EGFV UMR 1287

Ecophysiologie et Génomique Fonctionnelle de la Vigne

BIC

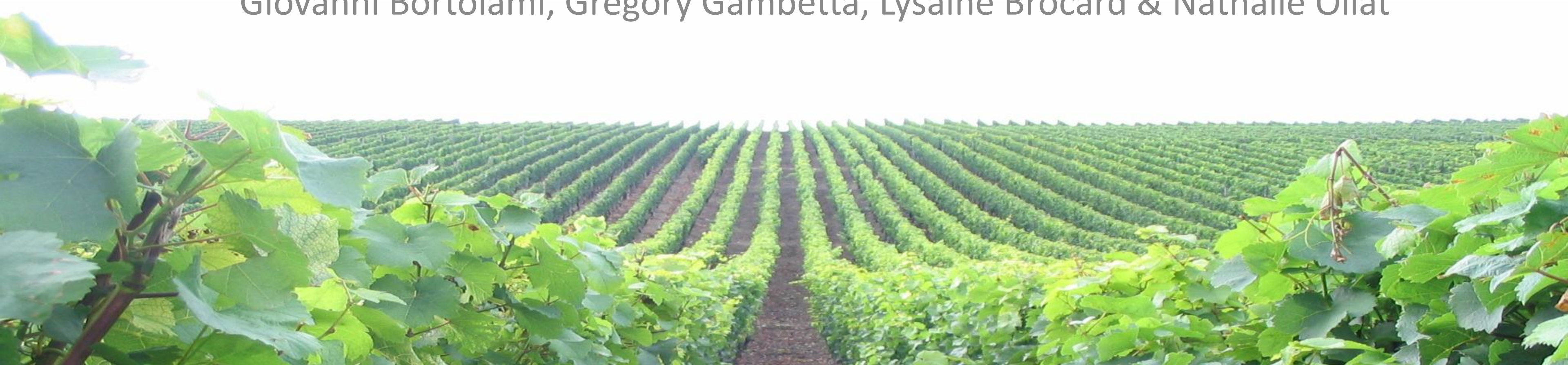
FRANCE-BIOIMAGING

Unité de recherche œnologie

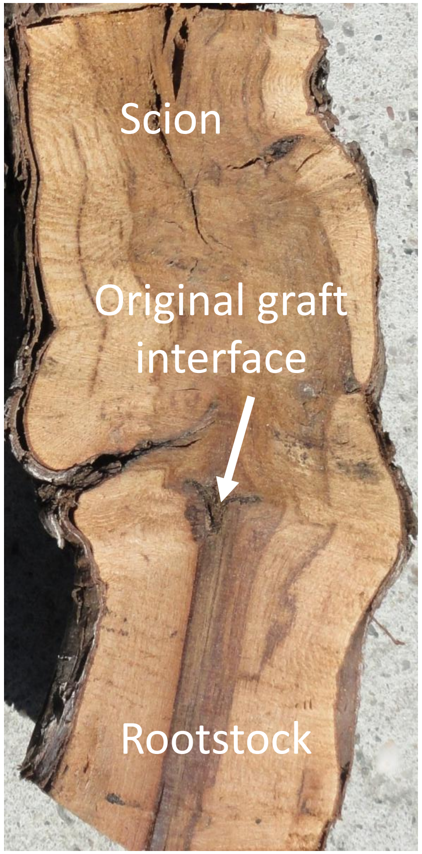
EA4577 - USC 1366 INRA

Understanding scion/rootstock interactions at the graft interface of grapevine

Sarah Jane Cookson, Duyen Prodhomme, Clément Chambaud, Cyril Hevin, Josep Valls Fonayet, Ghislaine Hilbert, Claudine Trossat-Magnin, Tristan Richard, Giovanni Bortolami, Gregory Gambetta, Lysaine Brocard & Nathalie Ollat



- In Europe, grapevines are grafted because of the Phylloxera outbreak of the end of the 19th century.
- Phylloxera is a soil dwelling aphid pest that is native to America & was introduced accidentally to Europe.
- American grapevine species have tolerance to Phylloxera & used as rootstocks
- Successful graft union formation is key to viticulture today



Transversal section of a graft interface many years after grafting (Photo: JP Tandonnet)

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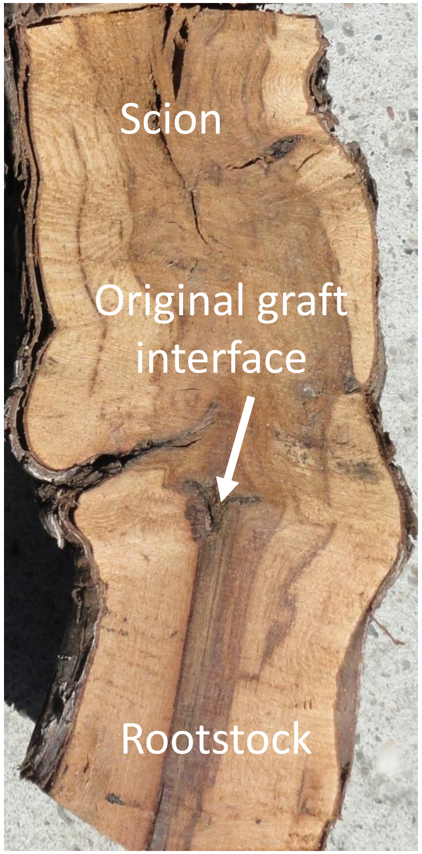
- Phylloxera is a soil dwelling aphid pest that is native to America & was introduced accidentally to Europe.
- American grapevine species have tolerance to Phylloxera & used as rootstocks
- Successful graft union formation is key to viticulture today

Although essential, producing grafted plants is not so easy.

215.3 millions grafts produced in France in 2015, 120-130 million grafts sold = 58 % sold[1]

This could be improved!

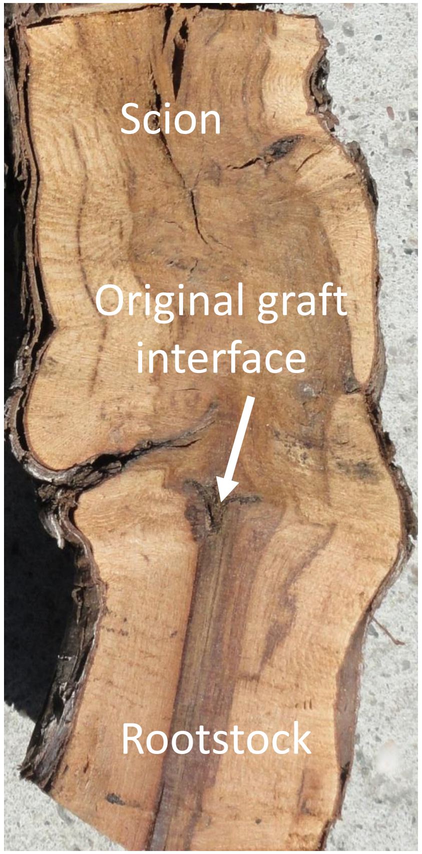


Transversal section of a graft interface many years after grafting (Photo: JP Tandonnet)

[1]www.ffpv.fr

Introduction | Cellular connections | Xylem connections | Transcripts & metabolites | Ongoing & Future work

Newly assembled grafted grapevine



Transversal section of a graft interface many years after grafting (Photo: JP Tandonnet)

1. **How does the graft union form?**
2. **Is hetero-grafting different from homo-grafting?**
3. **What are the causes of graft incompatibility?**

Graft union formation – wound responses & healing processes

Formation of a necrotic layer → Adhesion → Callus cell proliferation → Fragmentation of the necrotic layer → Contact between the cells of the two partners → Formation of **plasmodesmata** & differentiation of **xylem & phloem** → Development of functional connections between the scion & rootstock

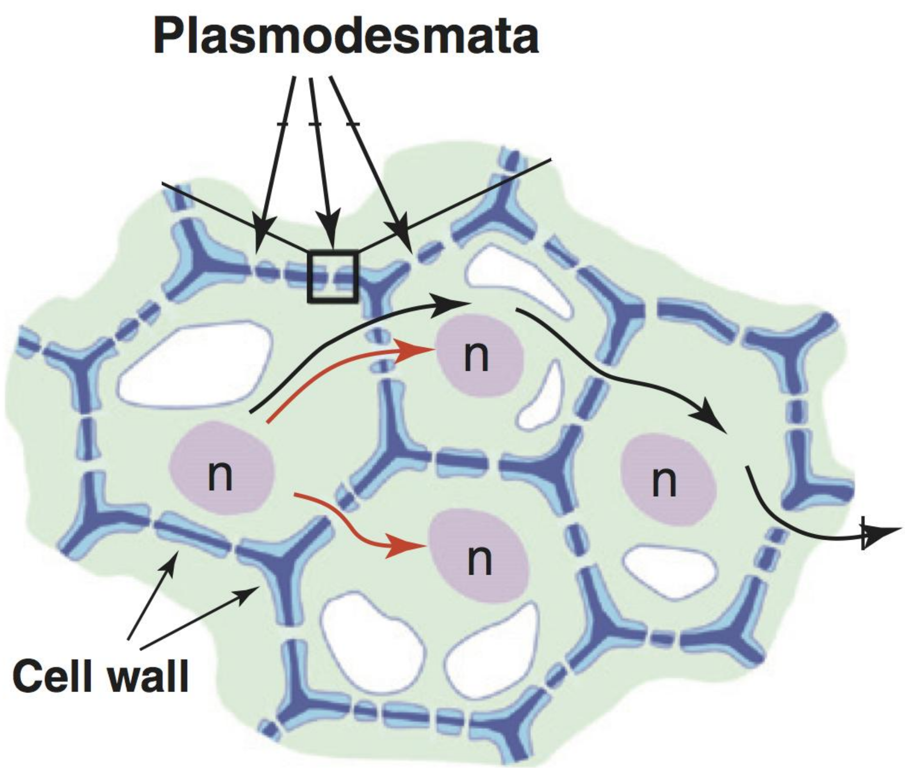
Grapevine graft interface 1 month after grafting

Outline

Grapevine graft interface 1 month after grafting

1. Physical connections between the scion & rootstock
    - Cellular connections
    - Xylem connections
2. Transcripts & metabolites involved
3. Ongoing & future work

Plasmodesmata – tiny channels connecting almost every cell

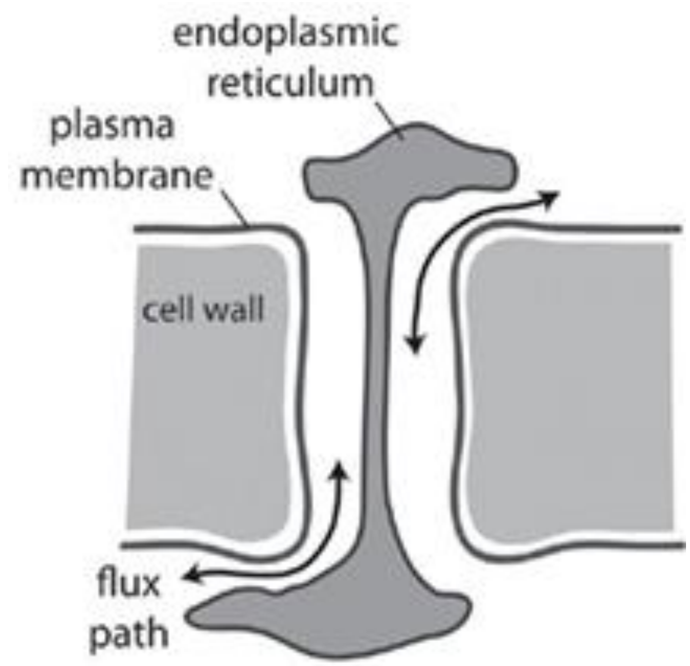


Plasmodesmata allowing communication (arrows) between different cells; n, nucleus.

- Key elements for cell to cell communication
- Transport proteins, RNAs…
- Permeability which is regulated

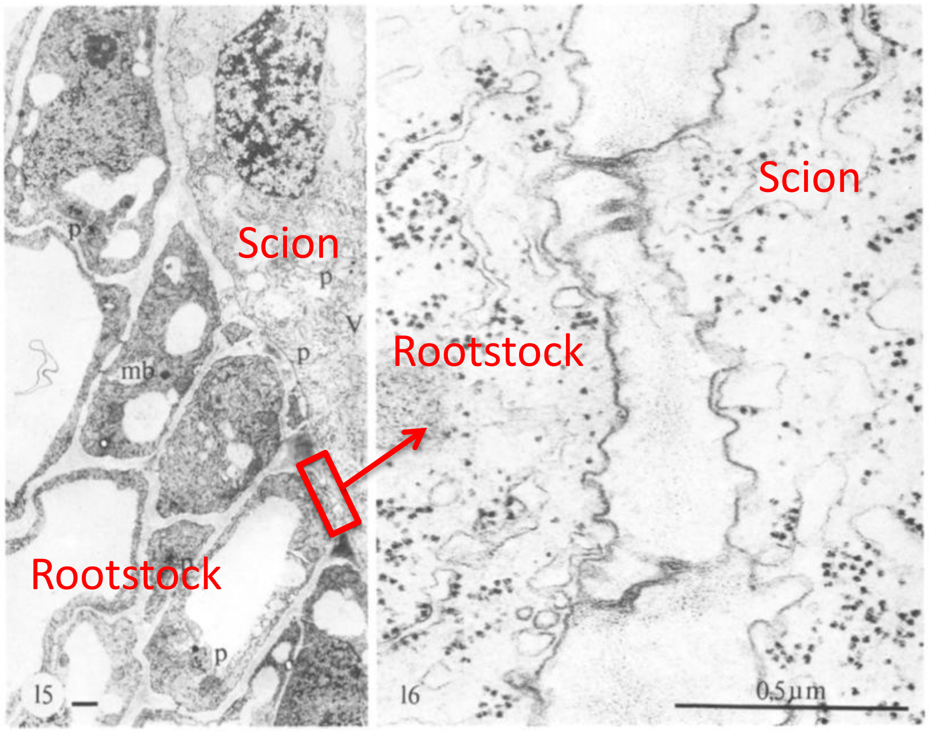
Plasmodesmata – tiny channels connecting almost every cell

- Flattened endoplasmic reticulum (ER) runs through them
- How plasmodesmata form across existing cell wall is a mystery
- Where does the ER in the middle come from?



Cross sectional image of a plasmodesmata[1]

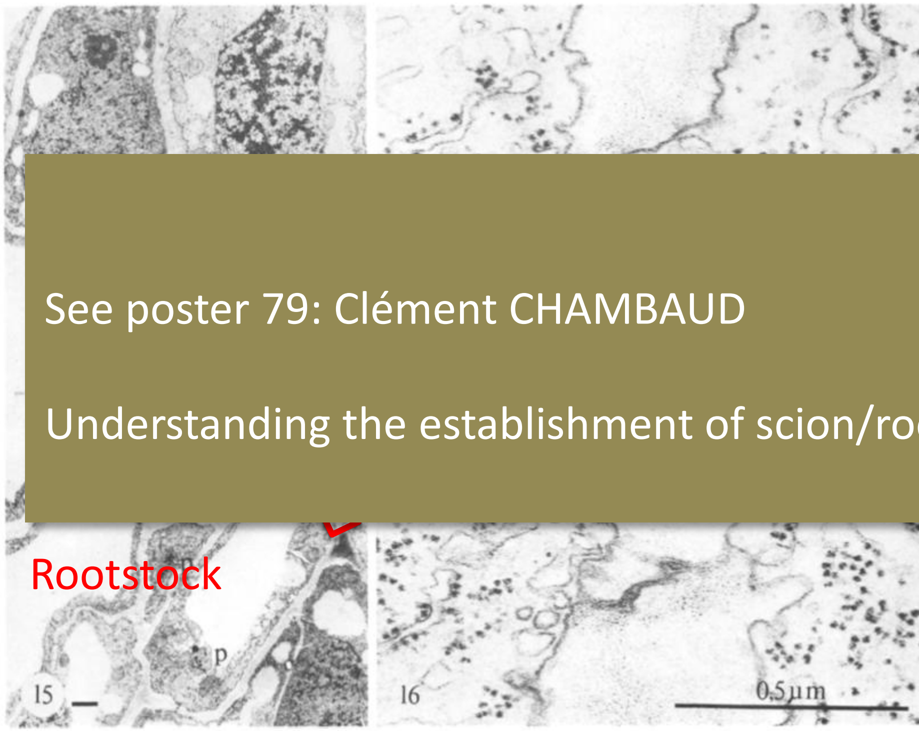
[1]Maule et al., 2012 Frontiers in Plant Science



Electron micrographs of interface of *Vicia faba*/*Helianthus annuus* grafts[1]

- Plasmodesmata have been shown to form at the graft interface
- Are they functional?
- Are they important for grafting success or graft incompatibility?

[1]Kollmann & Glockmann, 1984, Protoplasma



Electron micrographs of interface of *Vicia faba*/*Helianthus annuus* grafts[1]

See poster 79: Clément CHAMBAUD

Understanding the establishment of scion/rootstock interactions in grapevine

- Are they important for grafting success or graft incompatibility?

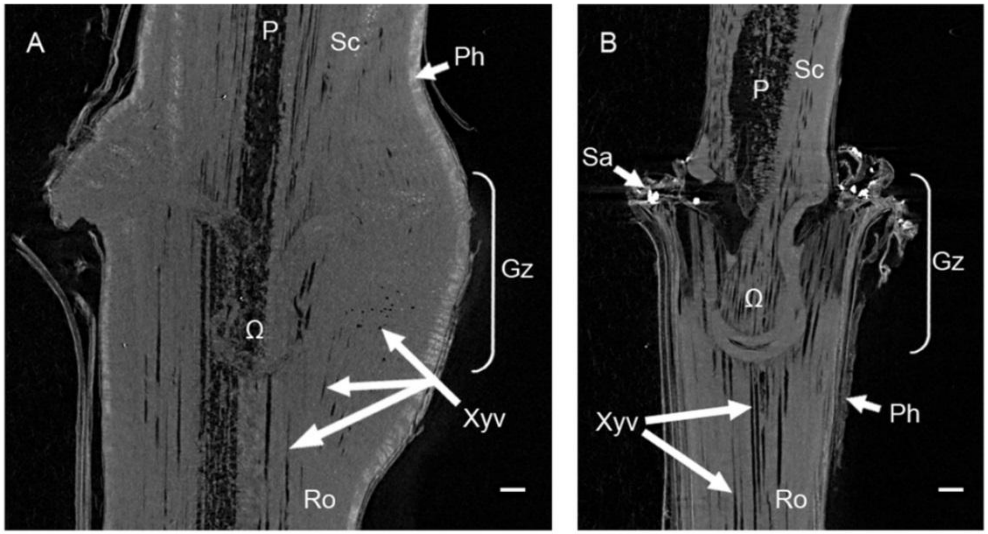
[1]Kollmann & Glockmann, 1984, Protoplasma

Xylem formation

- Have studied xylem formation at the graft interface
  1. Imaging xylem vessels
  2. Measuring hydraulic conductivity

Transport of blue stain from the rootstock to the scion 3 months after grafting

Imaging xylem formation – X-ray tomography

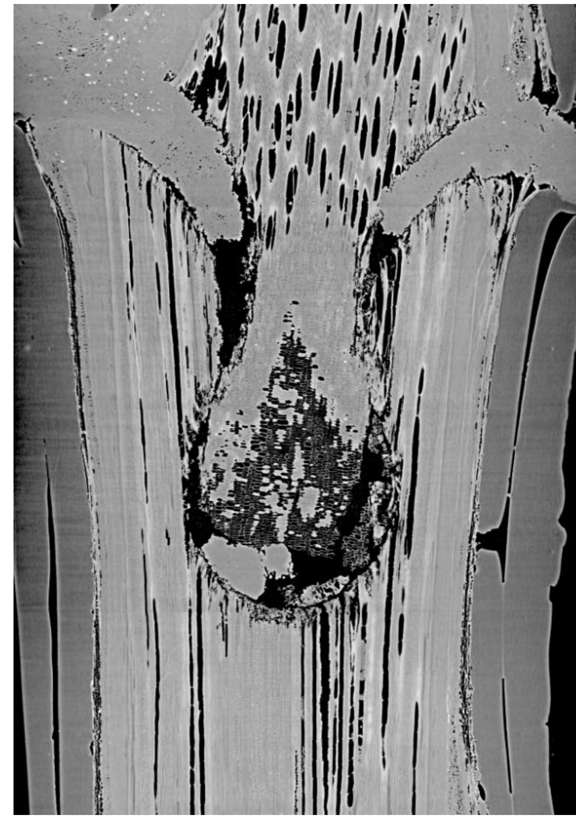


X-ray computed tomography (CT) with a relatively low resolution; scion, Sc; rootstock, Rc[1]

Origine

Anne Sophie SPILMONT, IFV

PLAN NATIONAL DÉPÉRISSEMENT DU VIGNOBLE



High resolution CT, but deadly & image size difficult to handle – 3D reconstruction to come…

[1]Milien et al., 2012. Sci. Hort.

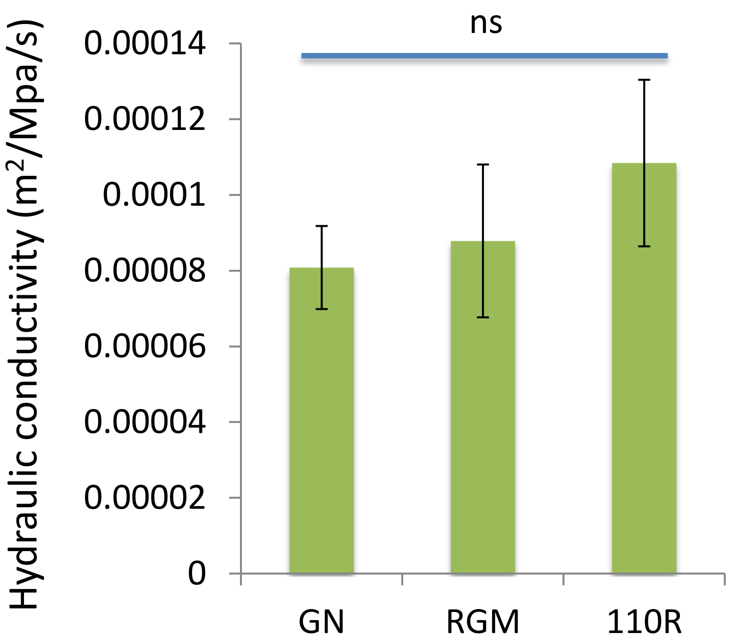
Hydraulic conductivity of the graft interface

- Hydraulic conductivity gives an indication of flux in the xylem for a given driving force
- Across the graft interface gives an indication of formation of xylem vessels
- We have used two techniques:

  1. High Pressure Flow Meter – not suitable for young grafts
  2. Low pressure flow meter (gravity) – can be used from 8 weeks after grafting

Hydraulic conductivity of different genotypes

Where measurements were made

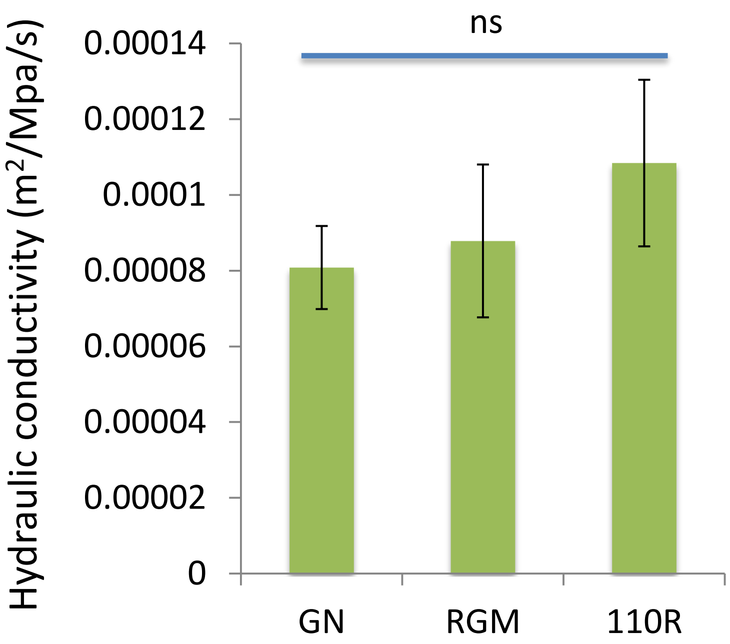


Hydraulic conductivity of an internode cutting during early stages of growth

GN, *Vitis vinifera* cv. Grenache; RGM, *V. riparia* cv Gloire de Montpellier; 110 R, *V. berlandieri* x *V. rupestris* cv. 110 Richter.

Hydraulic conductivity of different genotypes

Where measurements were made

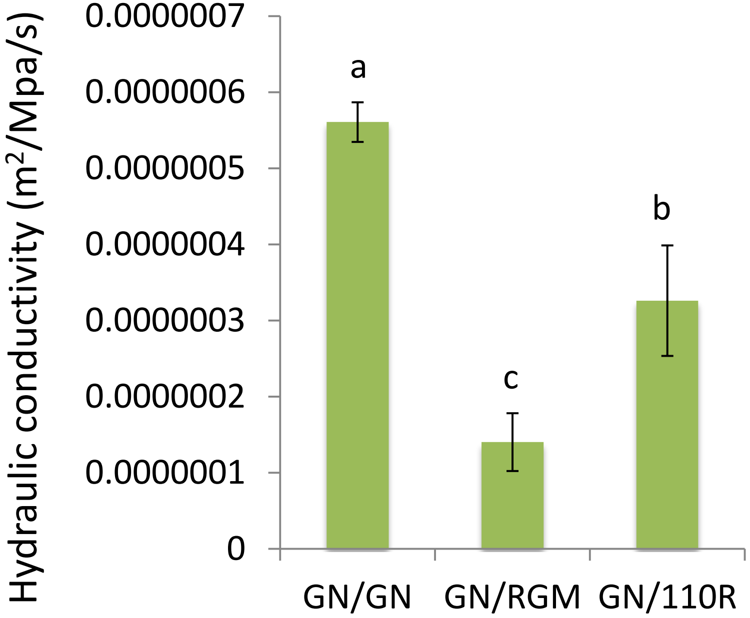


Hydraulic conductivity of an internode cutting during early stages of growth

No difference in hydraulic conductivity in cuttings of different genotypes studied

Hydraulic conductivity of the graft interface

Where measurements were made

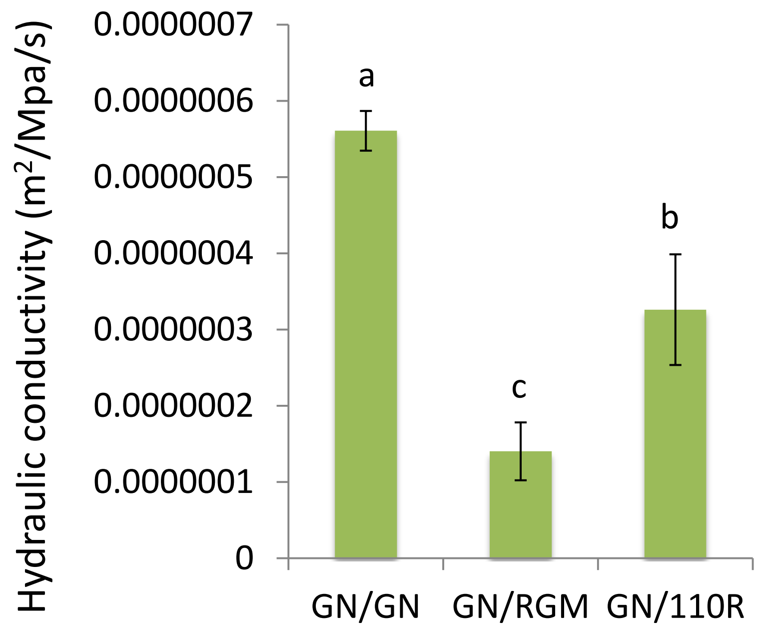


Hydraulic conductivity of the graft interface 8 weeks after grafting

GN, *Vitis vinifera* cv. Grenache; RGM, *V. riparia* cv Gloire de Montpellier; 110 R, *V. berlandieri* x *V. rupestris* cv. 110 Richter.

Hydraulic conductivity of the graft interface

Where measurements were made



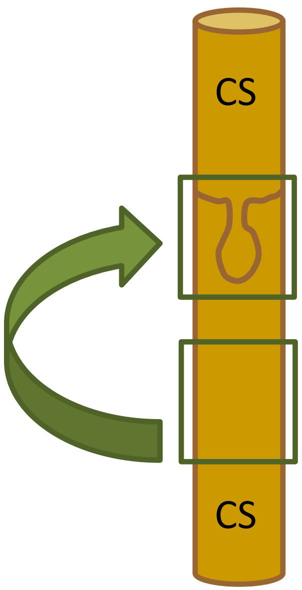
Hydraulic conductivity of the graft interface 8 weeks after grafting

Hydraulic conductivity reduced >100 times 8 weeks after grafting
Hydraulic conductivity is different between the scion/rootstock combinations

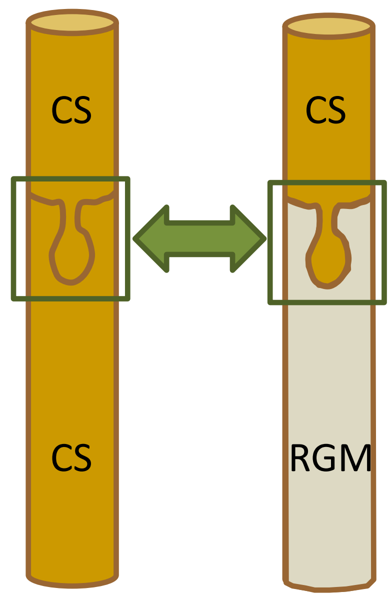
Future questions

- Can the measurements of hydraulic conductivity be linked to xylem formed at the graft interface?
- Are there differences in xylem connections between different scion/rootstock combinations?
- Is xylem connection involved in graft incompatibility &/or dieback?

Transcripts involved in graft union formation & heterografting?



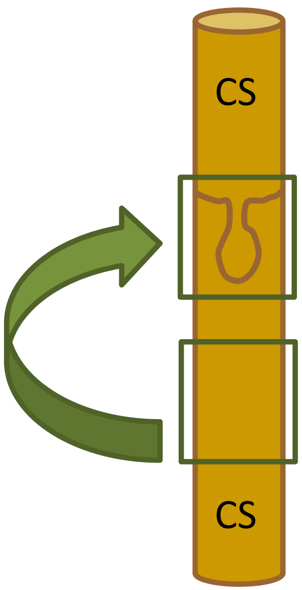
Homograft:
V. vinifera cv. Cabneret Sauvignon (CS)/CS



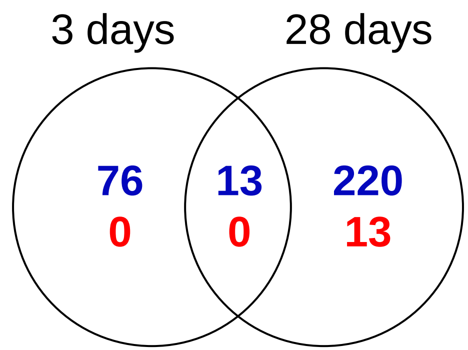
Homo- & heterografts:
CS/CS vs. CS/RGM

Cookson et al., 2013; 2014. J. Exp. Bot

Transcriptome of the rootstock & graft interface



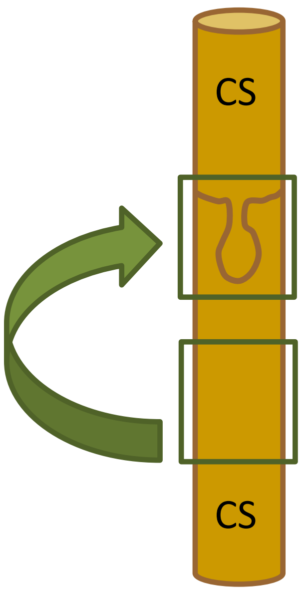
Number of transcripts differentially expressed between the **rootstock & graft interface**



Number of **UP** and **DOWN** regulated genes

Cookson et al., 2013. J. Exp. Bot

Transcriptome of the rootstock & graft interface



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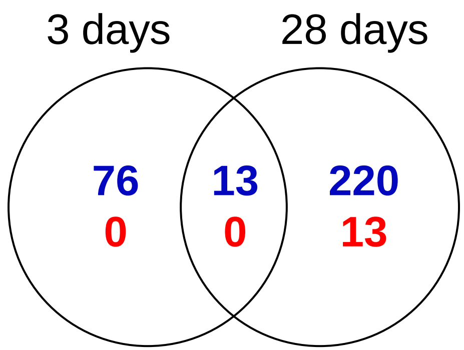


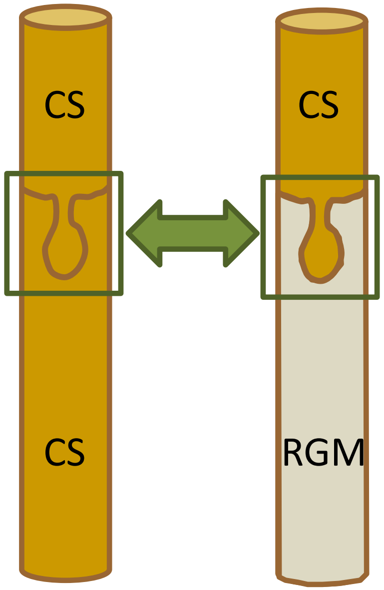
Number of **UP** and **DOWN** regulated genes

Graft interface is associated with the **UP-REGULATION** of gene expression

Genes up-regulated at the graft interface associated with cell wall formation, secondary metabolism (stilbenes), auxin, the regulation of transcription (e.g. *MYB102*), oxidative stress, jasmonic acid…

Cookson et al., 2013. J. Exp. Bot

Transcriptome of homo- & heterografts



Homo- & hetero-graft: CS/CS vs. CS/RGM

Time course: 3, 7, 14 & 28 days after grafting

>4000 genes were differentially expressed between the scion/rootstock combinations

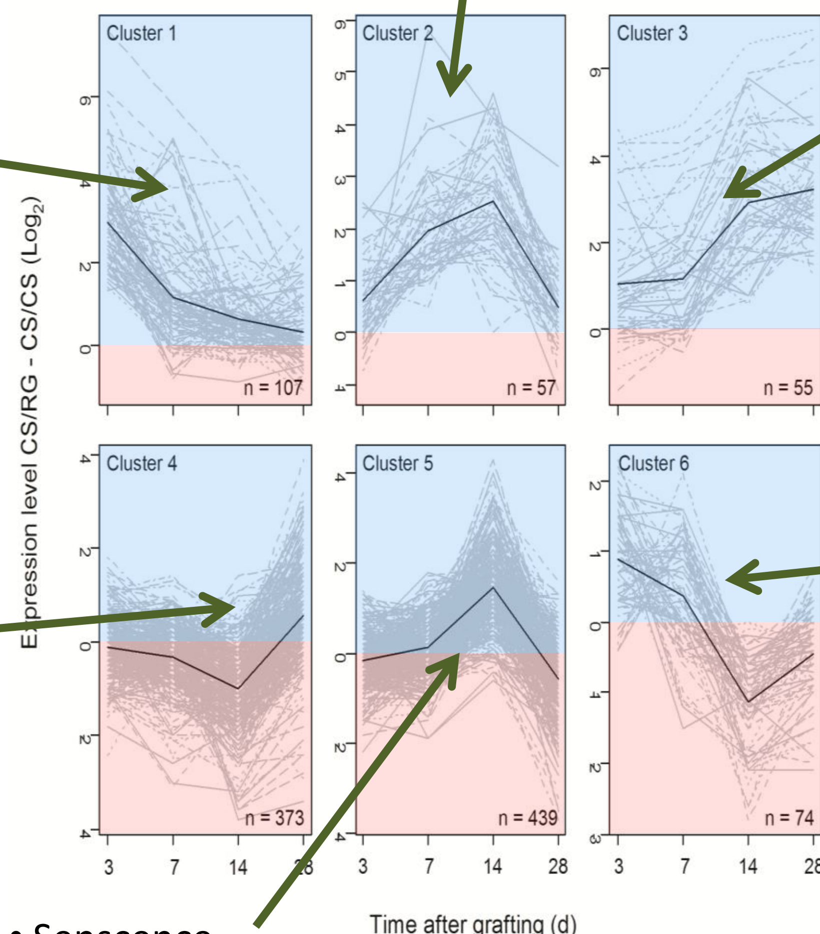
~1100 genes showed a rootstock genotype x time after grafting interaction

Cookson et al., 2014. J. Exp. Bot

- Polyamine oxidase

- Jasmonic acid
- Pathogenesis-related (PR) proteins
- Oxidative stress

- Secondary metabolism
- Cell organisation

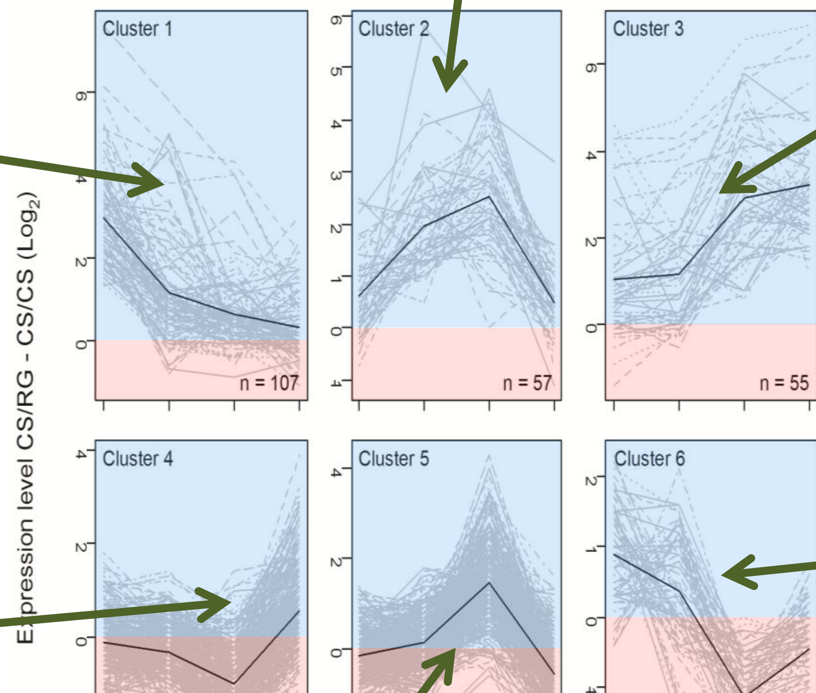


- Secondary metabolism
- Cell wall
- Receptor kinases

- Stress
- Jasmonic acid
- Transcription factors

- Senscence associated genes

Cookson et al., 2014. J. Exp. Bot



• Polyamine oxidase

• Jasmonic acid
• Pathogenesis-related (PR) proteins
• Oxidative stress

• Secondary metabolism
• Cell organisation

• Secondary metabolism
• Cell wall
• Receptor kinases

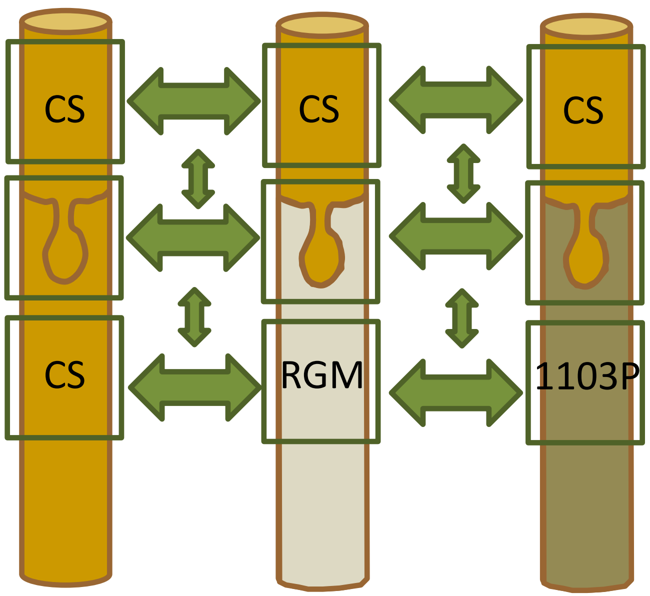
• Stress
• Jasmonic acid
• Transcription factors

Hetero-grafting induces the expression of genes involved in oxidative stress, jasmonic acid, secondary metabolism … → **STRESS** & **DEFENSE** responsive transcripts

28 days after grafting many transcripts associated with secondary metabolism accumulated – what about metabolites?

Cookson et al., 2014. J. Exp. Bot

Metabolomics of interface & wood of homo- & heterografts

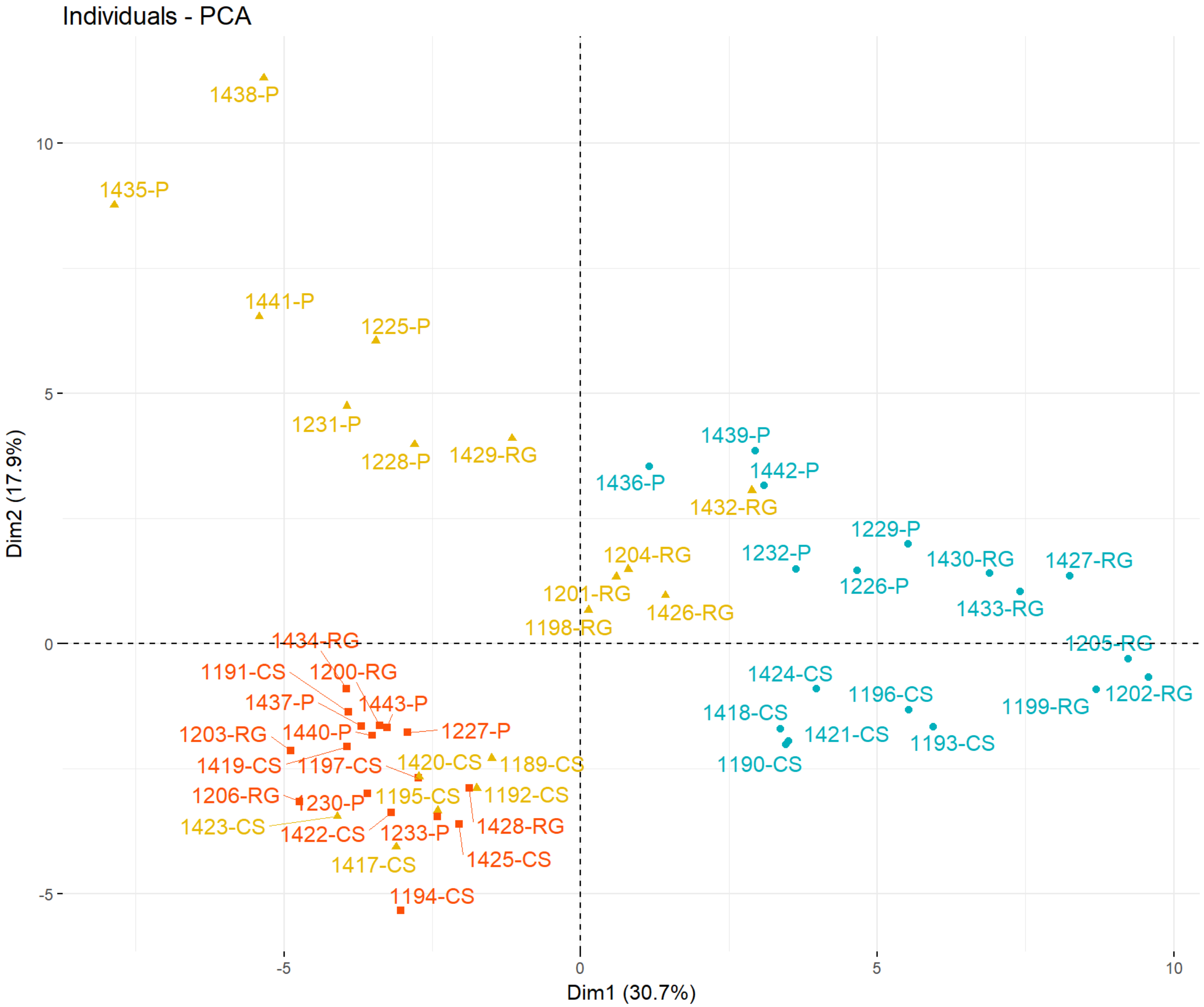


Homo- & hetero-grafts:
CS/CS vs. CS/RGM & CS/1103P

Measured:

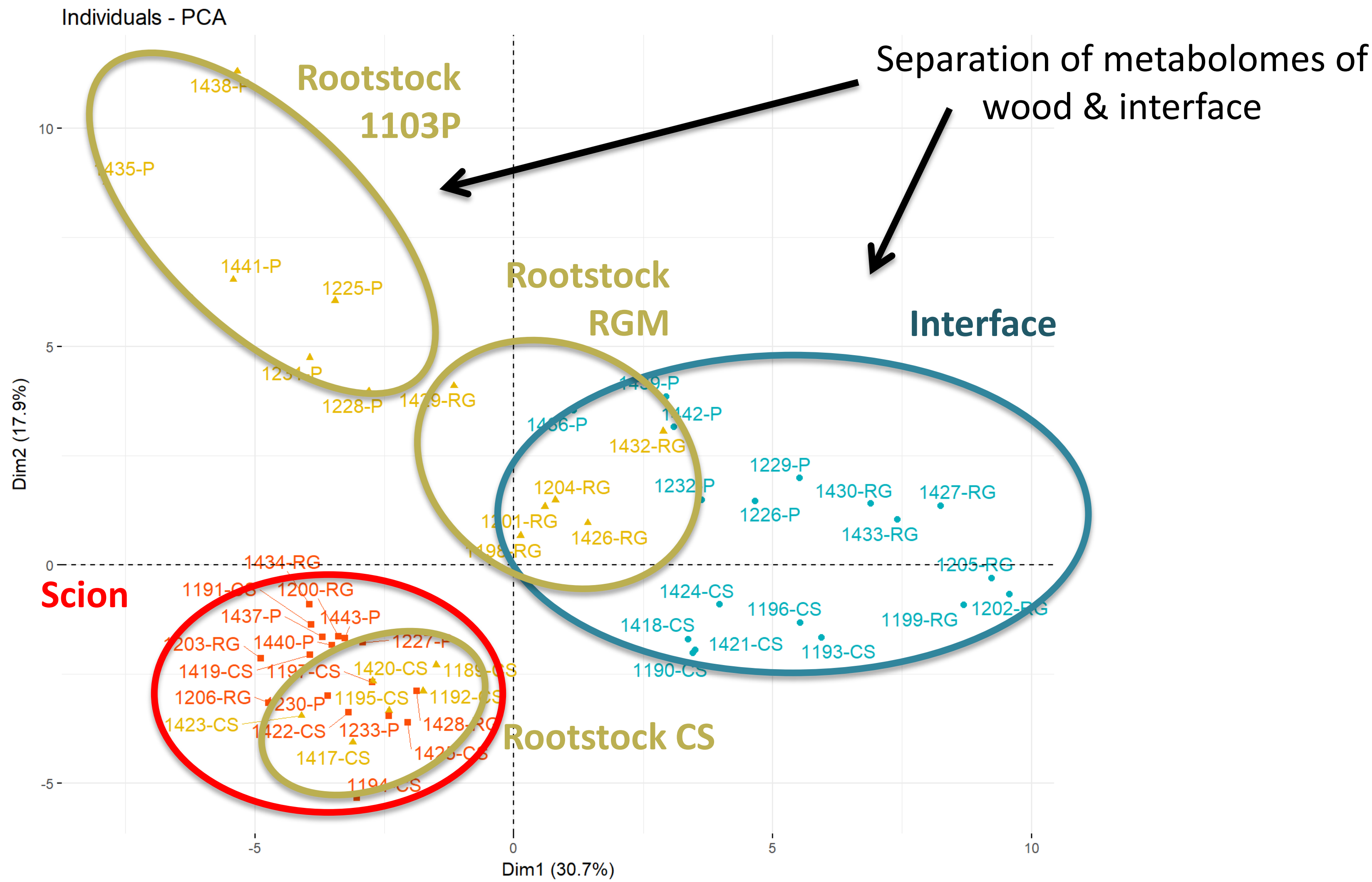
- Activity of phenylalanine ammonia lyase (PAL)
- Stilbenes
- Flavanols
- Amino acids
- Sugars
- Starch
- Protein

PCA of metabolite profiles of CS/CS, CS/RGM & CS/1103P

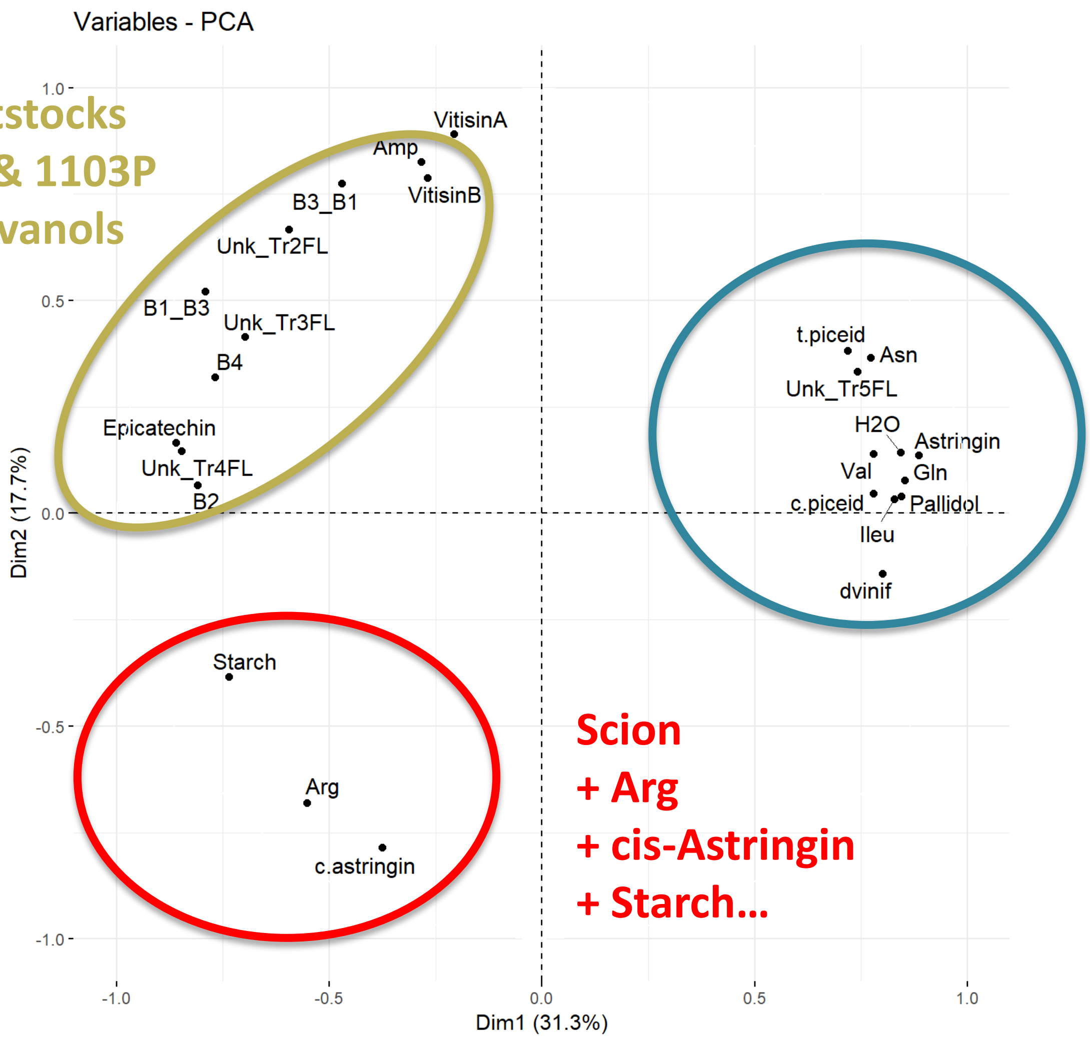


Interface
Rootstock
Scion

PCA of metabolite profiles of CS/CS, CS/RGM & CS/1103P



PCA of metabolite profiles of CS/CS, CS/RGM & CS/1103P



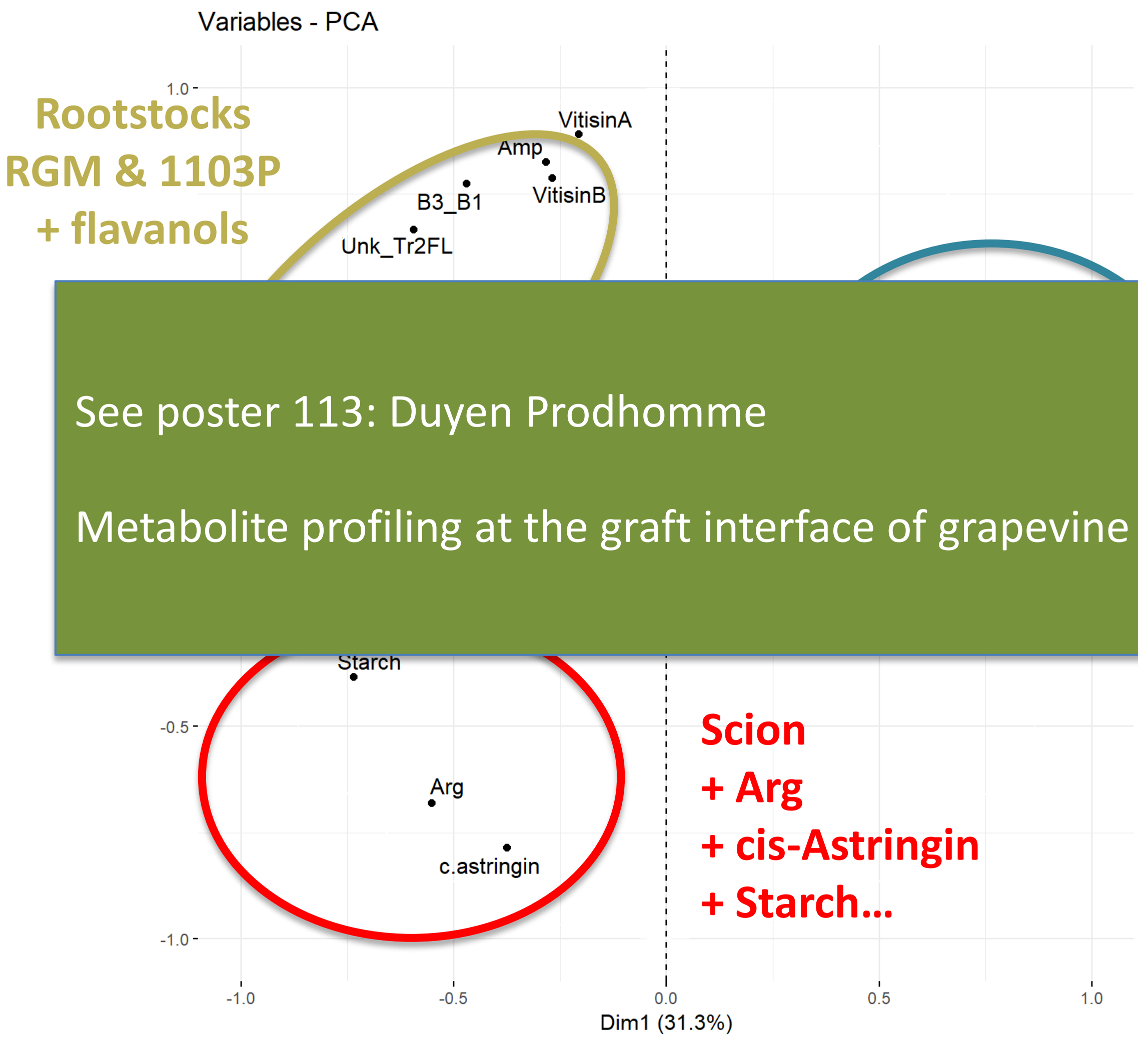
Rootstocks
RGM & 1103P
+ flavanols

Interface
+ Gln, Ileu, Val…
+ PAL activity
+ Piceid, Astringin…

Scion
+ Arg
+ cis-Astringin
+ Starch…

Introduction | Cellular connections | Xylem connections | Transcripts & metabolites | Ongoing & Future work

PCA of metabolite profiles of CS/CS, CS/RGM & CS/1103P



See poster 113: Duyen Prodhomme

Metabolite profiling at the graft interface of grapevine

Cellular connections

- Are plasmodesmata functional across the graft interface?
- Is plasmodesmata formation or function linked to grapevine incompatibility &/or dieback?

Xylem connections

- Are these differences in the formation of xylem in different scion/rootstock combinations?
- Are xylem connections involved in graft incompatibility &/or dieback?

Transcripts & metabolites

- Limitations to previous studies in perennial crops
  - Lack of appropriate controls (homo-grafts, & un-grafted scions & rootstocks)
  - Insufficient sampling density in time course
  - Graft interface sample a mixture of wood, callus & cells from both partners
  - Microarrays rather than RNAseq
  - No previous studies have measured stilbenes
- Future transcriptomics project – understand how each grafting partner functions using RNAseq in perfectly controlled experiment ($$$$$)
- Future metabolomics project - study a wider range of scion/rootstock combinations of different levels of compatibility with the objective of identifying metabolite markers of incompatibility

Goals

- Understand the mechanisms of graft union formation in woody plants
- Identify the origins of incompatibility responses in grapevine
- Use this knowledge to improve grafting success – applying chemicals? Antioxidants?
- One day study the genetic architecture of graft compatibility

Acknowledgements

Our colleagues at the EGFV & BIC

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Funding

Anne-Sophie SPILMONT

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Origine

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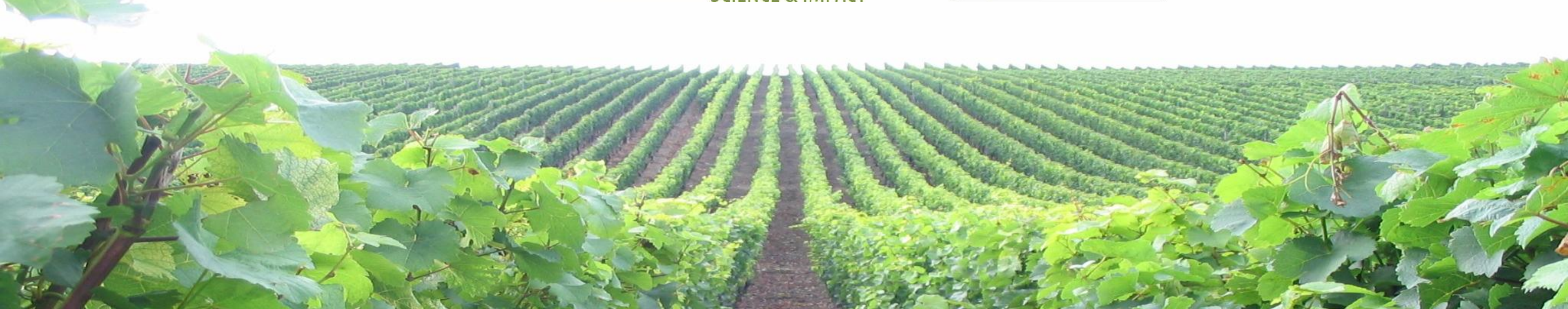

Thank you for your attention