

Metabolomic Changes in Grapevine Leaves of Resistant Varieties after Infection with *Plasmopara viticola*



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Targeted analysis

Necessary but not sufficient, the advantages and disadvantages



The current knowledge of chemical processes in plants, animals and humans are mainly based on conventional studies in which profiles of metabolites involve “targeted” metabolites or “targeted” classes of metabolites.



As a consequence the majority (80-90%) of plant metabolites remain unknown.



Metabolome



Metabolome:

all organic compounds of the specific plant (vitamins, amino acids, antioxidants, hormones, sugars, aromatic compounds, ...)

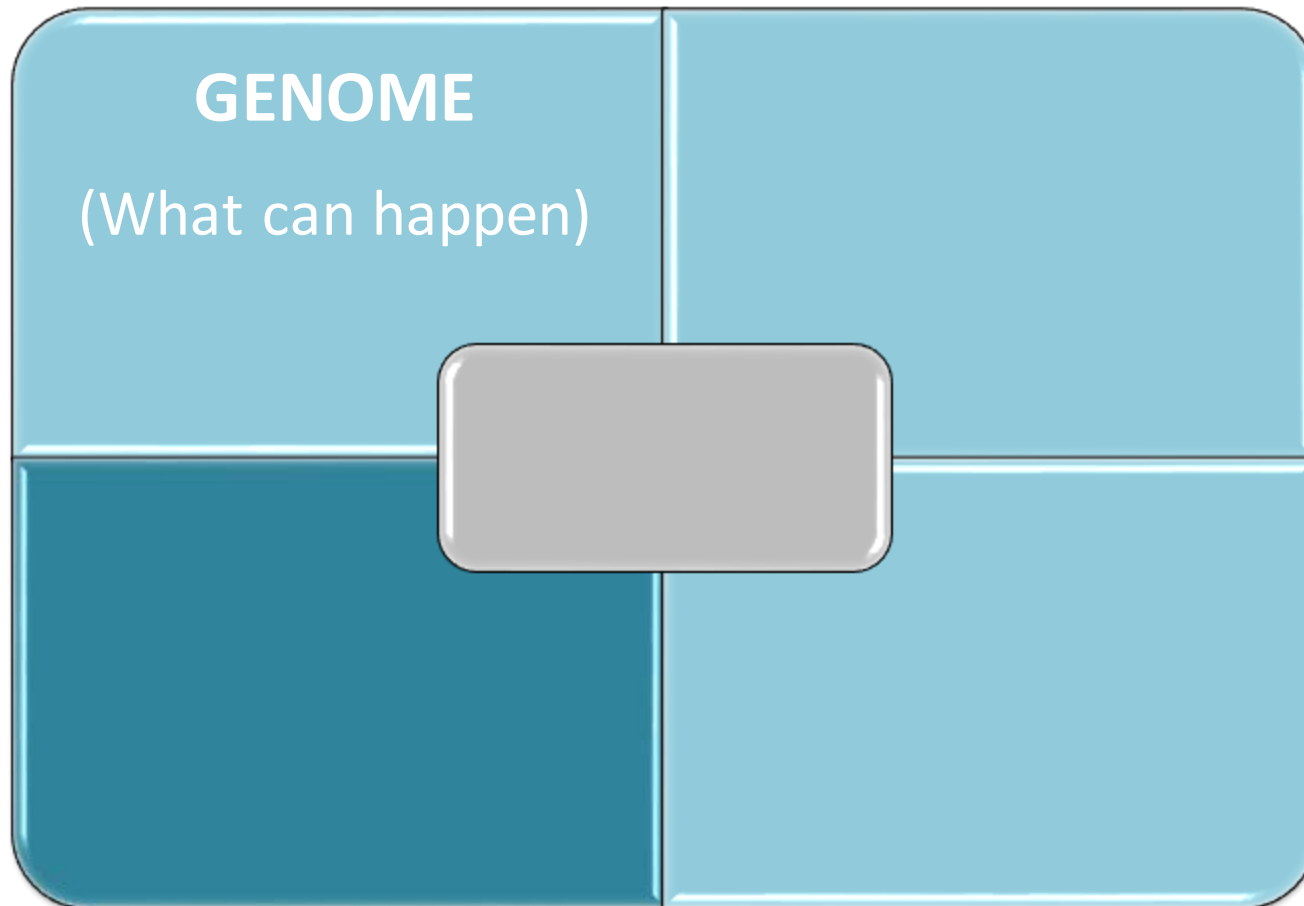
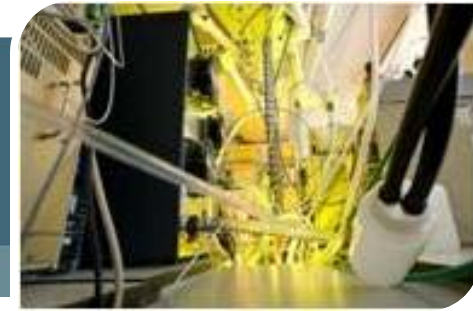
How big is metabolome:

- microorganisms > 600 metabolites
- human > 2.500 metabolites + food + drugs, ...
- **plants 200.000 metabolites, per species 5.000-10.000**

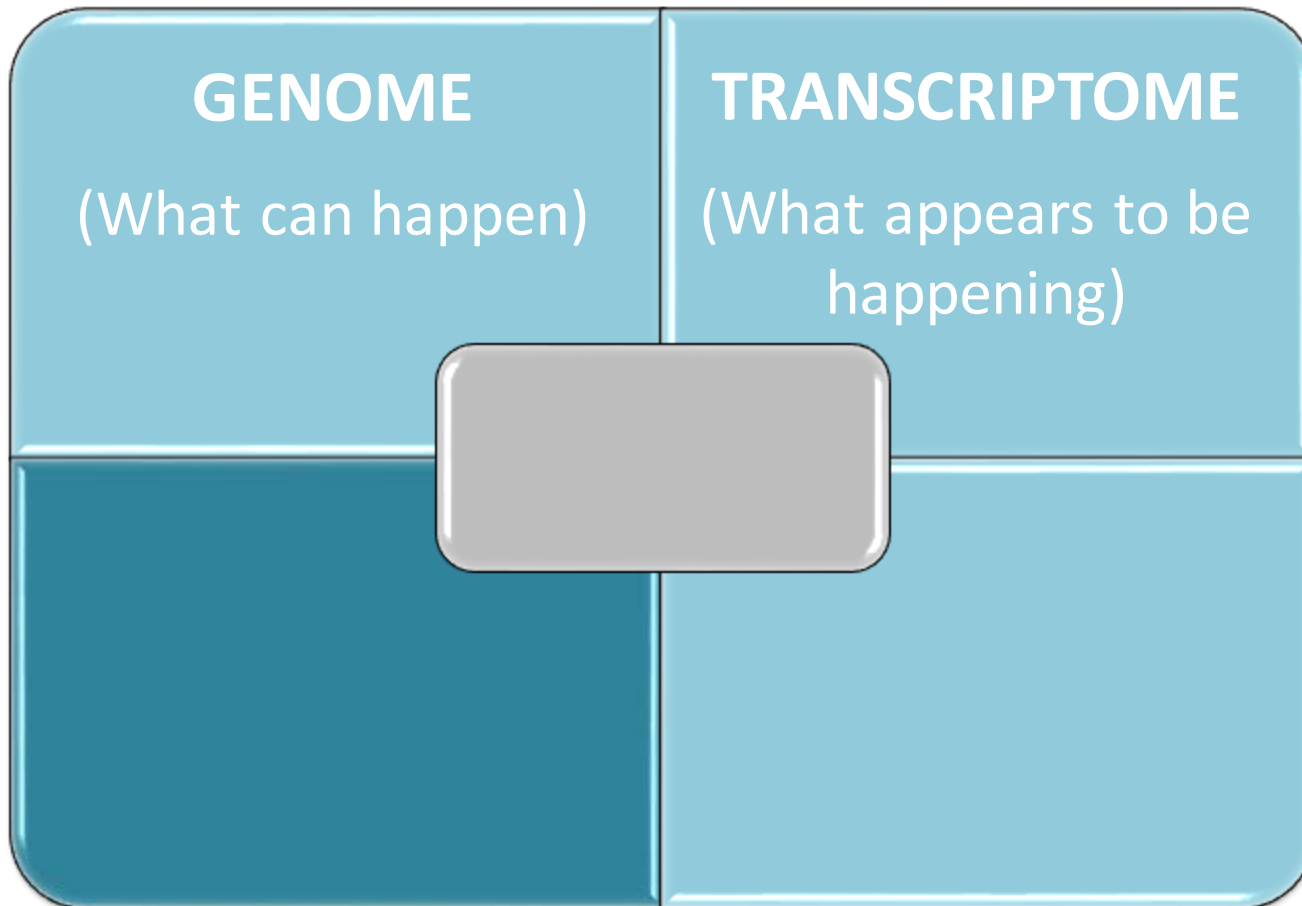
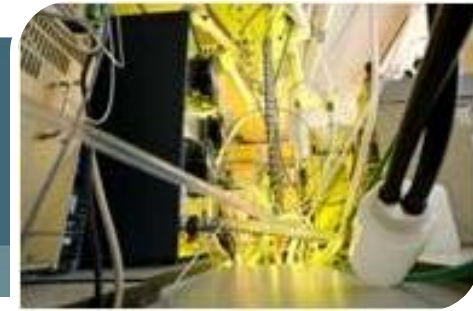
Known compounds:

- grape, apple: estimate 5.000-10.000 metabolites, **known ca. 10%**
- human diagnostics: 2% of endogenous metabolites

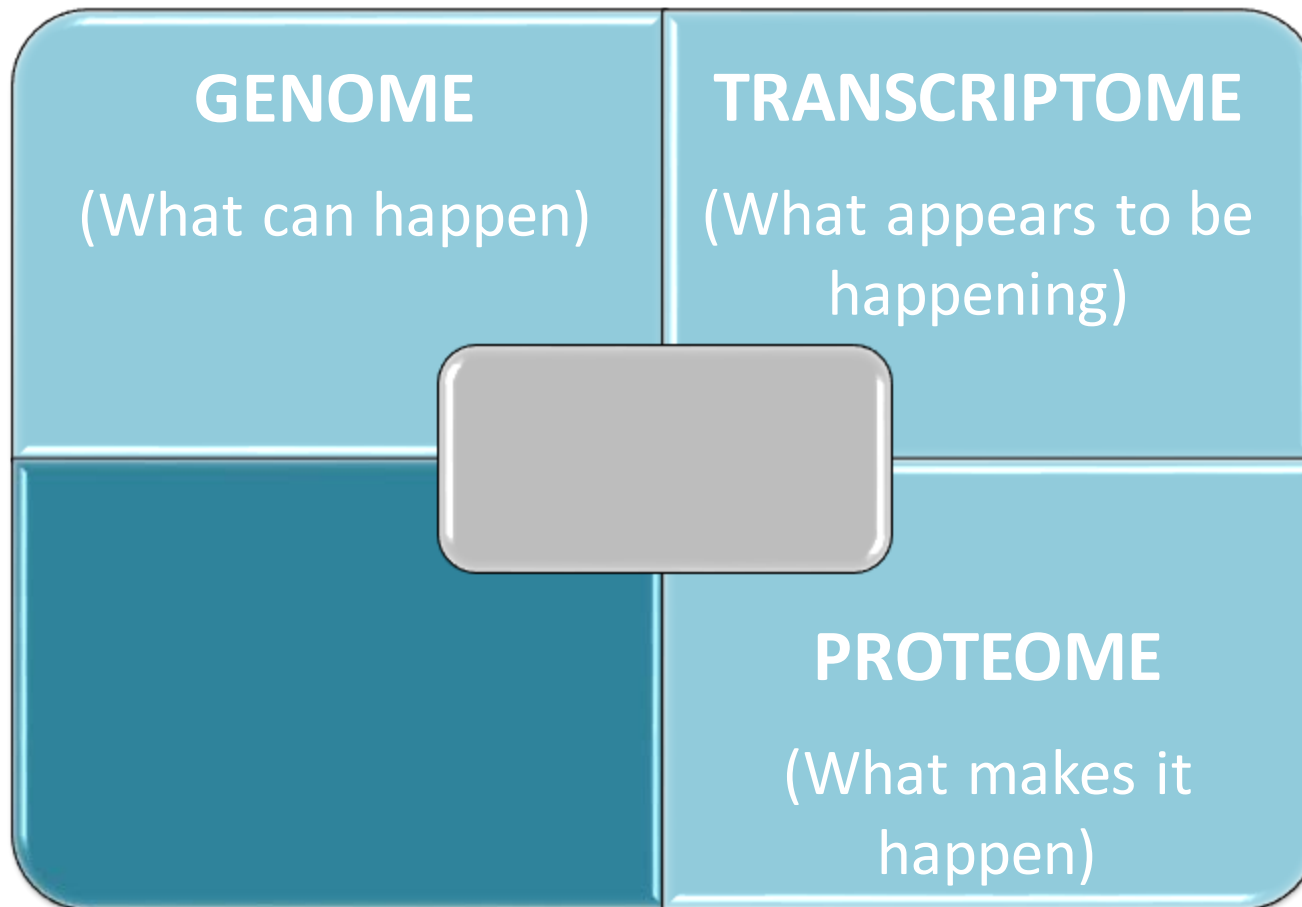
The sequence of "Omics" ...



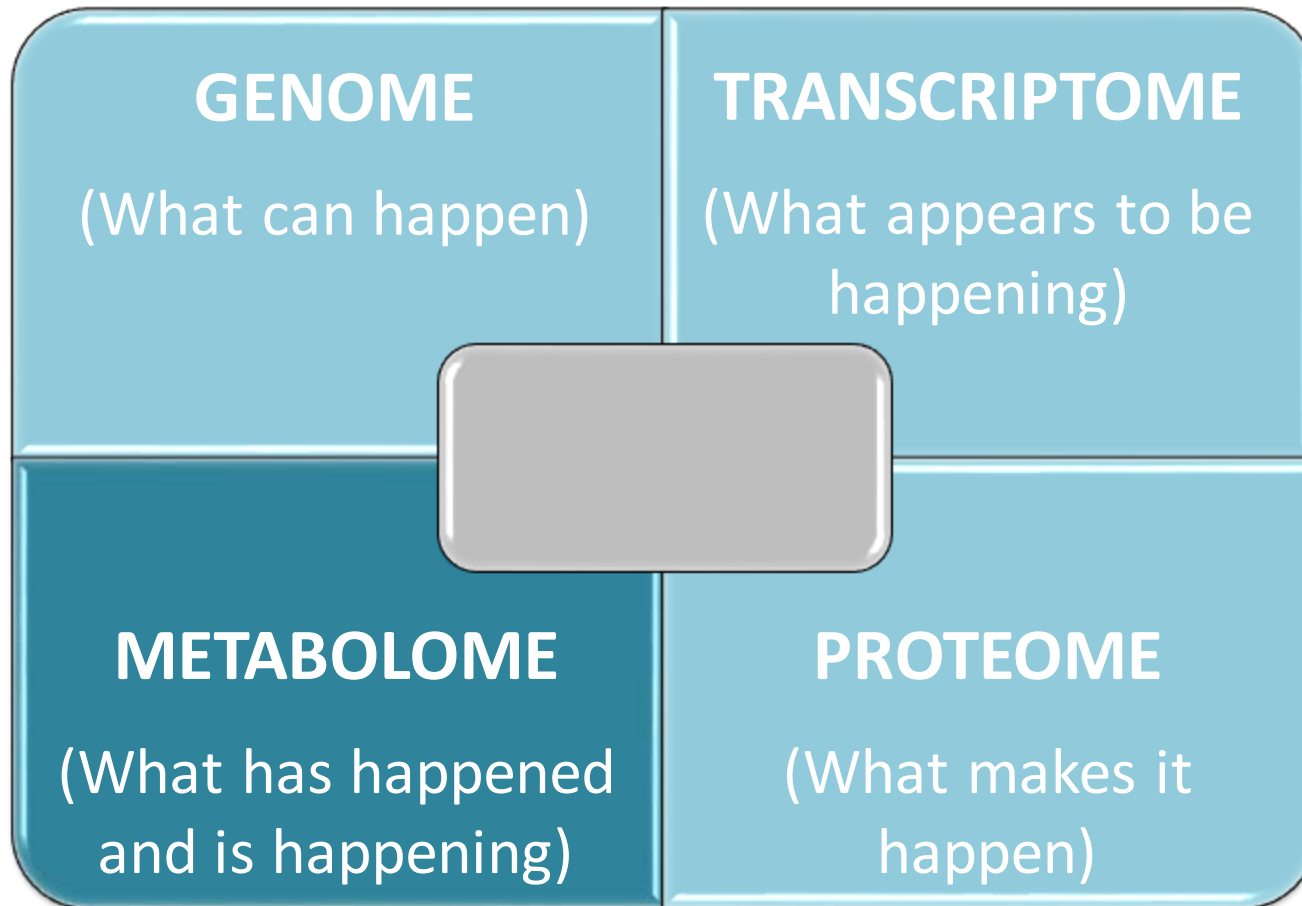
The sequence of "Omics" ...



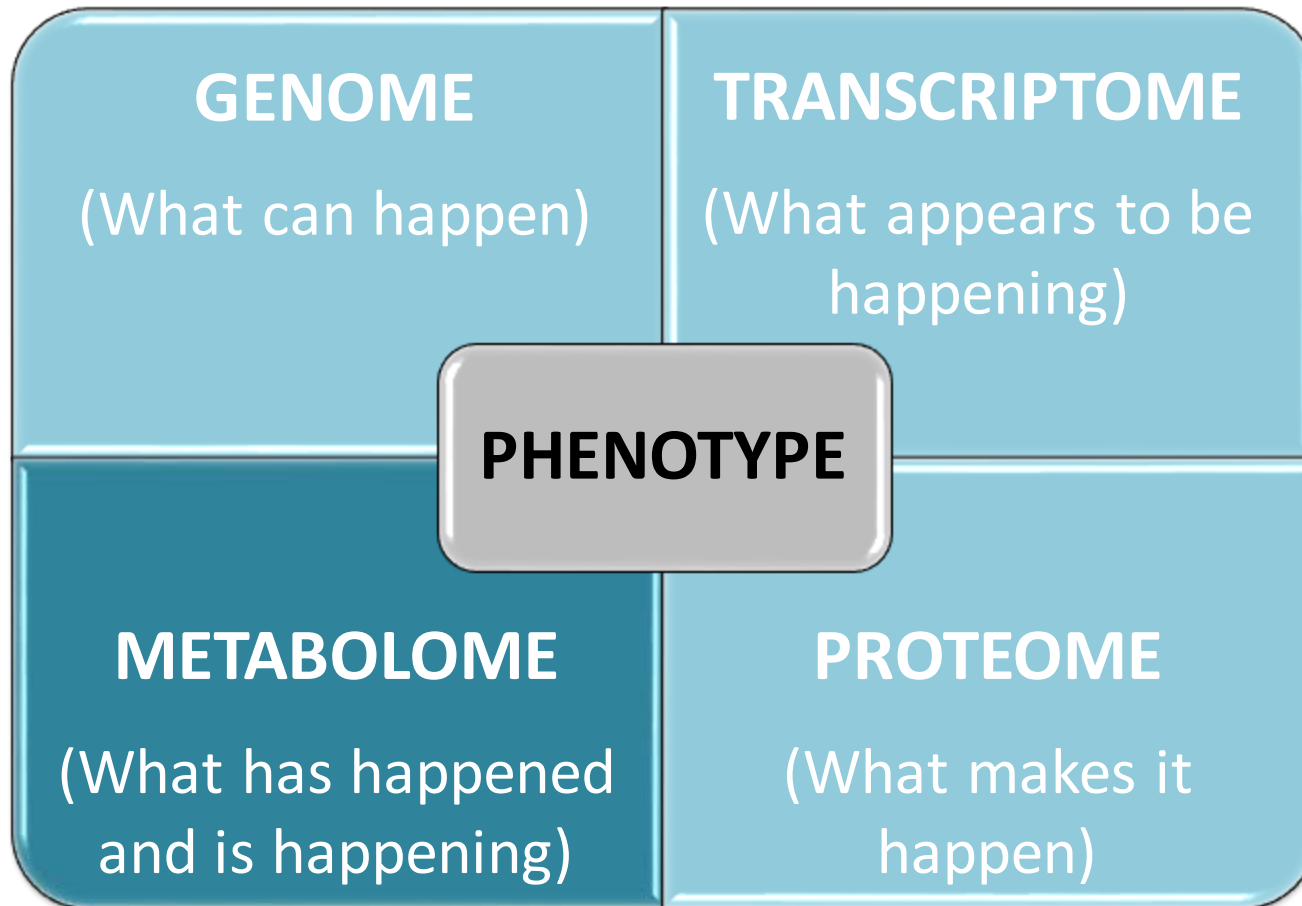
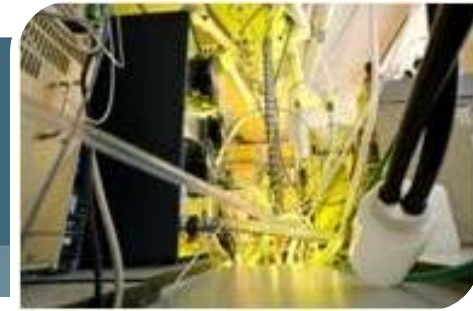
The sequence of "Omics" ...



The sequence of "Omics" ...



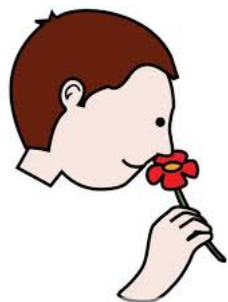
The sequence of "Omics" ...



Chemical classes of plant metabolites



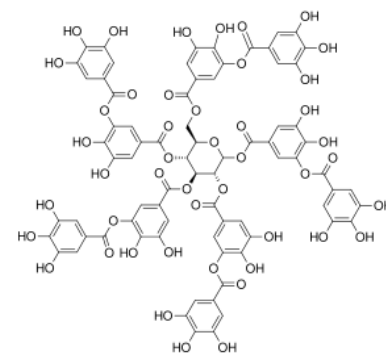
Volatile compounds



Primary metabolites



Secondary metabolites



Lipids

Study

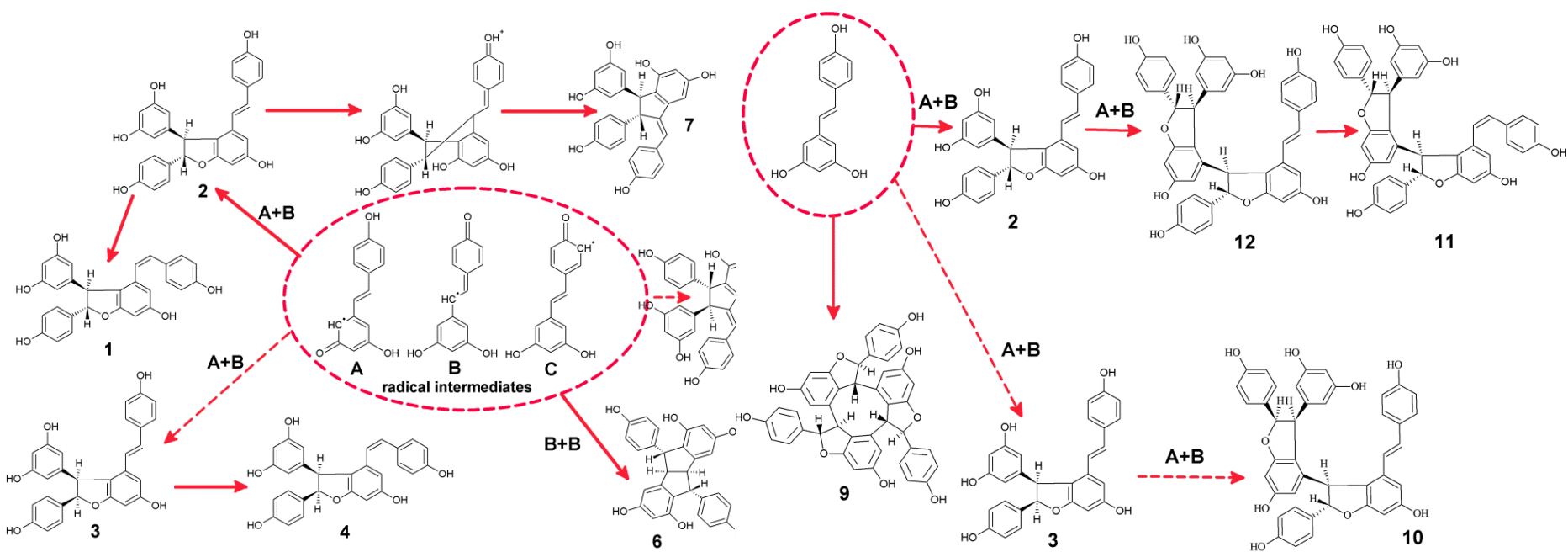


- Different fonts of resistance → different metabolites:
Infection of grapevine results into the production of **different metabolites**, in particular in the group of **viniferins**.

In this study, different metabolites in the leaves of plants from different species **resistant to downy mildew** were defined with markers *RPV* 1; 3; 10; 12.

- Number of metabolites analysed: >100

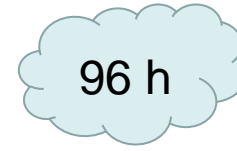
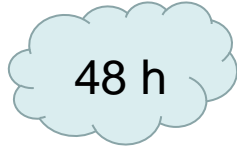
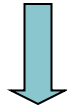
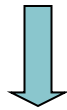
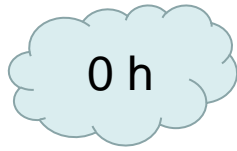
Formation of viniferins in the leaves of resistant Merzling x Teroldego grapes infected with *Plasmopara viticola*



Study



Time sampling

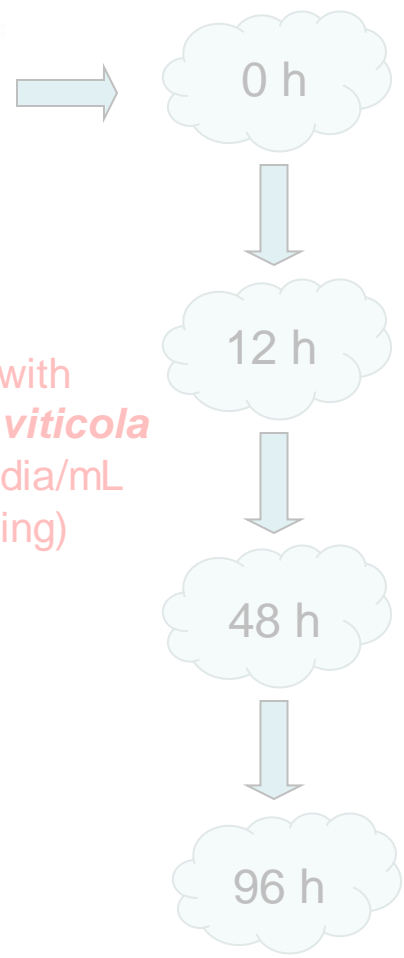


Infection with
Plasmopara viticola
 1×10^{-6} conidia/mL
(by spraying)

Study



Time sampling



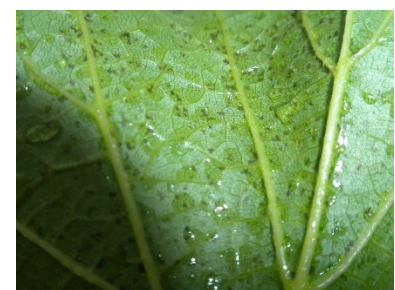
Infection with *Plasmopara viticola*
 1×10^{-6} conidia/mL
(by spraying)

Symptoms at 6 DPI

Bianca (*RPV 3*)



Jasmine (*RPV 12*)



Peverella
Vitis vinifera



BC4 (*RPV 1*)



Solaris (*RPV 10*)



A Versatile Targeted Metabolomics Method for the Rapid Quantification of Multiple Classes of Phenolics in Fruits and Beverages

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Supporting Information

ABSTRACT: Compelling evidence of the health benefits of phenolic compounds and their impact on food quality have stimulated the development of analytical methods for the identification and quantification of these compounds in different matrices in recent years. A targeted metabolomics method has been developed for the quantification of 135 phenolics, such as benzoates, phenylpropanoids, coumarins, stilbenes, dihydrochalcones, and flavonoids, in fruit and tea extracts and wine using UPLC/Qq2-MS/MS. Chromatography was optimized to achieve separation of the compounds over a period of 15 min, and MRM transitions were selected for accurate quantification. The method was validated by studying the detection and quantification limits, the linearity ranges, and the intraday and interday repeatability of the analysis. The validated method was applied to the analysis of apples, berries, green tea, and red wine, providing a valuable tool for food quality evaluation and breeding studies.

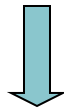
KEYWORDS: metabolite profiling, polyphenols, food analysis, UPLC/Qq2-MS/MS, mass spectrometry

A new RP-LC-MS/MS method for the determination of secondary metabolites

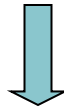
Method development



c.a. 150 phenolics selected for their importance and/or relevance, covering the major classes

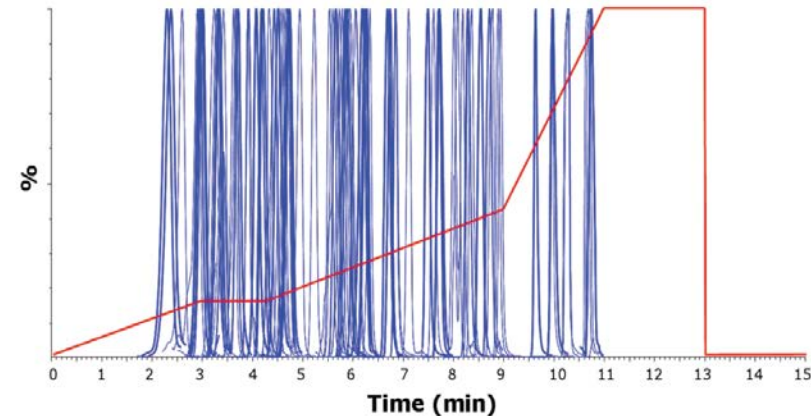


Optimization of chromatography and MS/MS conditions



Sensitivity, linearity and stability

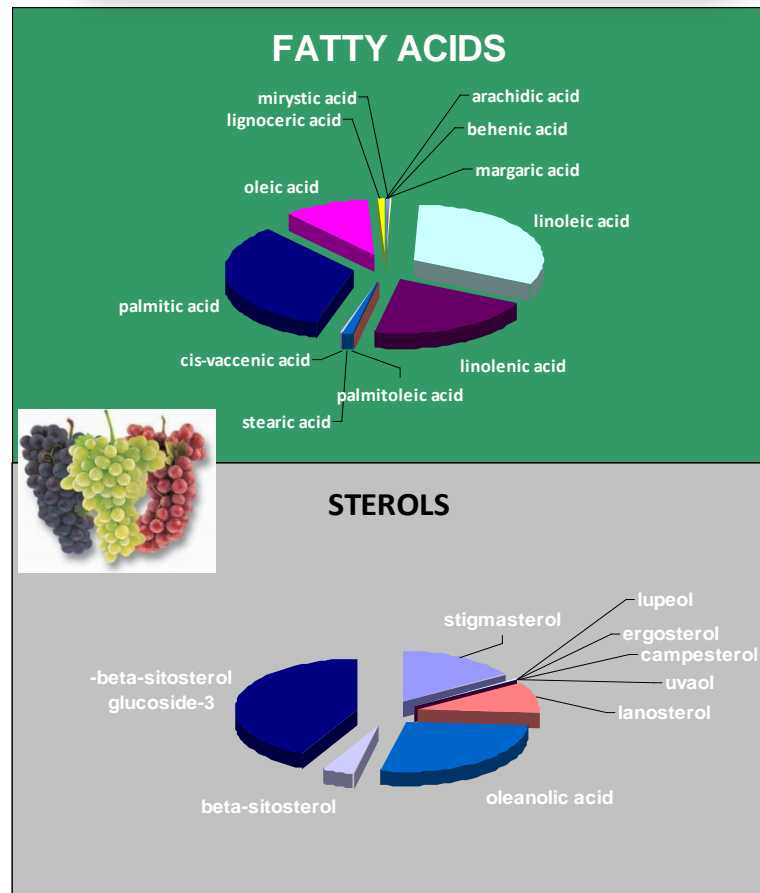
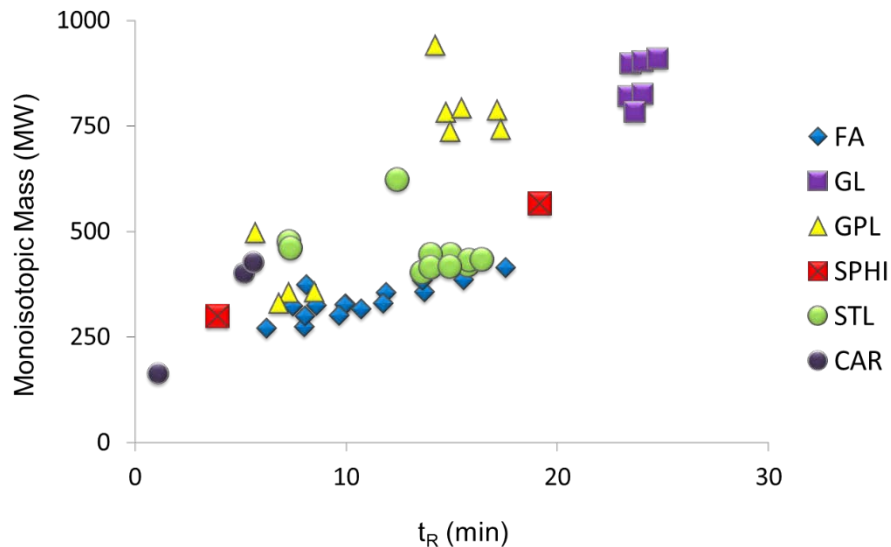
- Chalcones
- Flavanones
- Flavan-3-ols
- Flavones
- Isoflavones
- Flavonols



Targeted LC-MS/MS analysis of grape lipids (fatty acyls, glycerolipids, sphingolipids, sterols, glicerophospholipids)



The method allowed us to identify 35 lipids



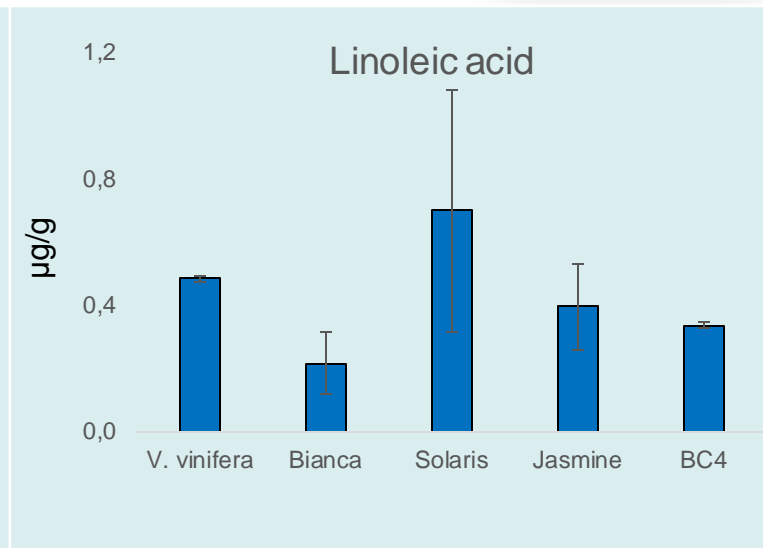
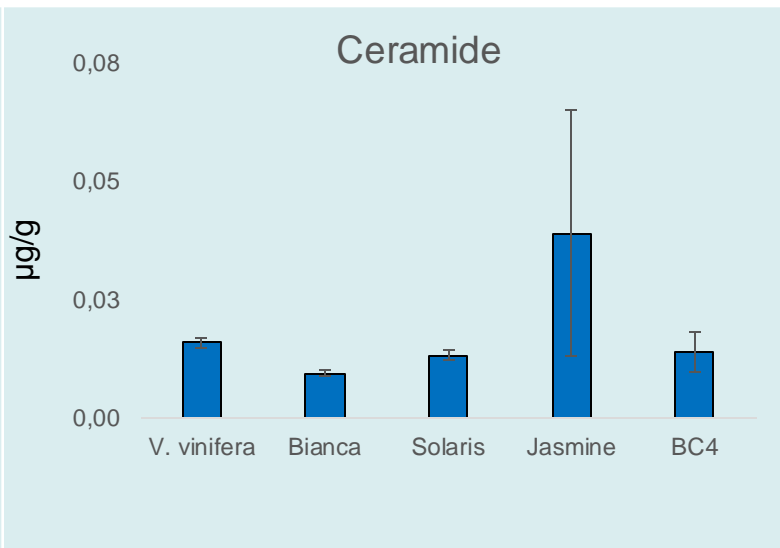
Results – Basal levels



Control non infected

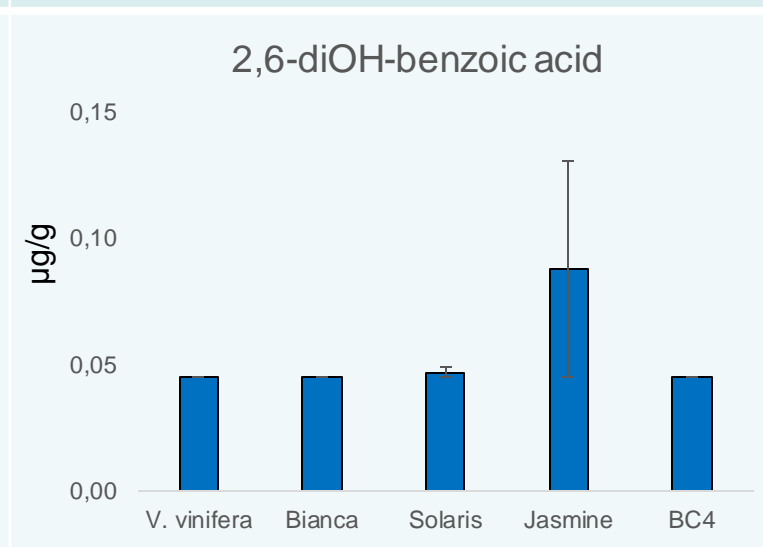
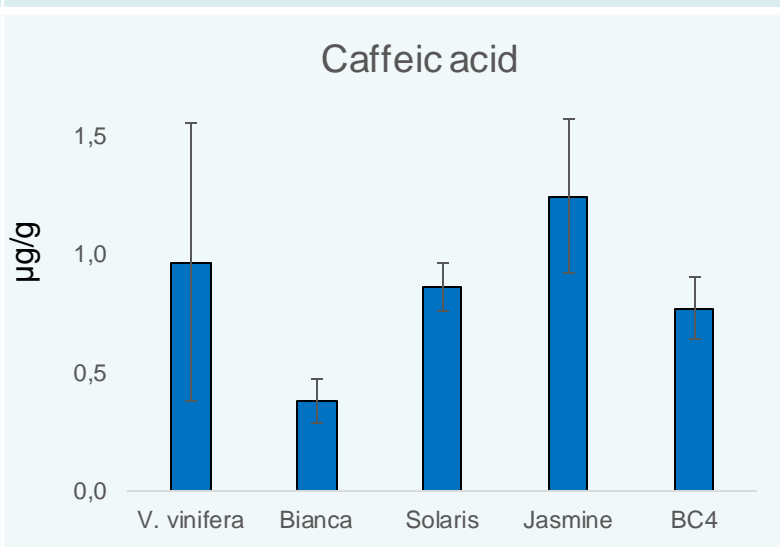
Lipids

0 h



Polyphenols

0 h



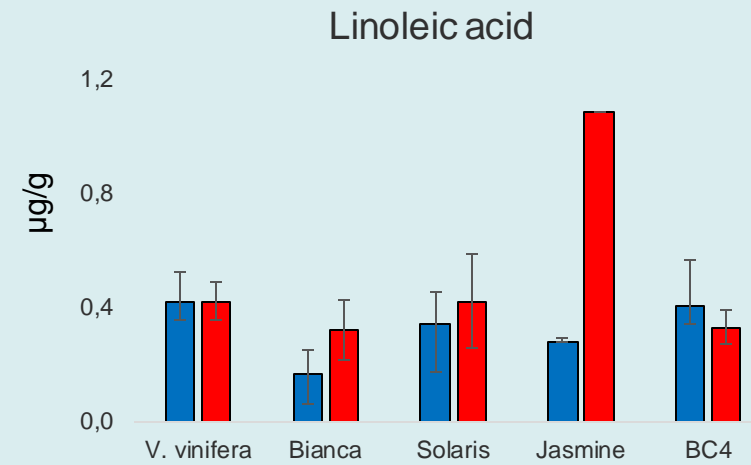
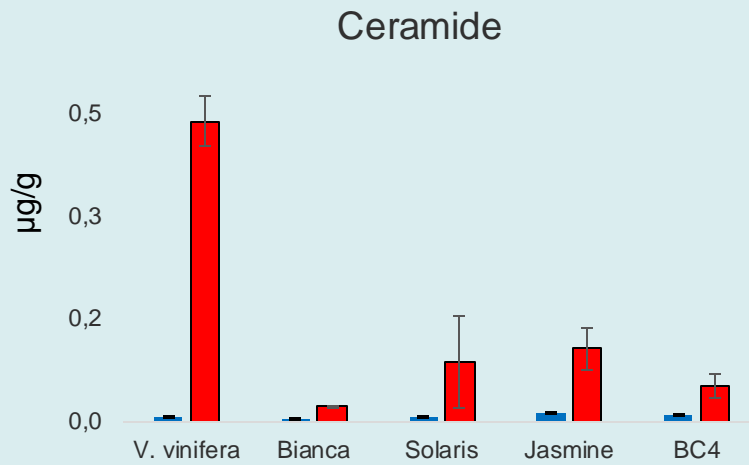
Results – Influence of VARIETY



Control non infected
Infected

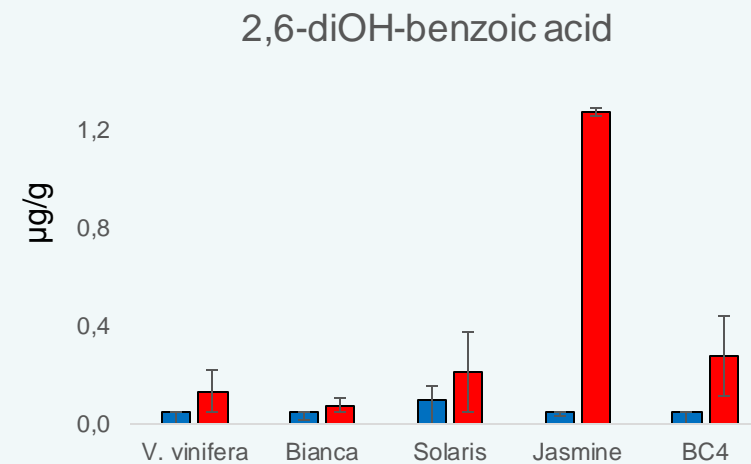
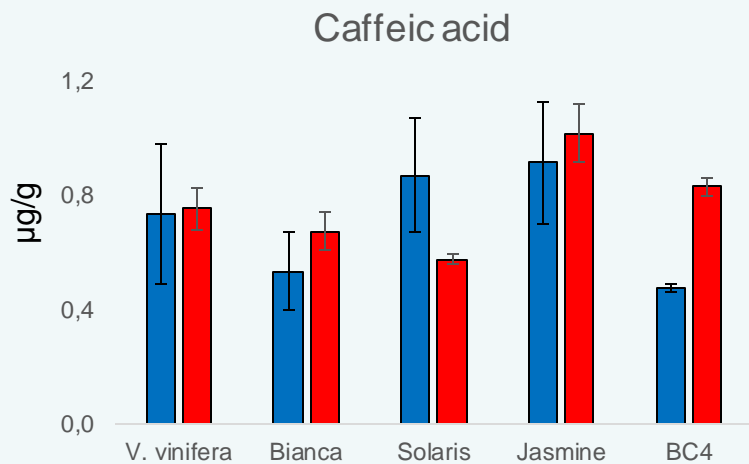
Lipids

96 h



Polyphenols

96 h



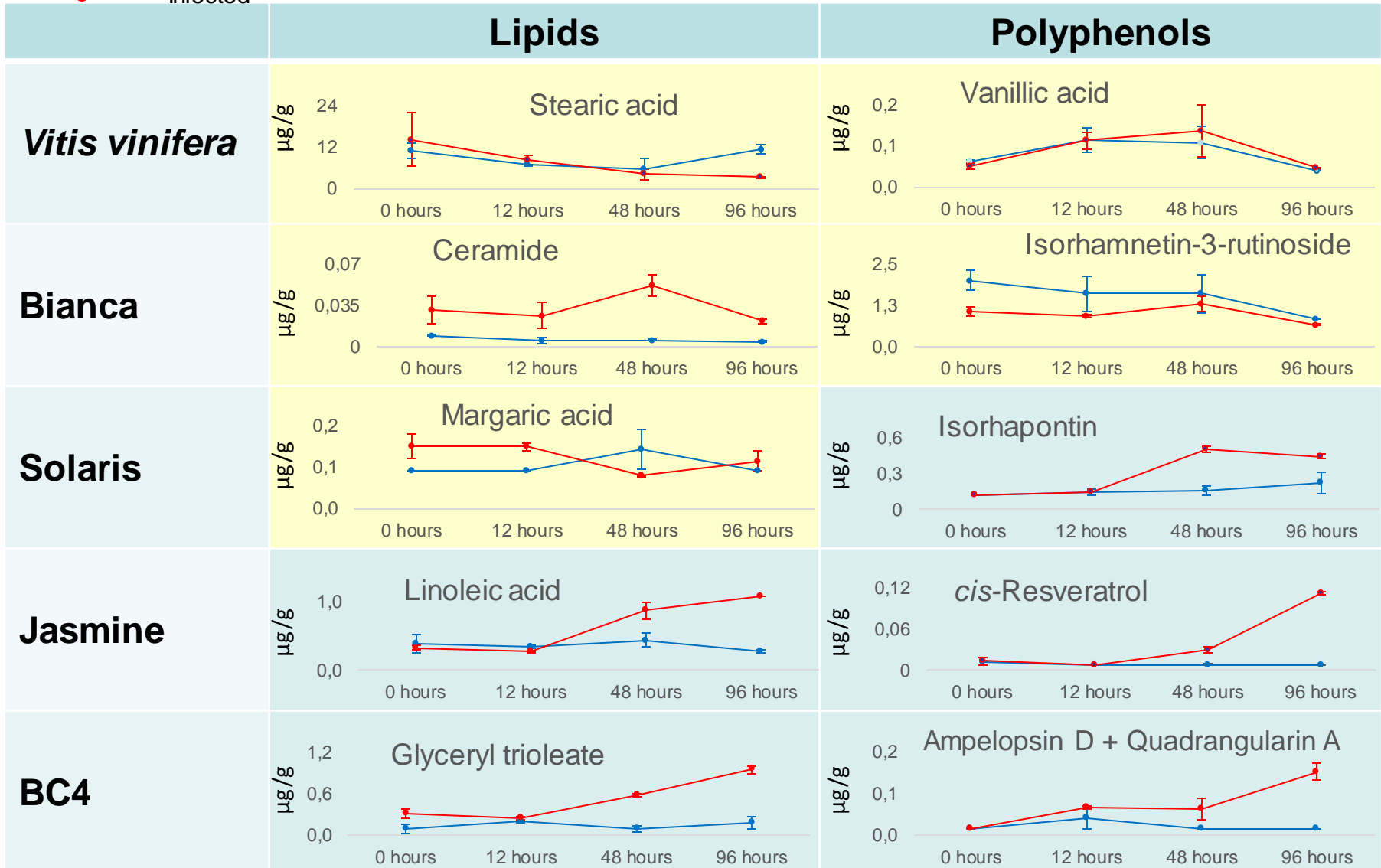
Results – Influence of TIME



—●— Control non infected
—●— Infected

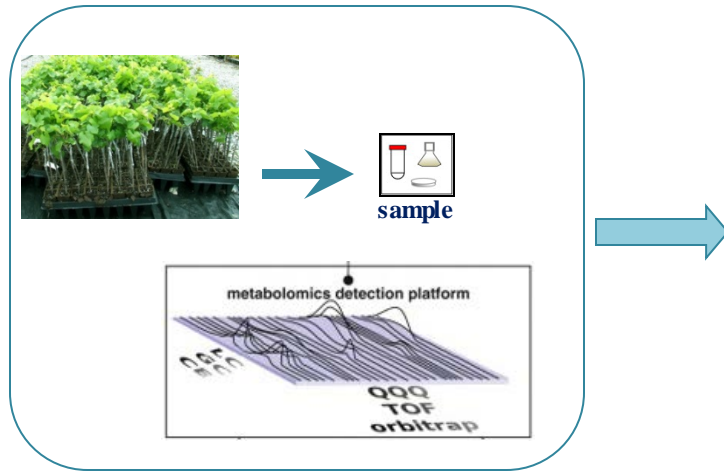
Not significant

Significant



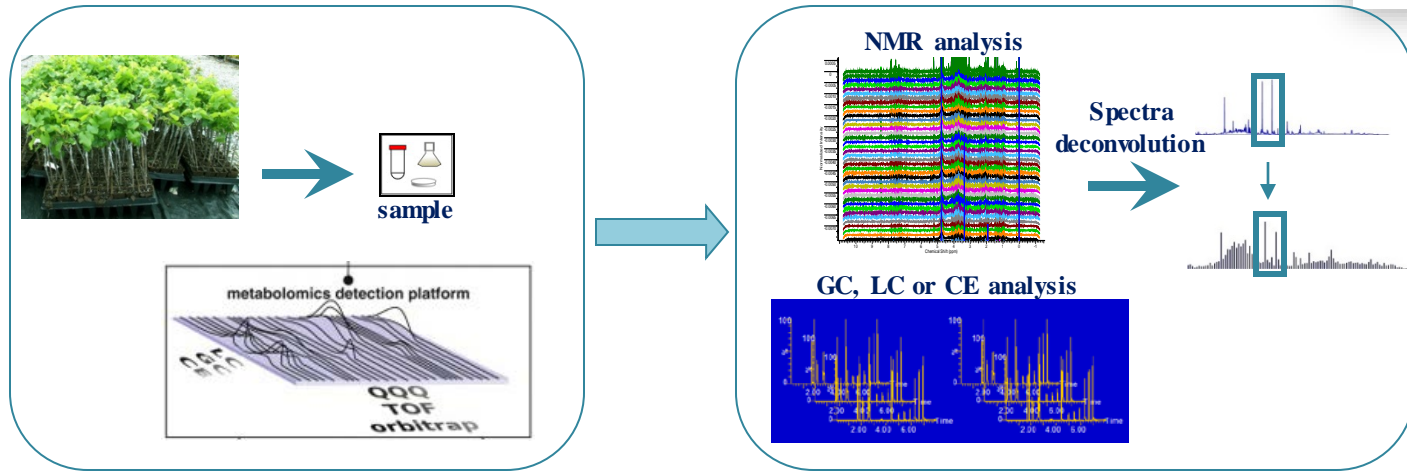
Population studies gene/metabolite

THE NEXT
STEP



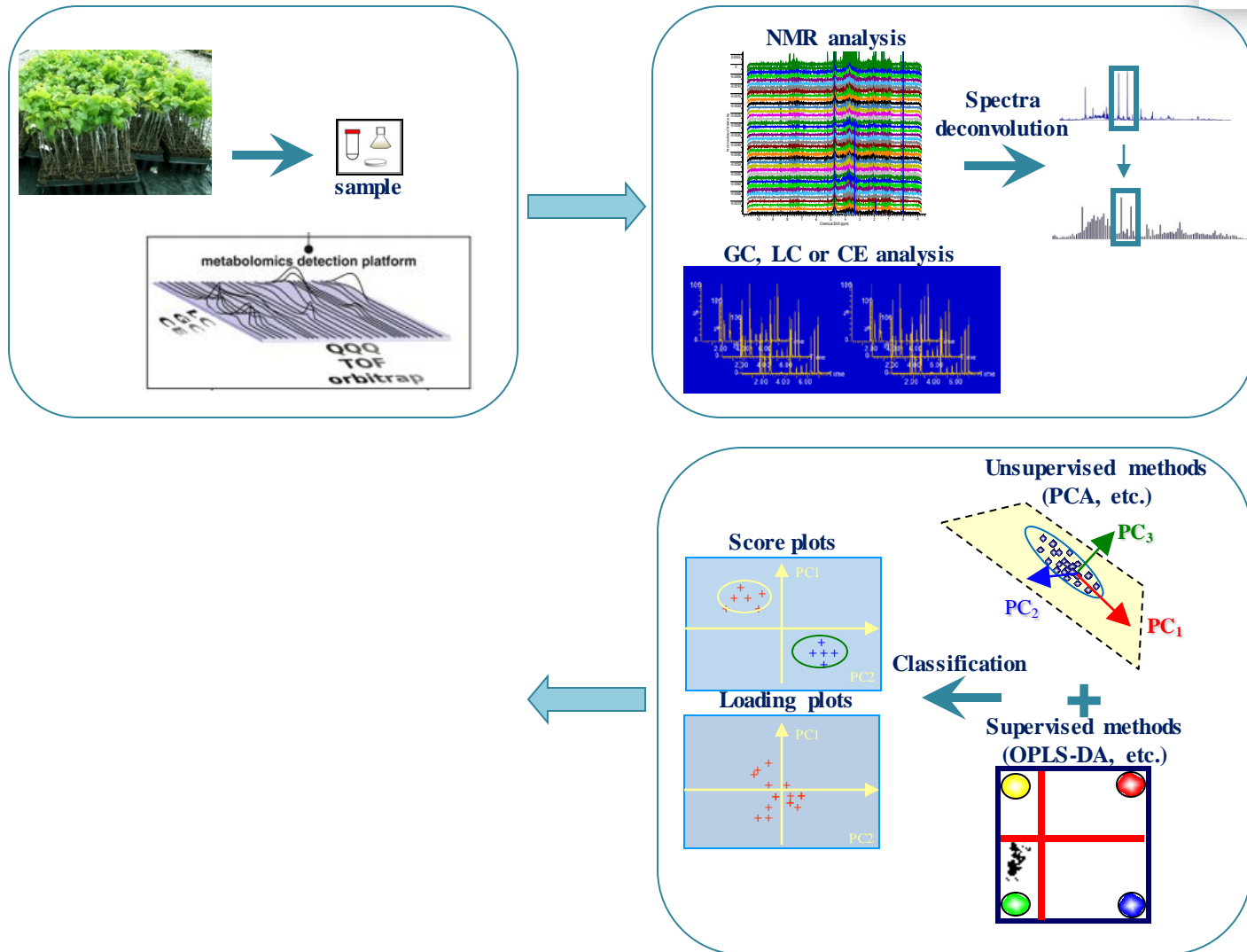
Population studies gene/metabolite

THE NEXT STEP



Population studies gene/metabolite

THE NEXT STEP

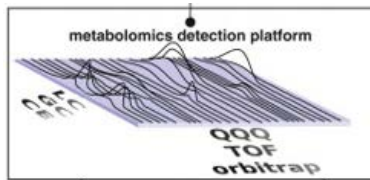


Population studies gene/metabolite

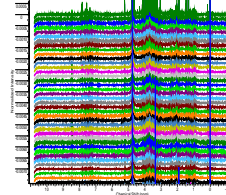
THE NEXT STEP



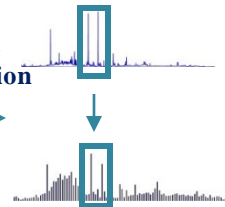
sample



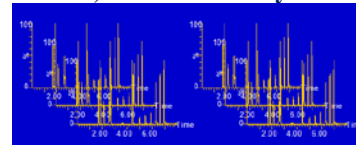
NMR analysis



Spectra deconvolution



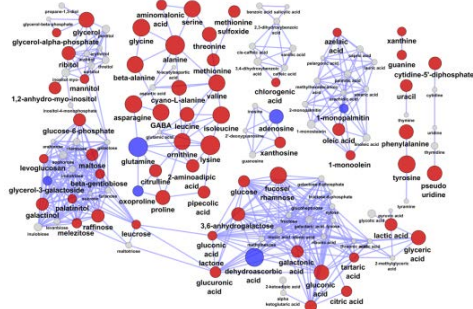
GC, LC or CE analysis



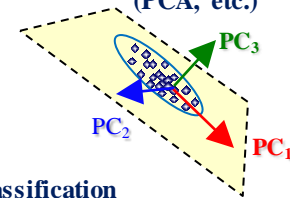
Field trials



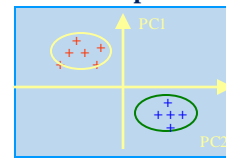
Metabolic pathways



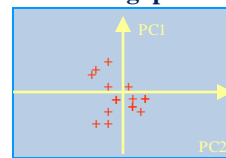
Unsupervised methods
(PCA, etc.)



Score plots

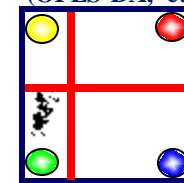


Loading plots



Classification

Supervised methods
(OPLS-DA, etc.)



VitisCyc: overview of the *Vitis vinifera* Metabolic Map (courtesy of Sushma Naithani)



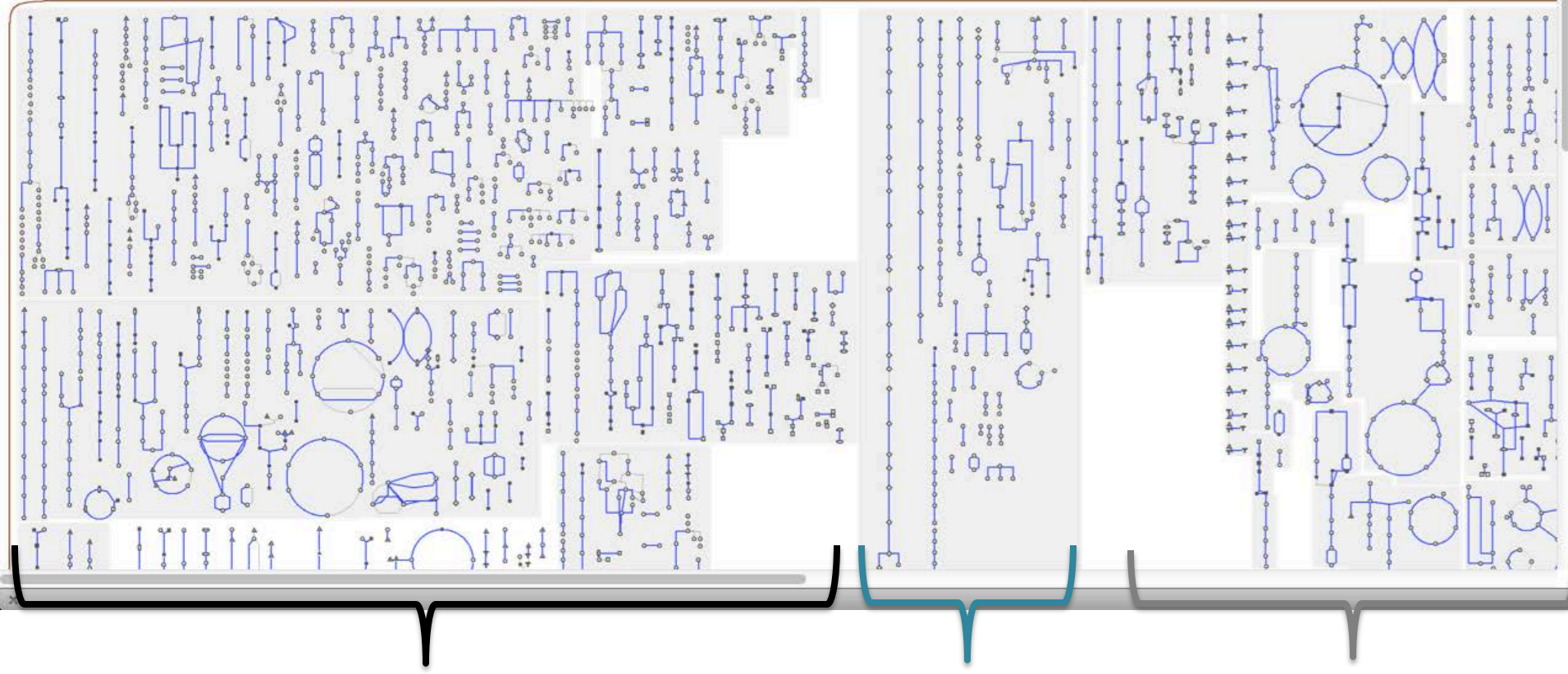
Home Search Tools Help

Note: there is a new Cellular Overview Implementation, please see [new Cellular Overview](#). The new Cellular Overview is accessible by the top menu bar command Tools--Cellular Overview.

Overview of the *Vitis vinifera* Metabolic Map

This diagram provides a schematic of all pathways of *Vitis vinifera* metabolism. Nodes represent metabolites, with shape indicating class of metabolite (see key to right). Lines represent reactions. Move the mouse over a metabolite icon to identify it. Click on a metabolite icon to navigate to the metabolite page or a related pathway page.

- [Instructions](#)
- [Omics Viewer: Paint omics data onto this diagram](#)
- [Species Comparison: Highlight reactions shared with other organisms](#)



Biosynthesis

**Generation of Precursor
Metabolites and Energy**

Degradation

Thank you for your attention!

