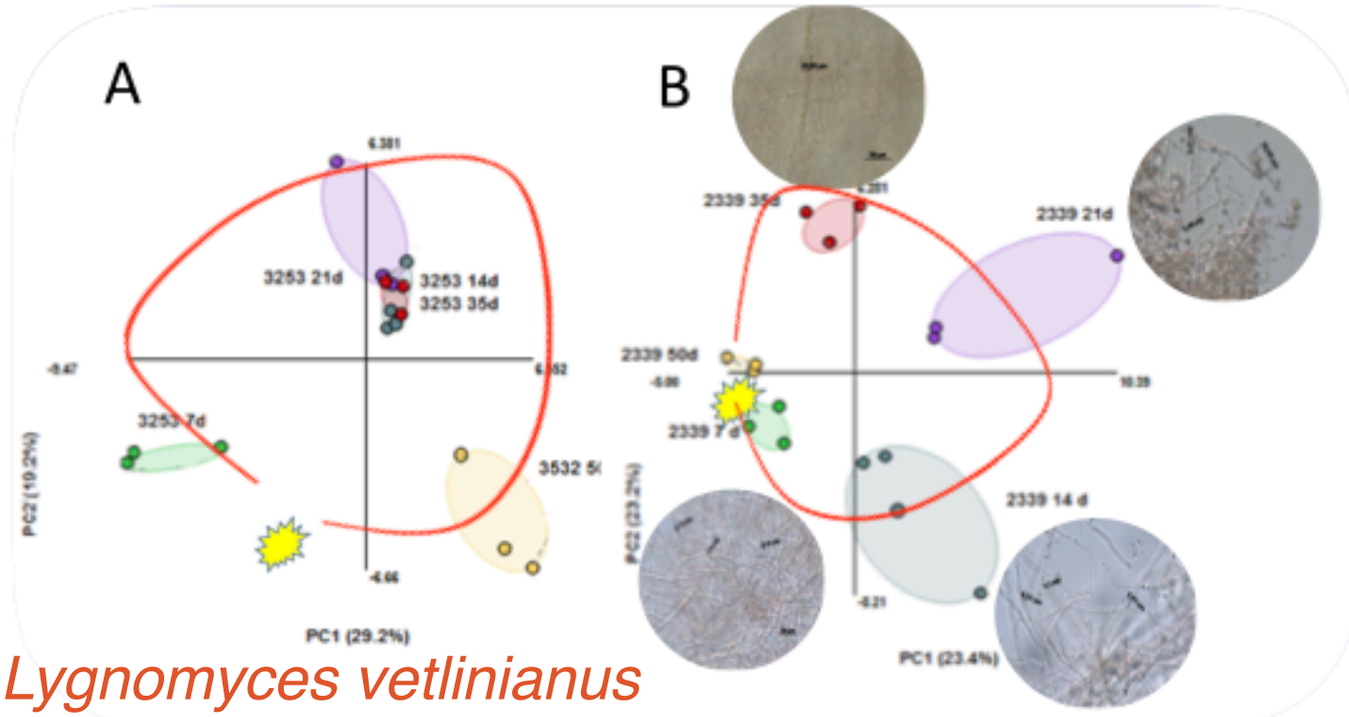
≈ 50 million years ago

Profile is depleted with
di- & trisaccharides

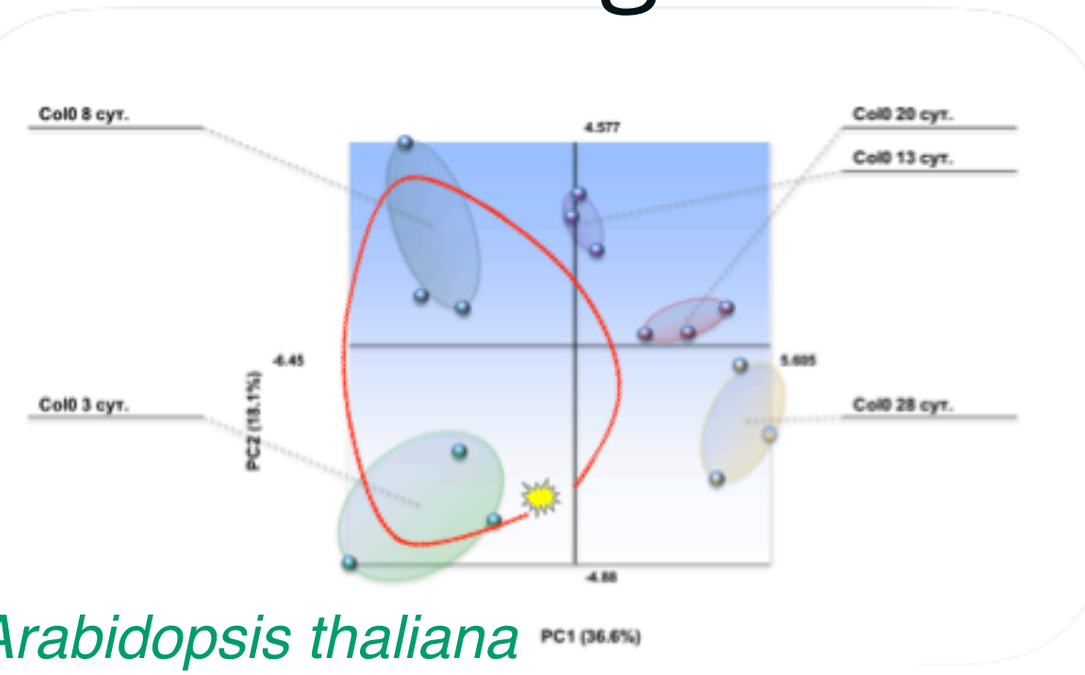


Shavarda, Ozerov, unpublished data

Metabolite profile adjustment in onthogenesis

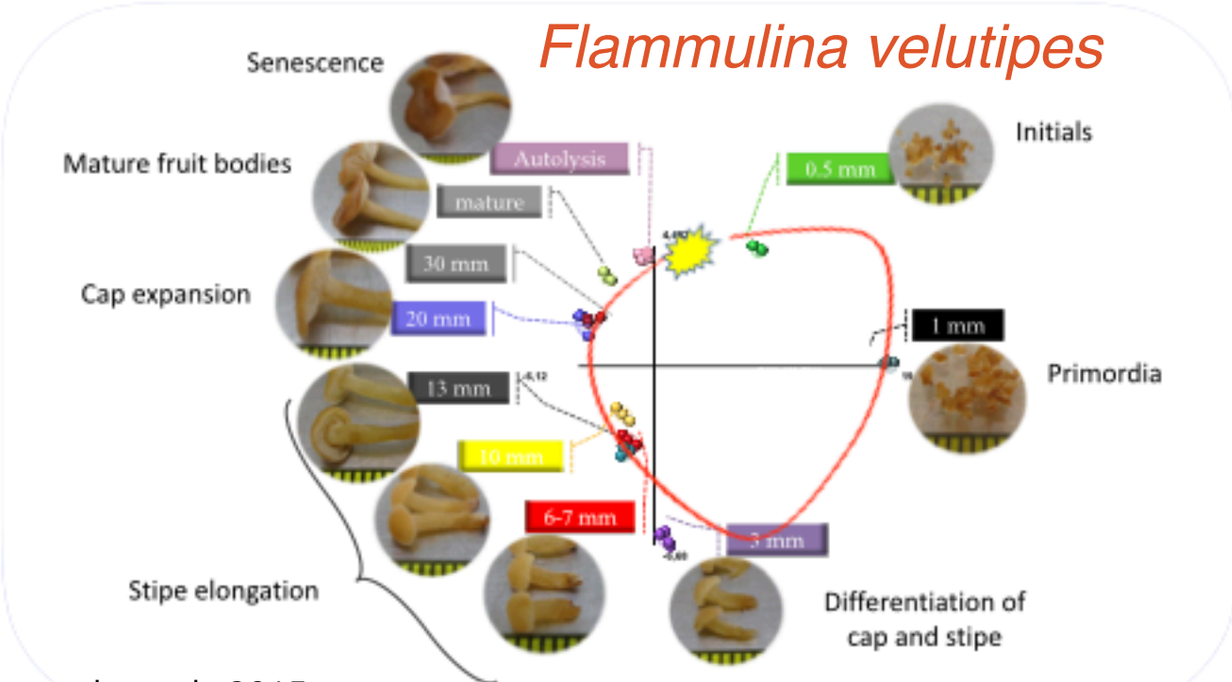


Lygnomyces vetlinianus

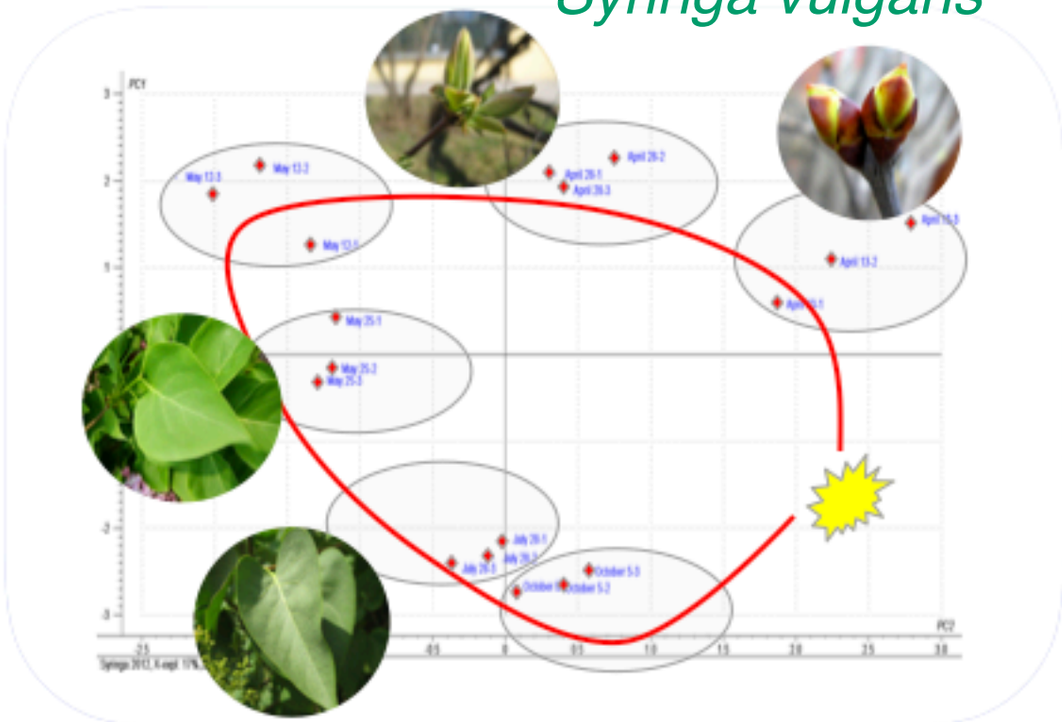


Arabidopsis thaliana

Flammulina velutipes

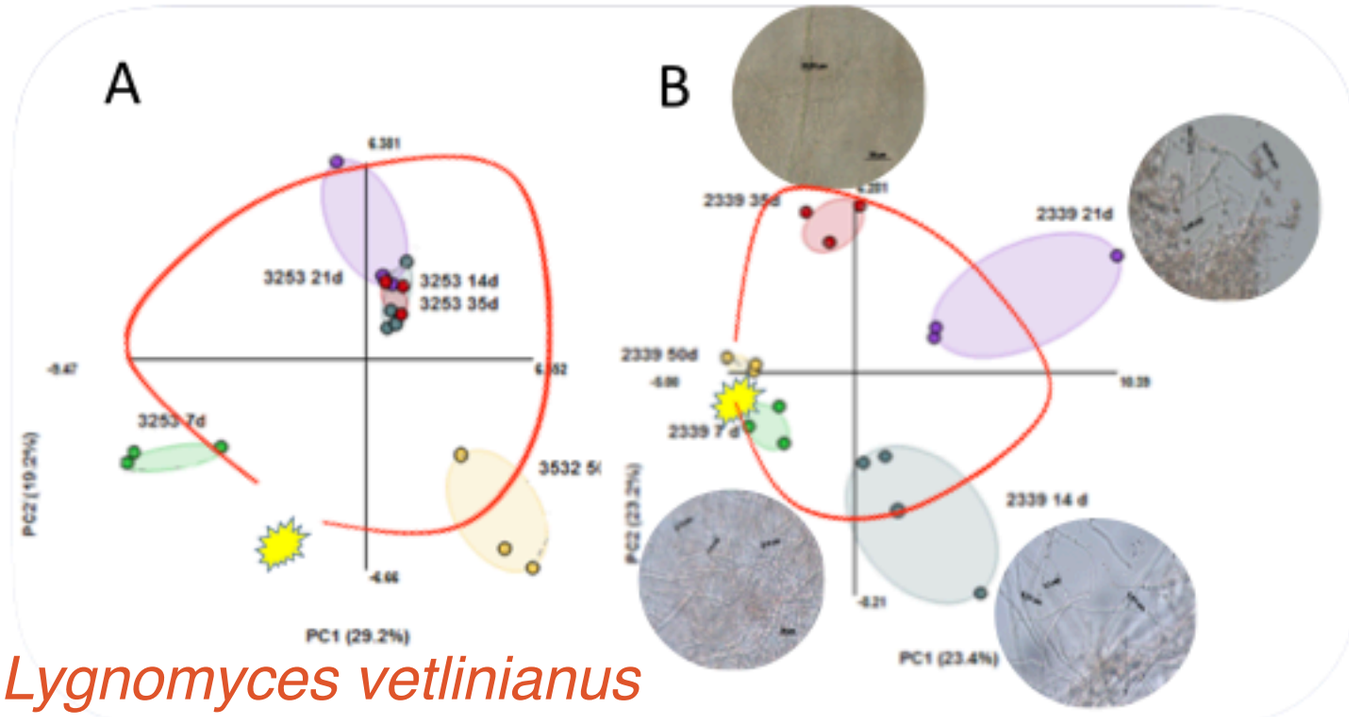


Syringa vulgaris

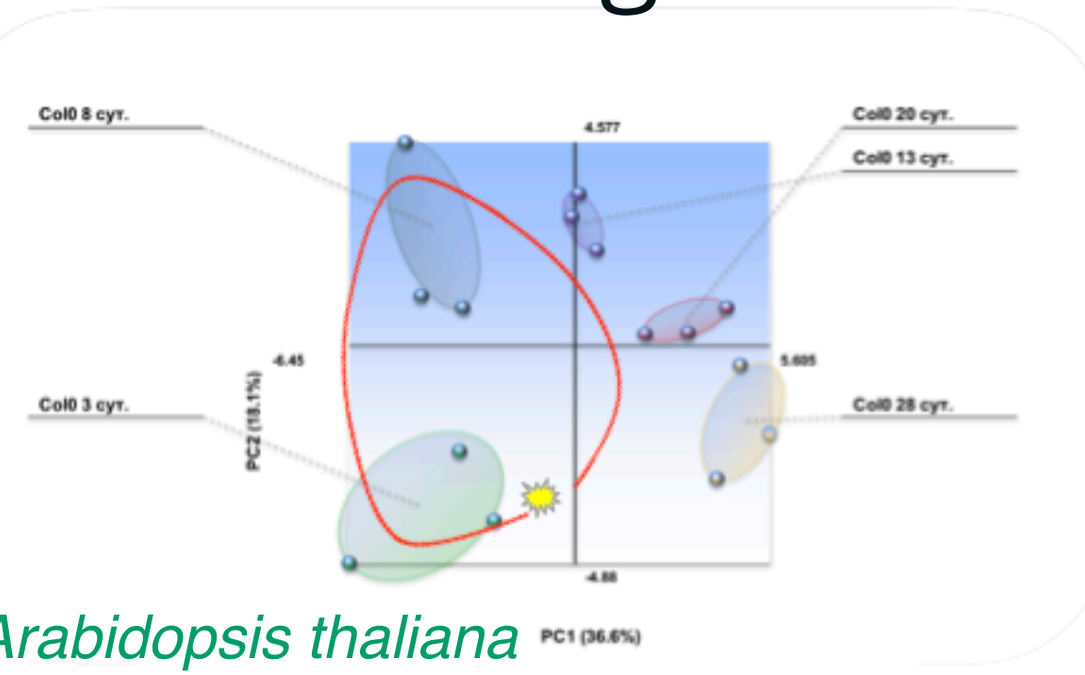


Shavarda et al., 2015

Metabolite profile adjustment in onthogenesis

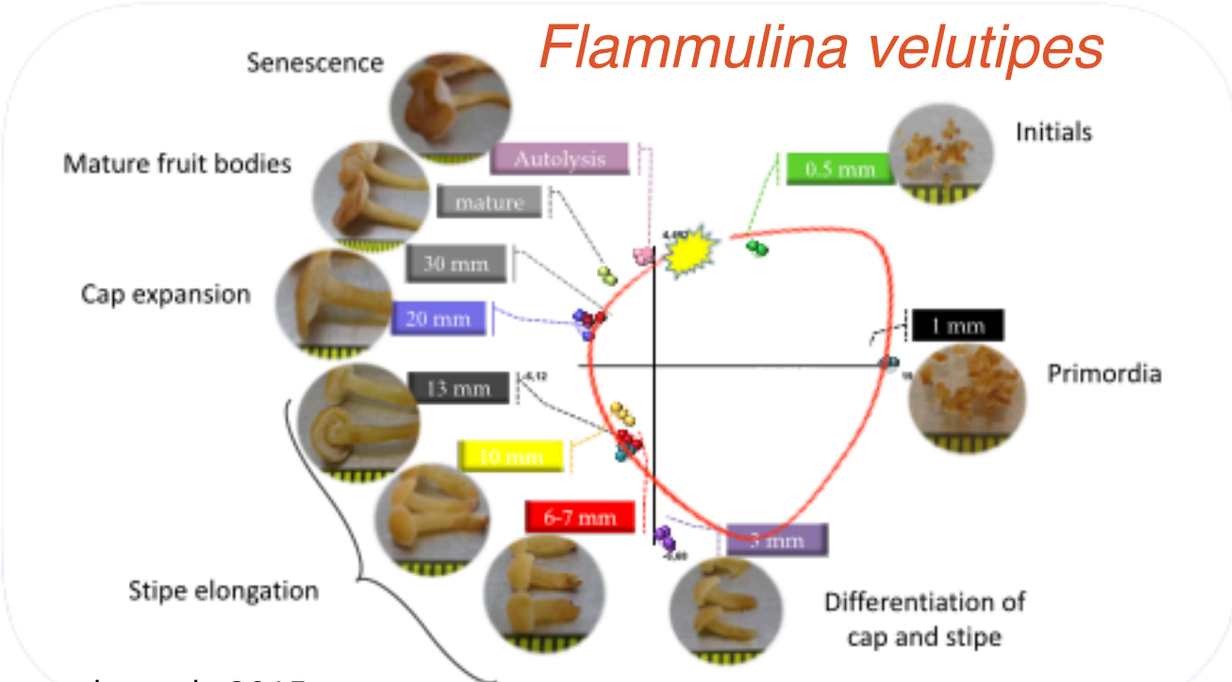


Lygnomyces vetlinianus

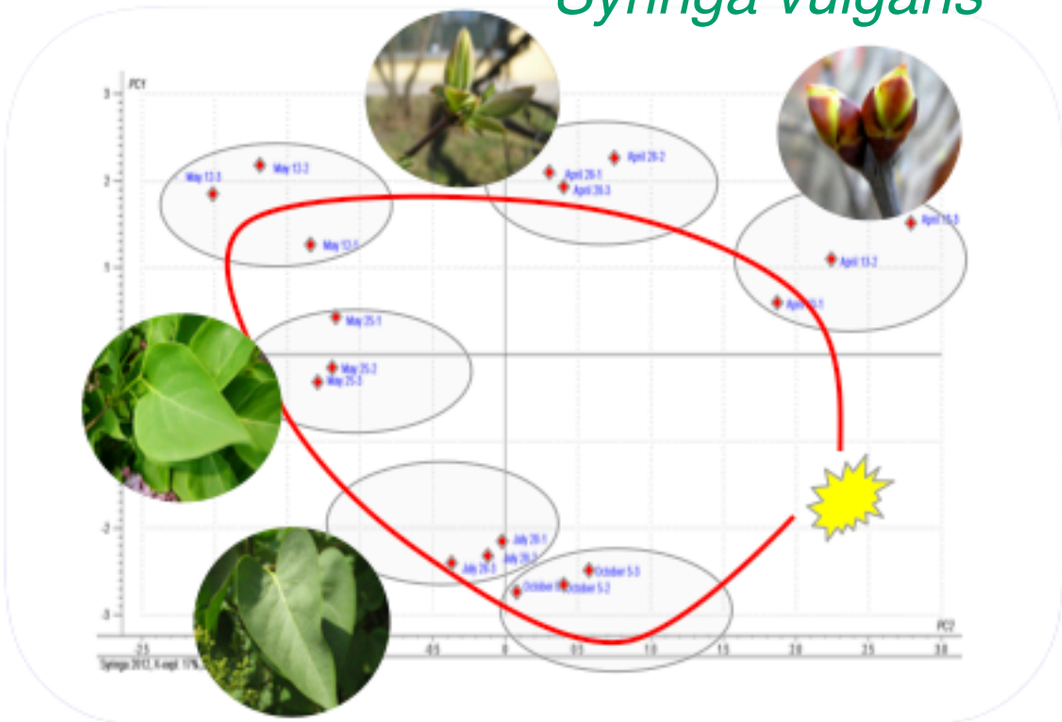


Arabidopsis thaliana

Flammulina velutipes

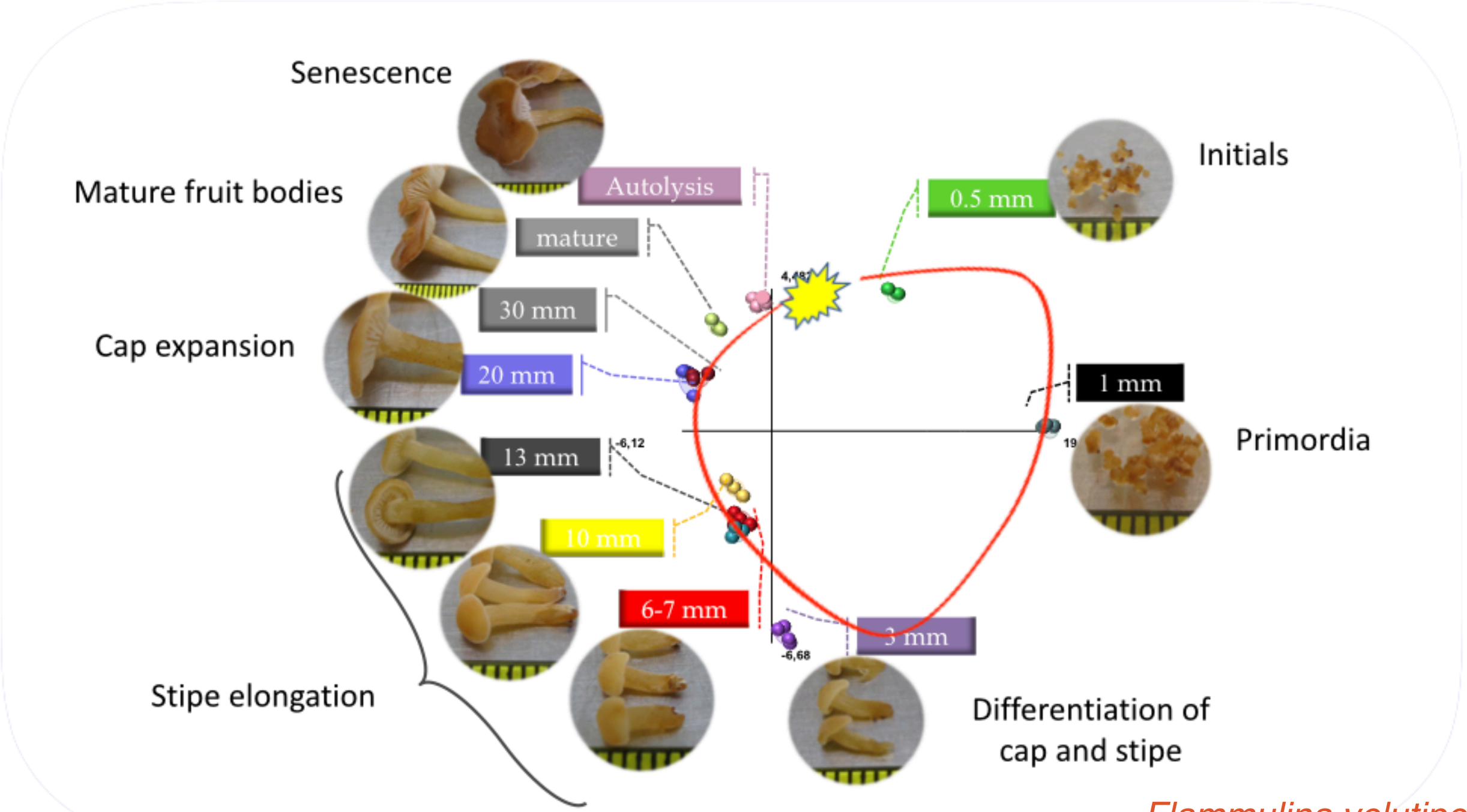


Syringa vulgaris



Shavarda et al., 2015

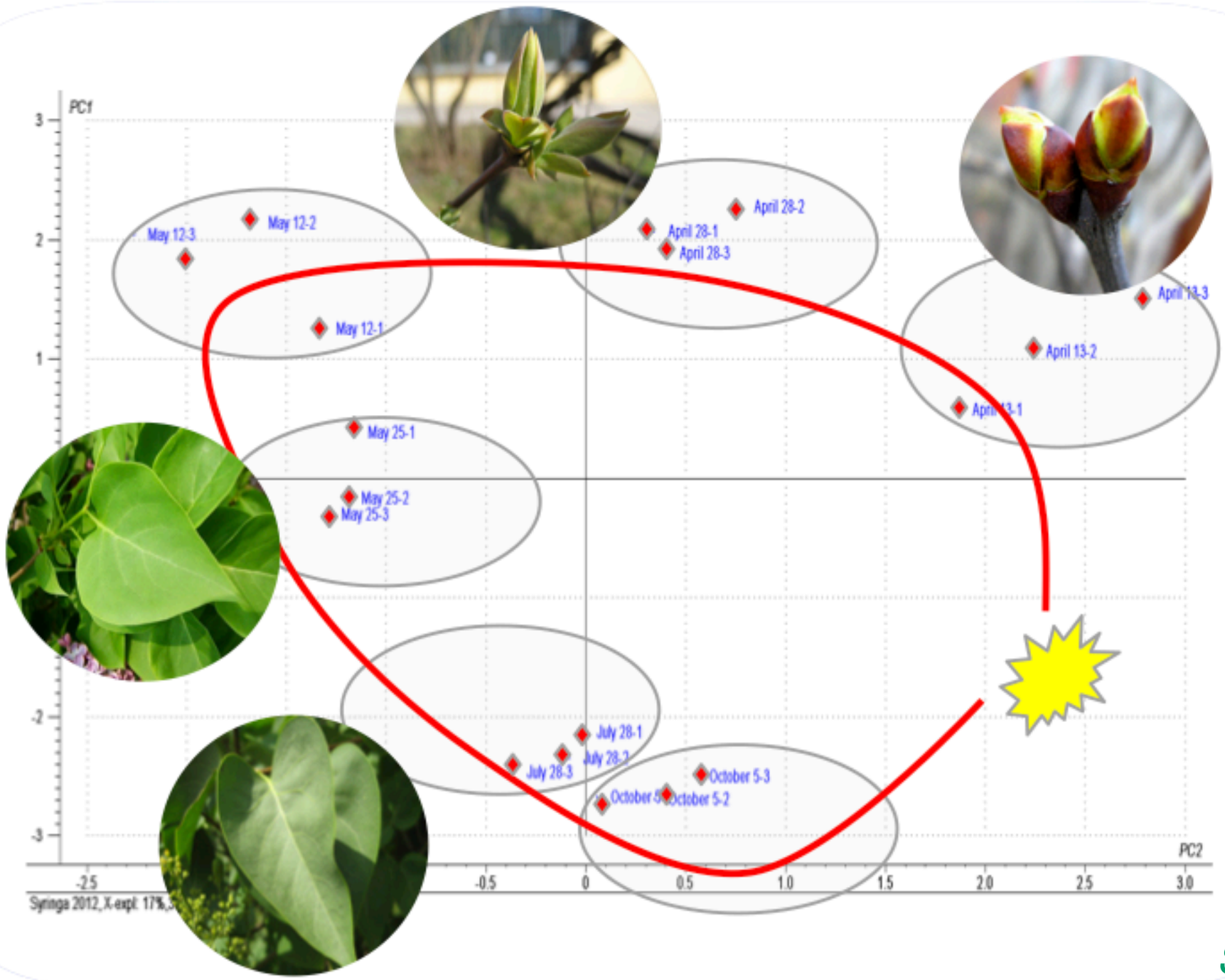
Metabolite profile adjustment in onthogenesis



Shavarda et al., 2015

Flammulina velutipes

Metabolite profile adjustment in onthogenesis



Syringa vulgaris

Shavarda et al., 2015

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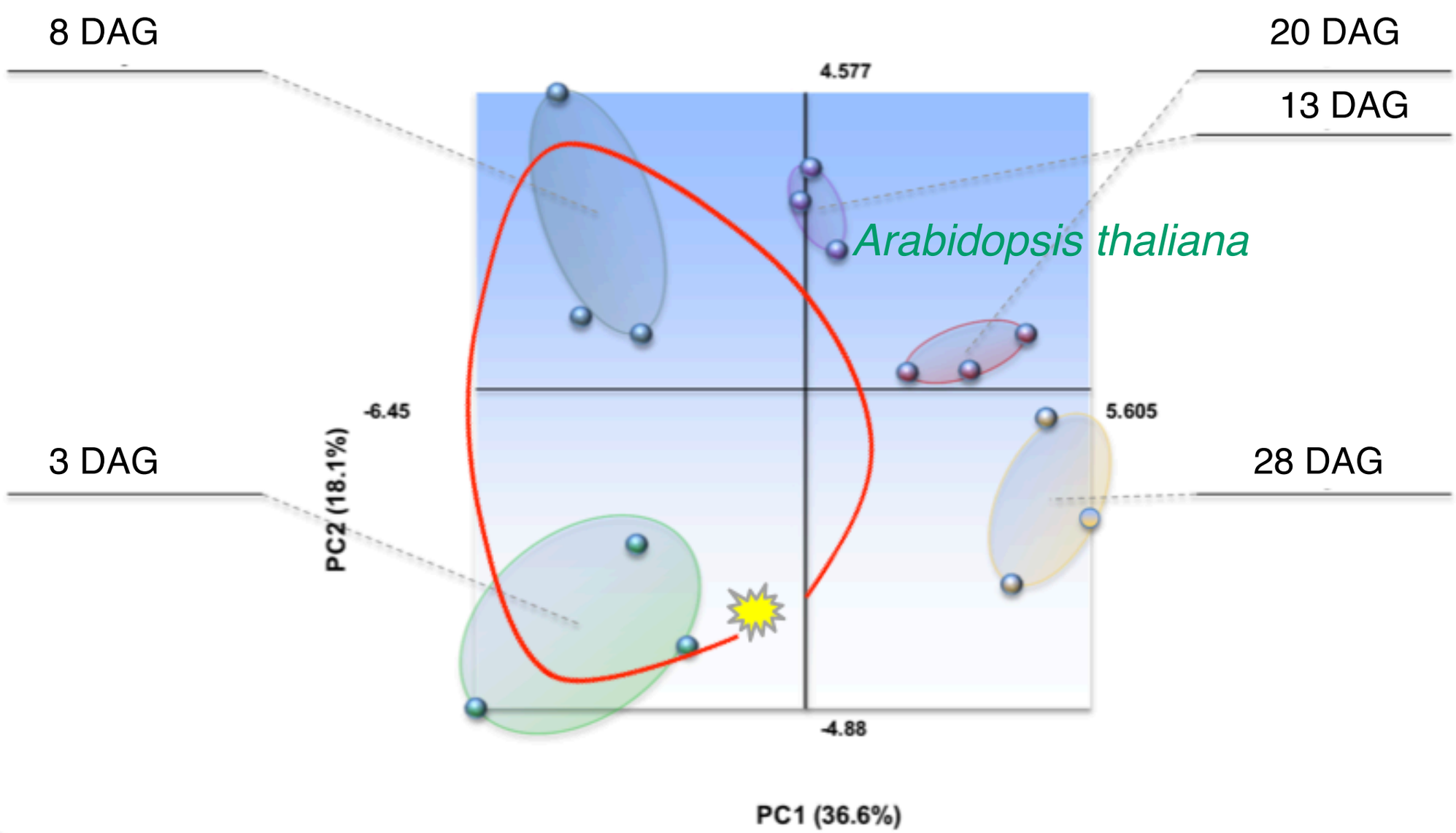

Shavarda et al., 2015

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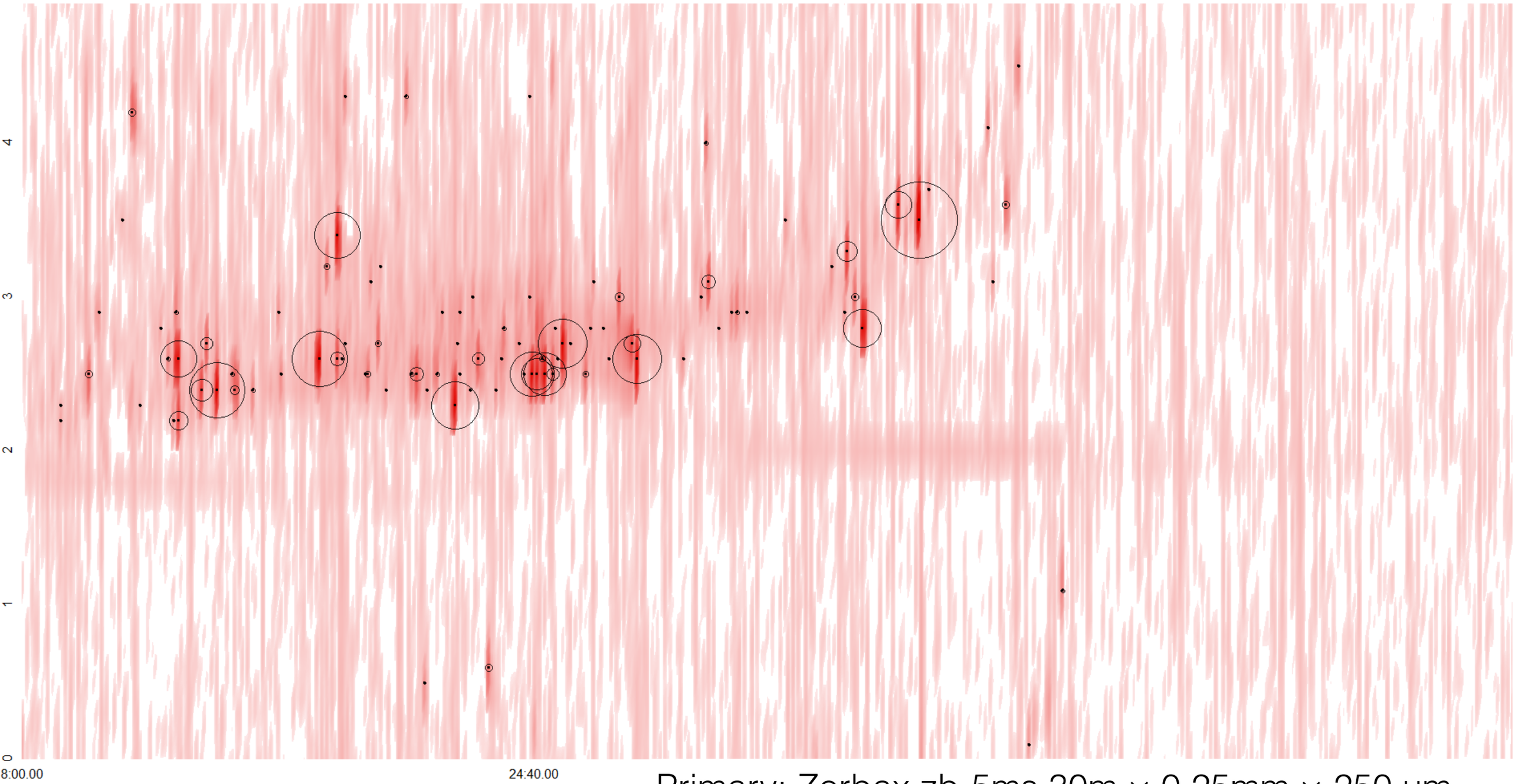
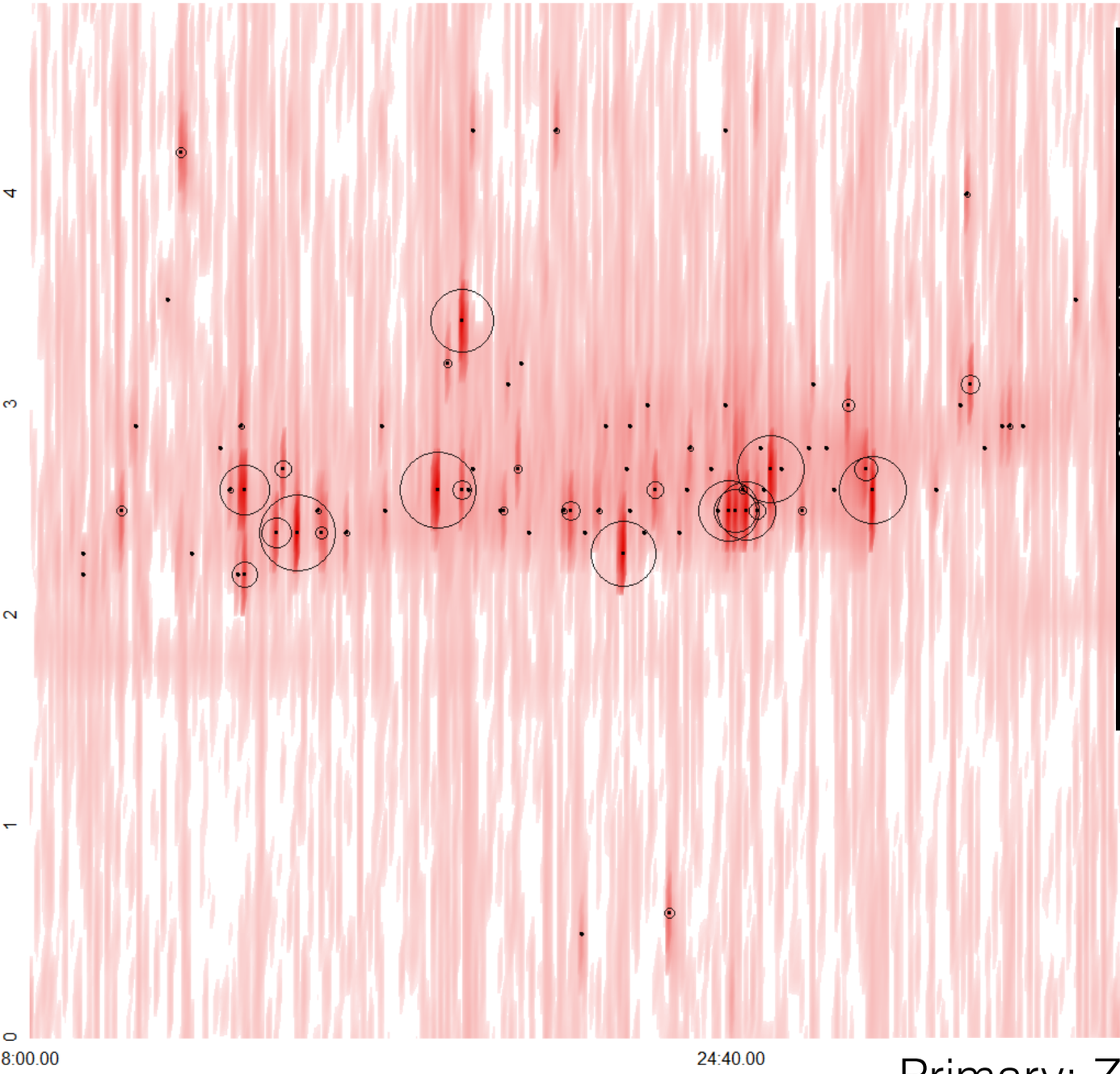
LECO Pegasus 4D GC×GC TOFMS

GC×GC mode overview



Primary: Zorbax zb-5ms 30m × 0.25mm × 250 μm
Secondary: Restek RXI17 1.5m × 0.15mm × 250 μm

GC×GC mode overview



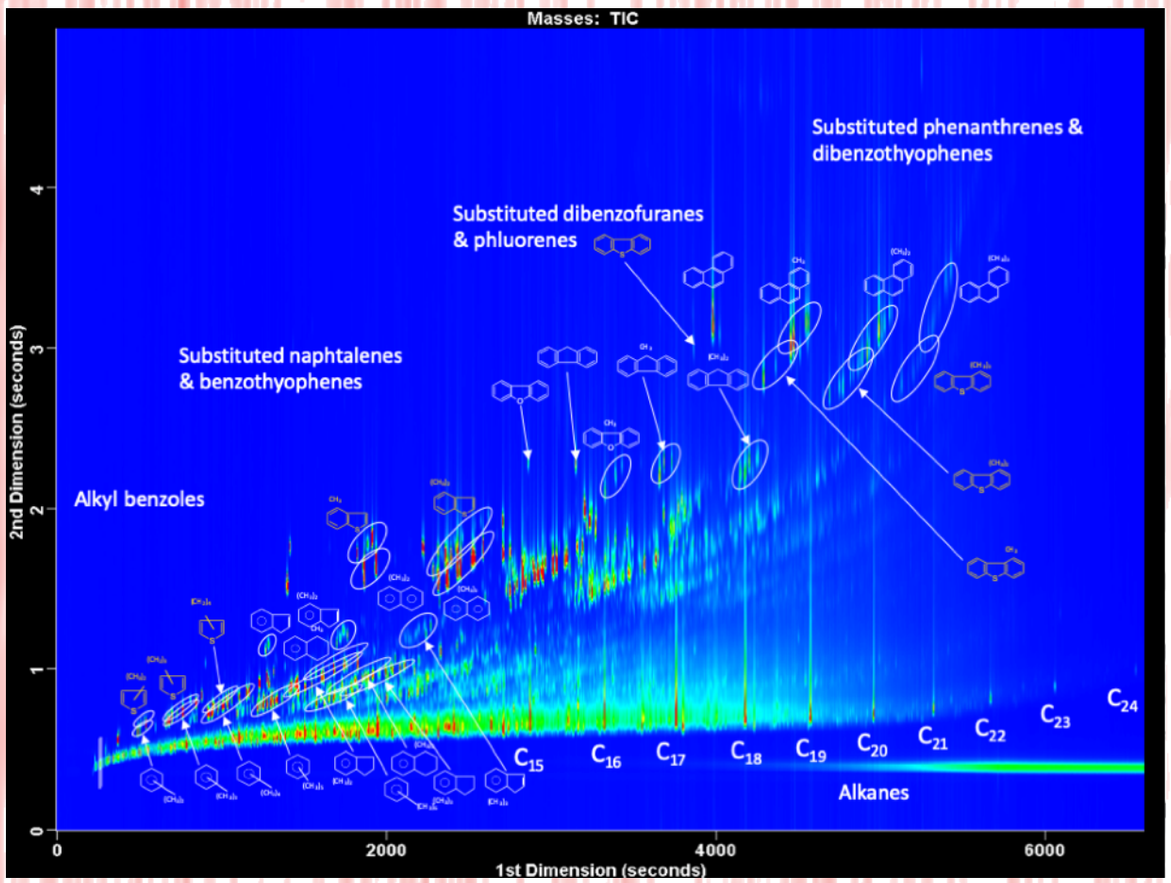


Image credit: LECO Europe

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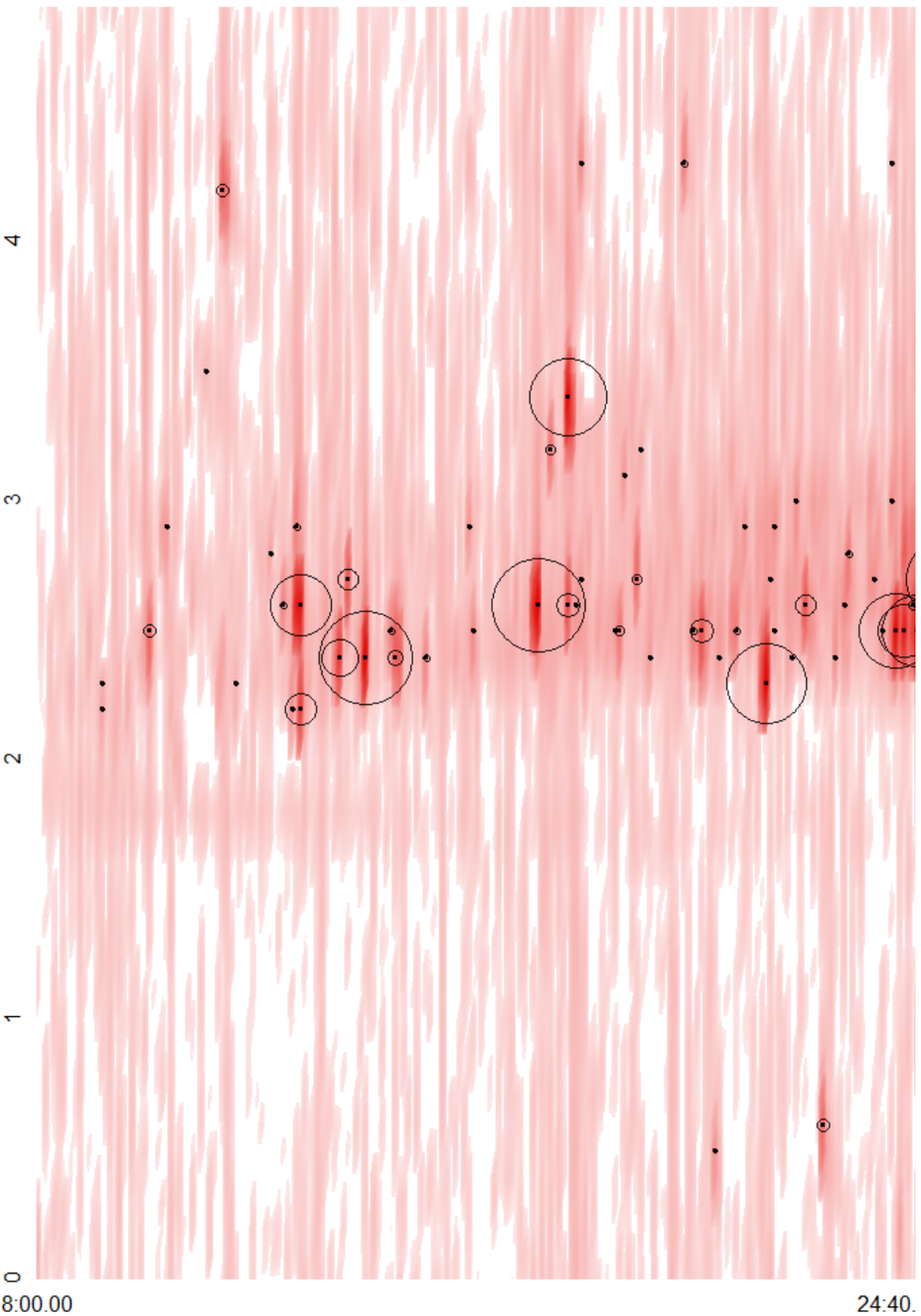
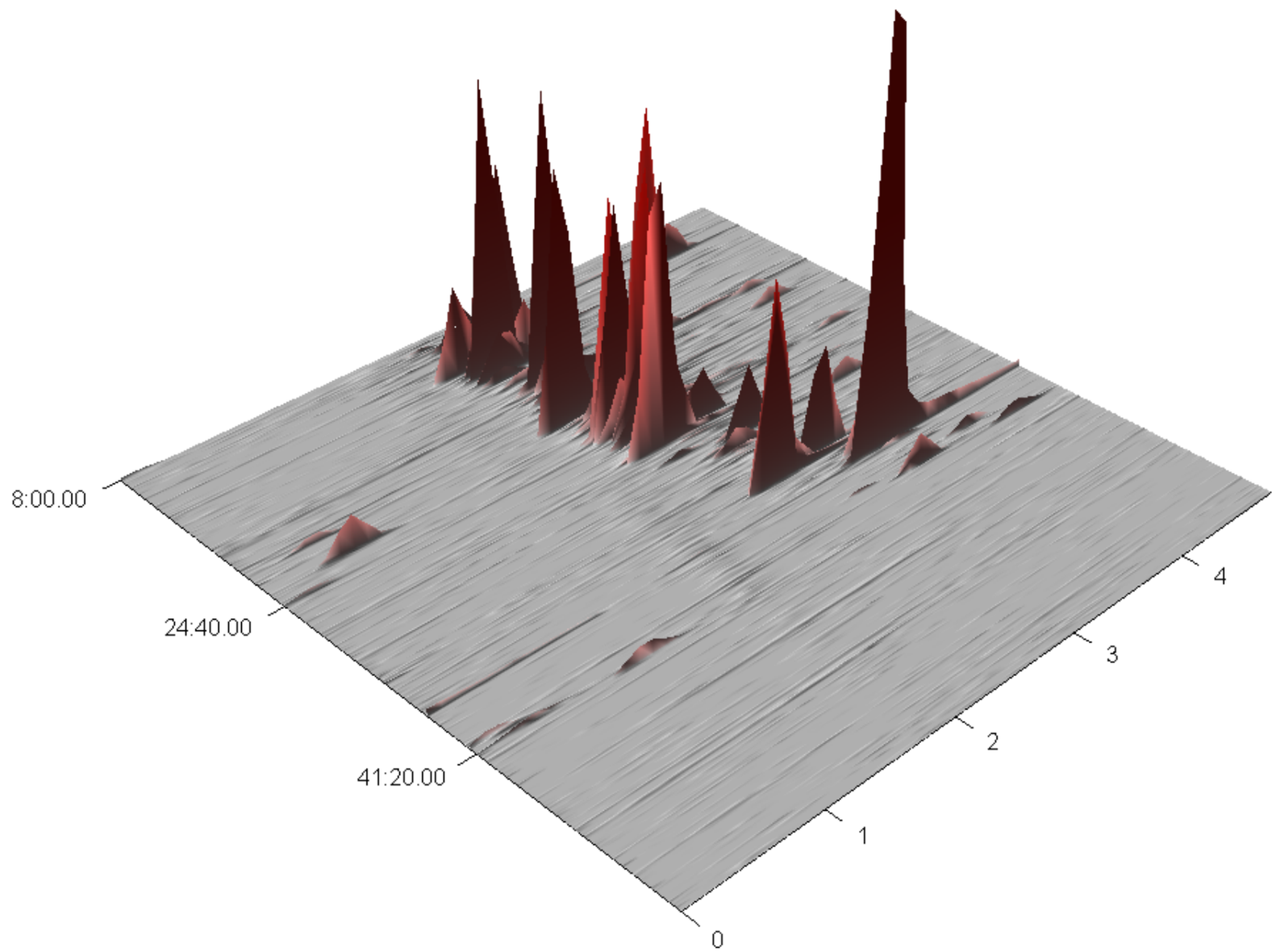
GC×GC mode overview





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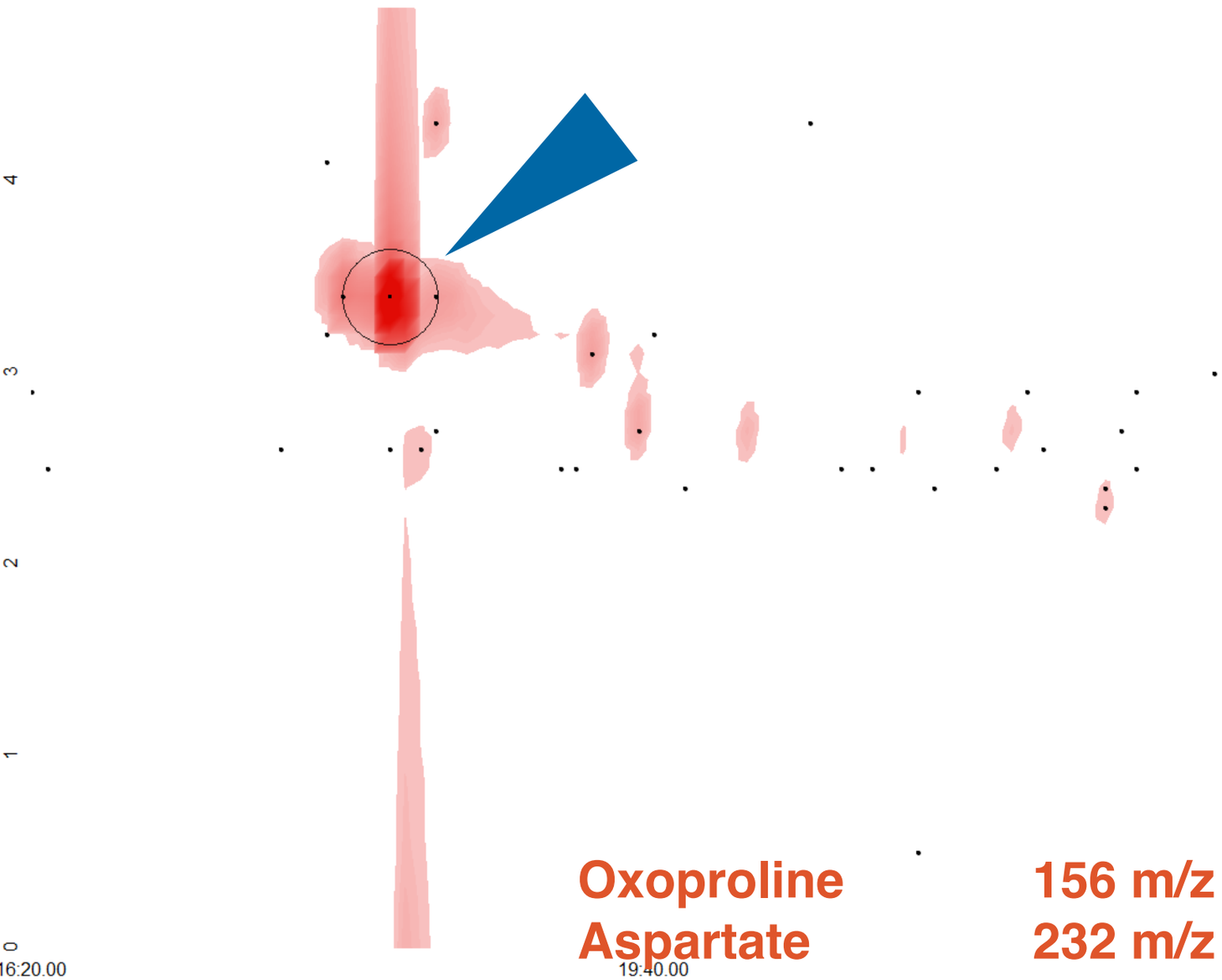
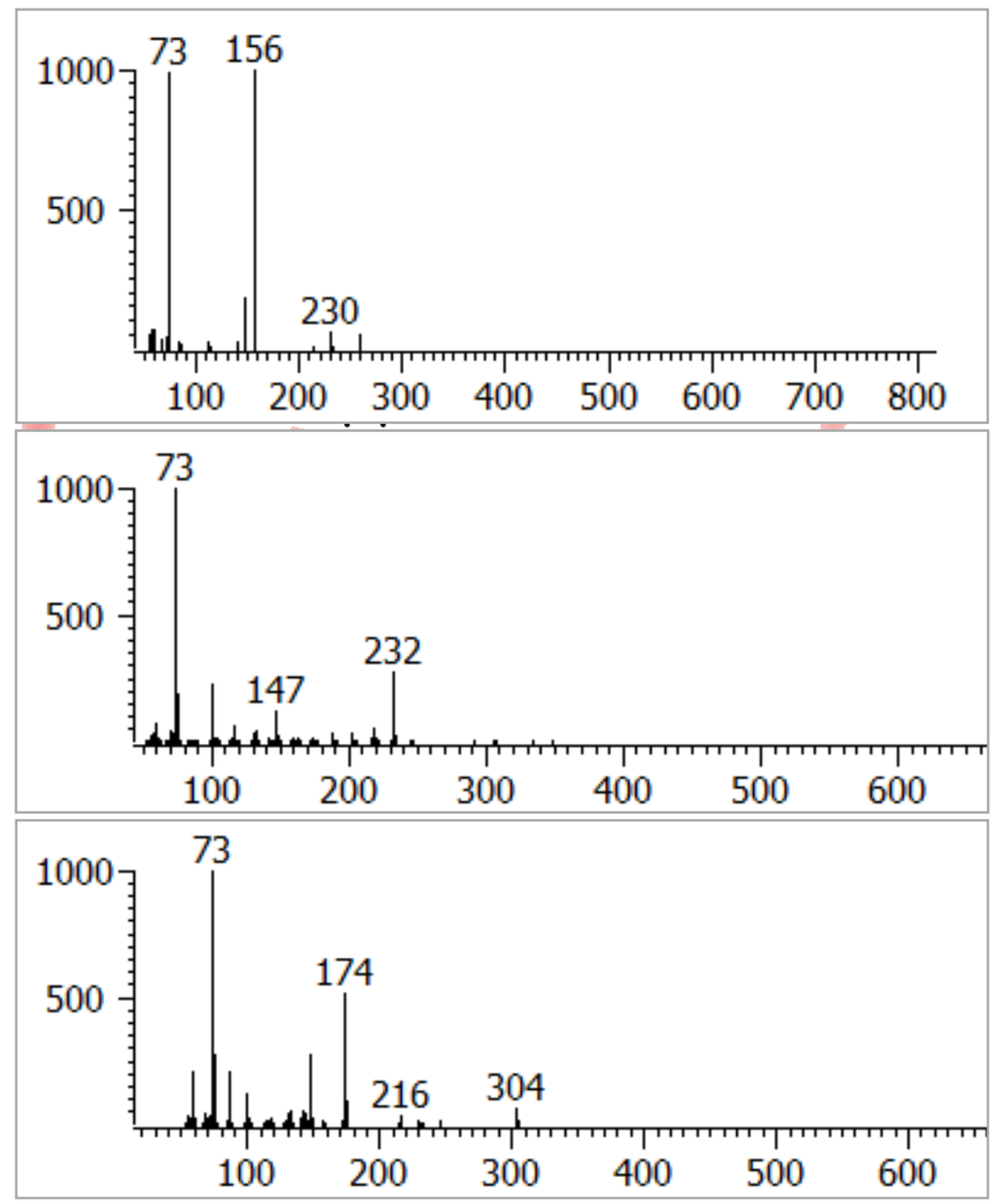
GC×GC mode advantages

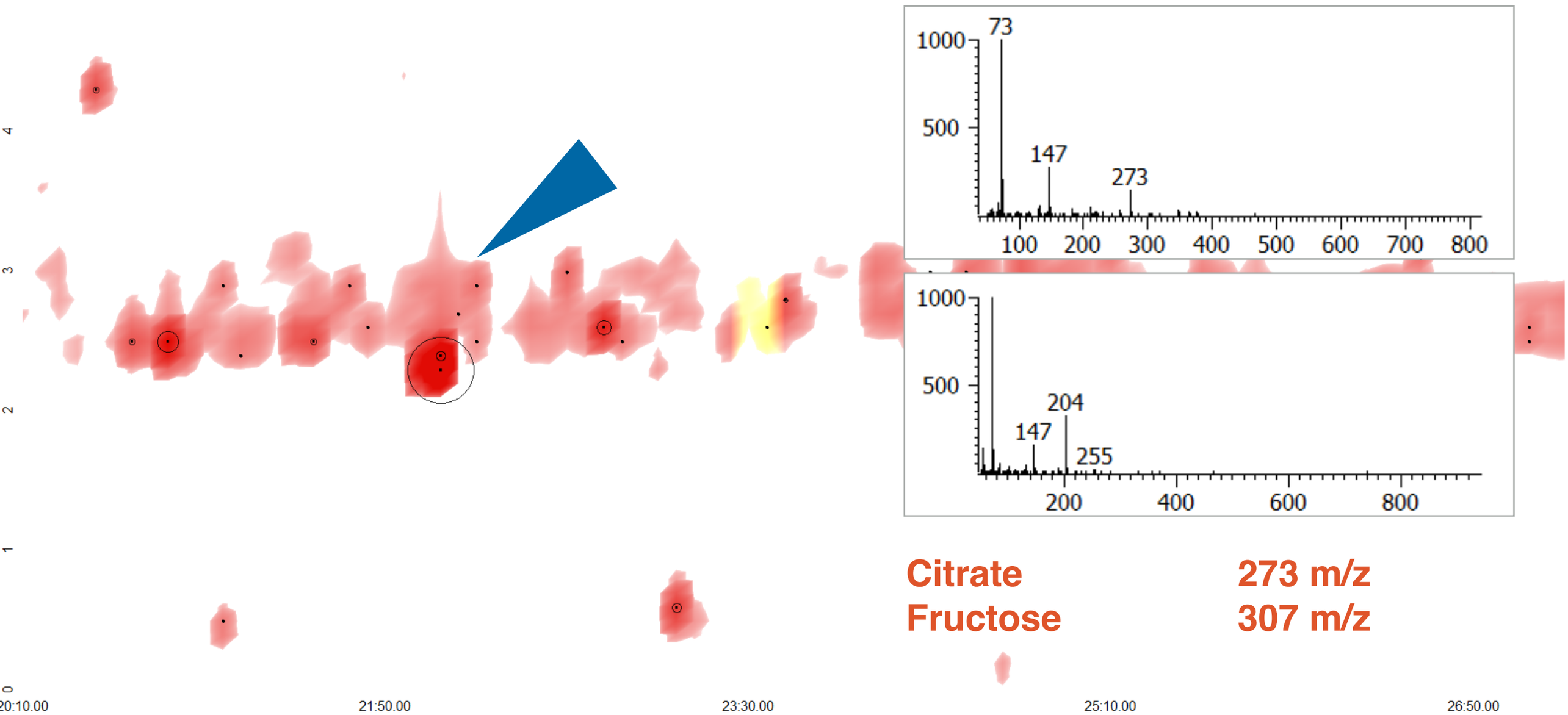




Oxoproline	**156 m/z**
Aspartate	**232 m/z**
α-aminobutyrate	**174 m/z**

Primary: Zorbax zb-5ms 30m × 0.25mm × 250 μm
Secondary: Restek RXI17 1.5m × 0.15mm × 250 μm

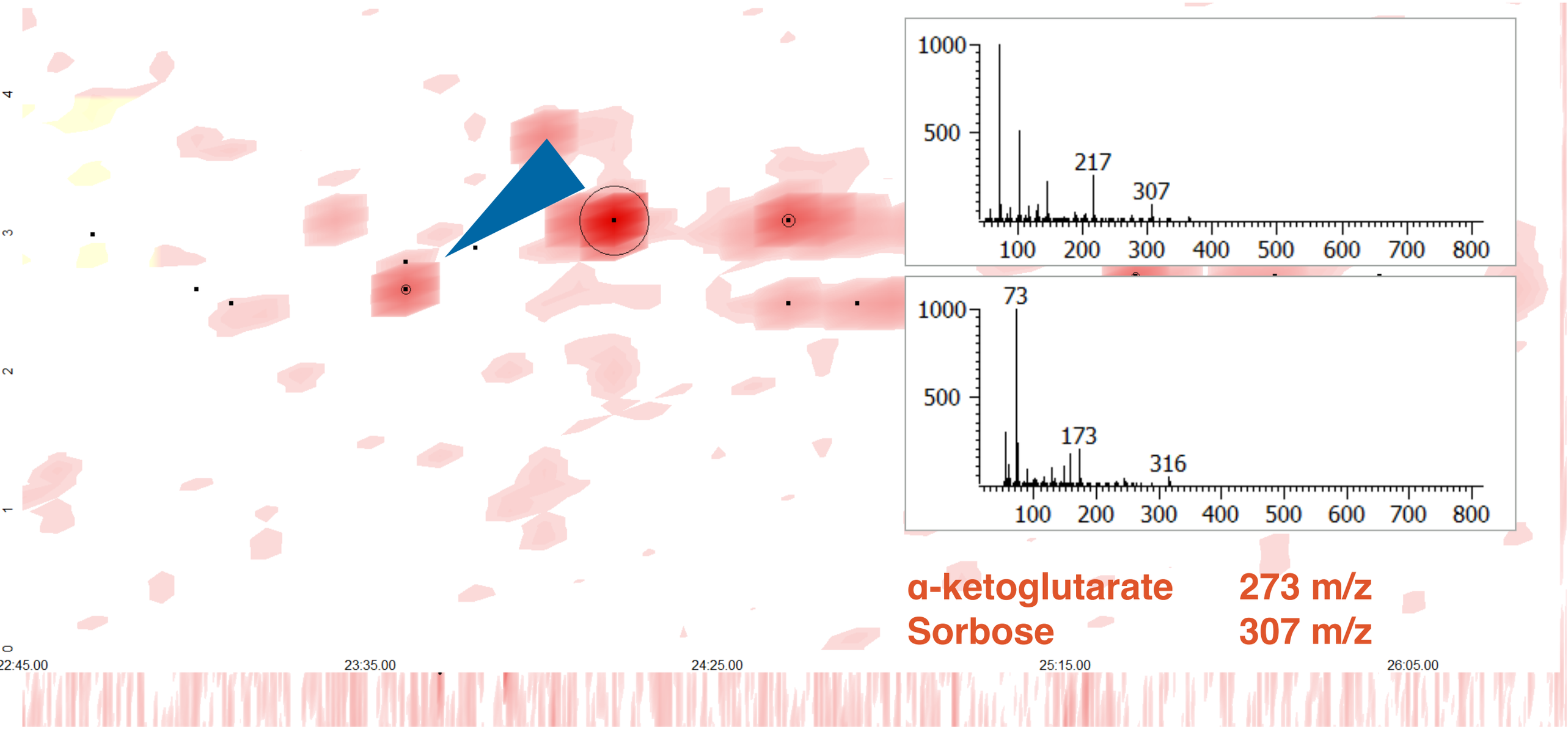
GC×GC mode advantages



Citrate **273 m/z**
Fructose **307 m/z**

Primary: Zorbax zb-5ms 30m × 0.25mm × 250 µm
Secondary: Restek RXI17 1.5m × 0.15mm × 250 µm

GC×GC mode advantages



α-ketoglutarate 273 m/z
Sorbose 307 m/z

Primary: Zorbax zb-5ms 30m × 0.25mm × 250 μm
Secondary: Restek RXI17 1.5m × 0.15mm × 250 μm

Conclusions

1. GC-TOFMS is essential to identification of osmotically active metabolites involved into regulation of gravity response.
2. GC×GC TOFMS benefits for analysis of complex metabolite profiles composed of similar metabolites.
3. GC×GC TOFMS increased sensitivity in addition to 2D separation allows to identify minor metabolites otherwise masked by more abundant compounds with same or similar Rt.

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Acknowledgements

Dr. Dmitry V. Suslov
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St. Petersburg State, VIR

Prof. **Sergei Medvedev**
St. Petersburg
State University

Dr. **Dmitry Suslov**
St. Petersburg
State University

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RC MICT of SPbSU 109-8088.