SafeGrape - Genomic of the grapevine - pathogen interactions: Botrytis cinerea virulence factors & molecular mechanisms of induced resistance

Benoît Poinssot, Jani-Petri Kelloniemi, Zsuzsanna Antal, Christophe Bruel, Agnes Cimerman, Xavier Daire, Berengere Dalmais, Marc Fermaud, Patrick Frettinger, Marie-Josèphe Gagey, et al.

CNIV comité national des interprofessions des vins d'appellation d'origine

AGENCE NATIONALE DE LA RECHERCHE ANR

"SafeGrape"

Genomics of the grapevine - pathogen interactions: *Botrytis cinerea* virulence factors & Molecular mechanisms of induced resistance

Seminar Plant Genomics 2012 – 3rd-4th-5th April 2012 – Pont Royal en Provence

afaq ISO 9001 Qualité AFNOR CERTIFICATION

SafeGrape - PARTNERS

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On the Vitis side:

UMR pme

UMR 1088, Dijon
Benoît Poinssot & Co

UNIVERSITÉ DE REIMS CHAMPAGNE-ARDENNE

UR VVC-SE, Reims
Fabienne Baillieul & Co

On the Botrytis side:

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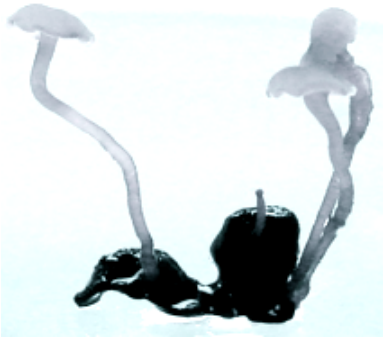


UR INRA 1290, Grignon
Muriel Viaud & Co

Microbiologie MAP Adaptation et Pathogénie

UMR 5240, Lyon
Mathias Choquer & Co

Fungal diseases in viticulture

- **Grapevine is a major crop for French agriculture**: 10.1 billion € (2011), 3% of the cultivated surface
- ***Vitis vinifera* susceptible to many diseases including:**

Grey mould
Botrytis cinerea
- Necrotroph
- Ascomycete

Downy mildew
Plasmopara viticola
- *Biotroph*
- *Oomycete*

- **Half of the fungicides used in France sprayed in vineyards** but they should be reduced by 50% by 2018 (Grenelle de l'environnement).

Extending our knowledge on the infection process is necessary to **reduce applications of chemicals**, but also to **develop alternative strategies:**

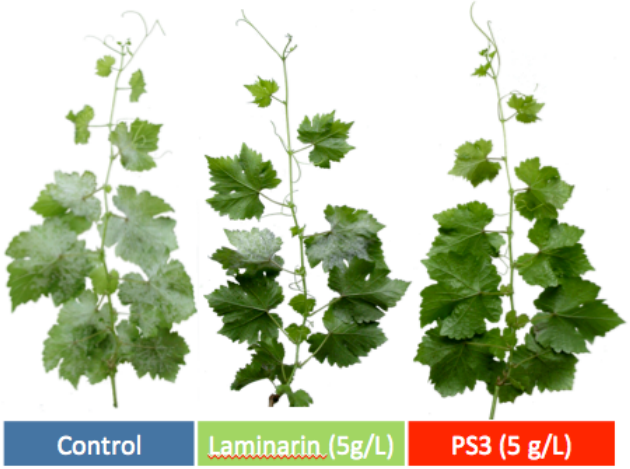
- Obtaining resistant cultivars (NB: no resistance gene for *B. cinerea*)
- **Elicitation of plant immunity by biomolecules …**

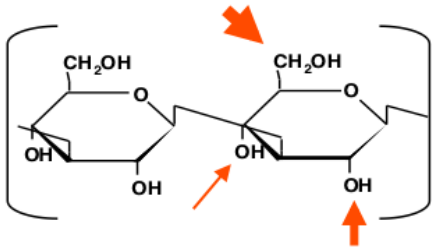
PAMP – triggered immunity (PTI) in grapevine

- **Several PAMP** (Pathogen Associated Molecular Pattern) **have been identified in grapevine:**

- **Ex: Laminarin (Lam):** Linear *β* -1,3-glucan polymer (*Laminaria digitata*) induces a low resistance against *B. cinerea* and *P. viticola by* triggering grapevine defense responses (Calcium, MAPK, ROS, PR2 …)

- Interestingly, chemical modification of this PAMP can improve its efficacy : **Sulfated Laminarin (PS3) induces a stronger resistance against *P. viticola*** but did not trigger grapevine defense responses as laminarin do.





What are the mechanisms involved?

Trouvelot et al., 2008

SafeGrape - OBJECTIVES

Molecular mechanisms of the interactions between grapevine and its major pathogens

The development of alternative ways to protect the grapevine from pathogens requires a better knowledge of the infection process and the mechanisms of defences. In this context, the aims of our project were:

- **To identify the pathways involved in induced resistance …**
  - against different pathogens (*B. cinerea versus* P. *viticola)*
  - in different organs (leaves, veraison and mature berries)

- **To elucidate the mode of action of sulfated laminarin (PS3)**

- **To characterise *B. cinerea* genes involved …**
  - in infection structures development
  - in the successful infection of berries

SafeGrape - APPROACHES

Transcriptomic view of the interaction

Different scenari of infections

t0 t1 t2 t3

Plant/fungal RNA

Hybridization on *Botrytis* chips
(~16 000 ORFs - 3 oligos/ORF)

PartnerChip

NimbleGen HIGH - DEFINITION GENOMICS

Hybridization on *Vitis* chips
(~30 000 ORFs - 4 oligos/ORF)

UNIVERSITÀ DEGLI STUDI DI VERONA

NimbleGen HIGH - DEFINITION GENOMICS

ANAIS*: Analysis of Nimblegen Arrays Interface

Gene KO in *B. cinerea*

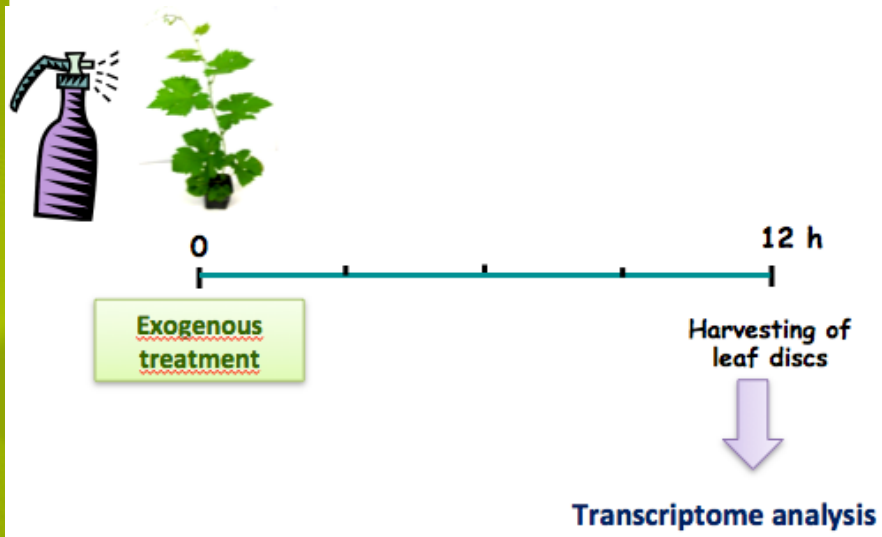
Gene silencing in *V. vinifera*

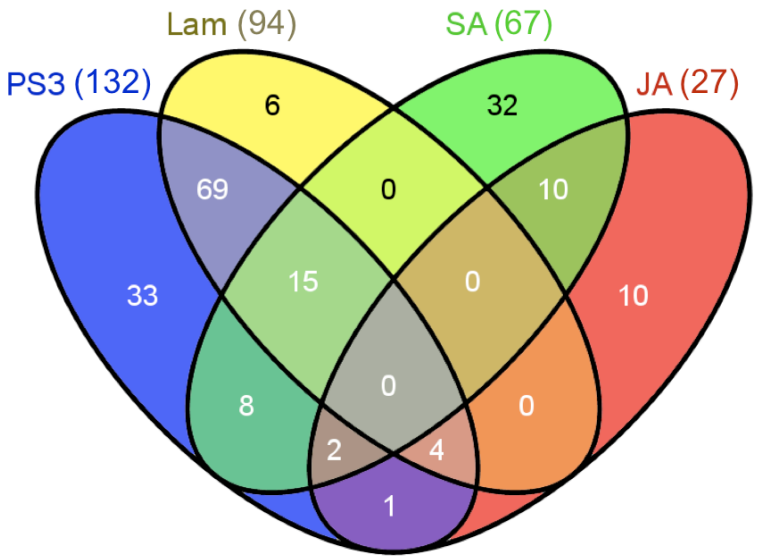
Coll. Plateforme Transformation vigne

INRA Institut National de la Recherche Agronomique COLMAR

* Simon & Biot, 2010

PS3 induced grape genes





Up –regulated transcripts at 12hpt
(FC > 2 with $p < 0.05$)

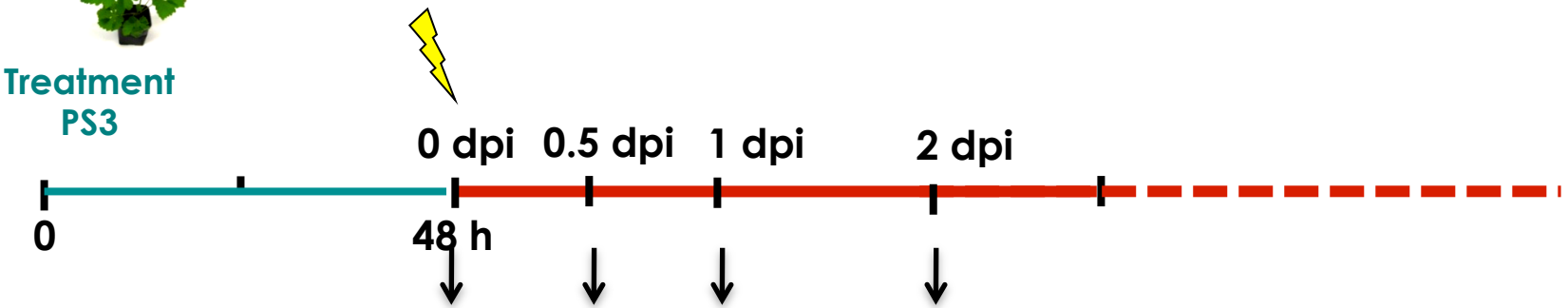
- The two β-glucans (PS3 and Lam) share only a part of their transcriptome
- **PS3 does not up-regulate SA-marker genes** (NRX1, WRKY40, PR-2) **or JA-marker genes** (13-LOXA, FAH Lyase, JAZ1, AOC1)
- **PS3 up-regulates genes involved in response to biotic and abiotic stresses, in glycolysis** …

Does PS3 prime grapevine defense reactions during the infection?

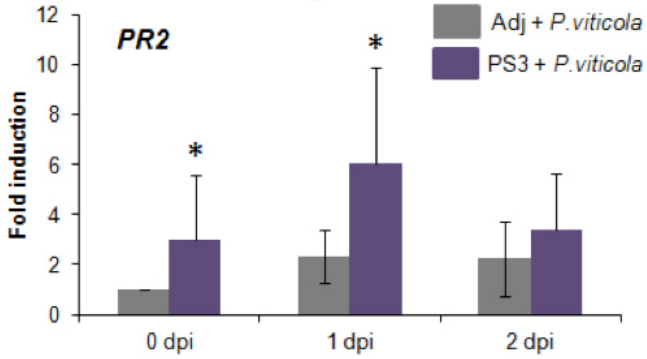
Gauthier et al., soumis

UMR pme

PS3 primes defense reactions



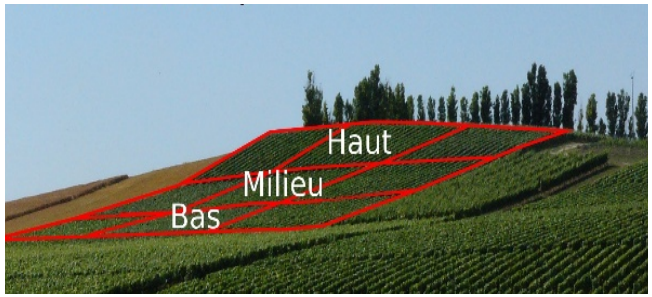
- Arrays data showed **that PS3 primes a faster induction of SA-marker genes (PR1, PR2, PR5, NRX1…) and *PRR* genes (PAMP perception)**
- Faster production of SA confirmed by metabolomics
- Origin of SA currently tested (phenypropanoic *versus* isochorismate pathway)
- NB: **PS3 also induce a faster activation of defence genes against *B. cinerea*.**



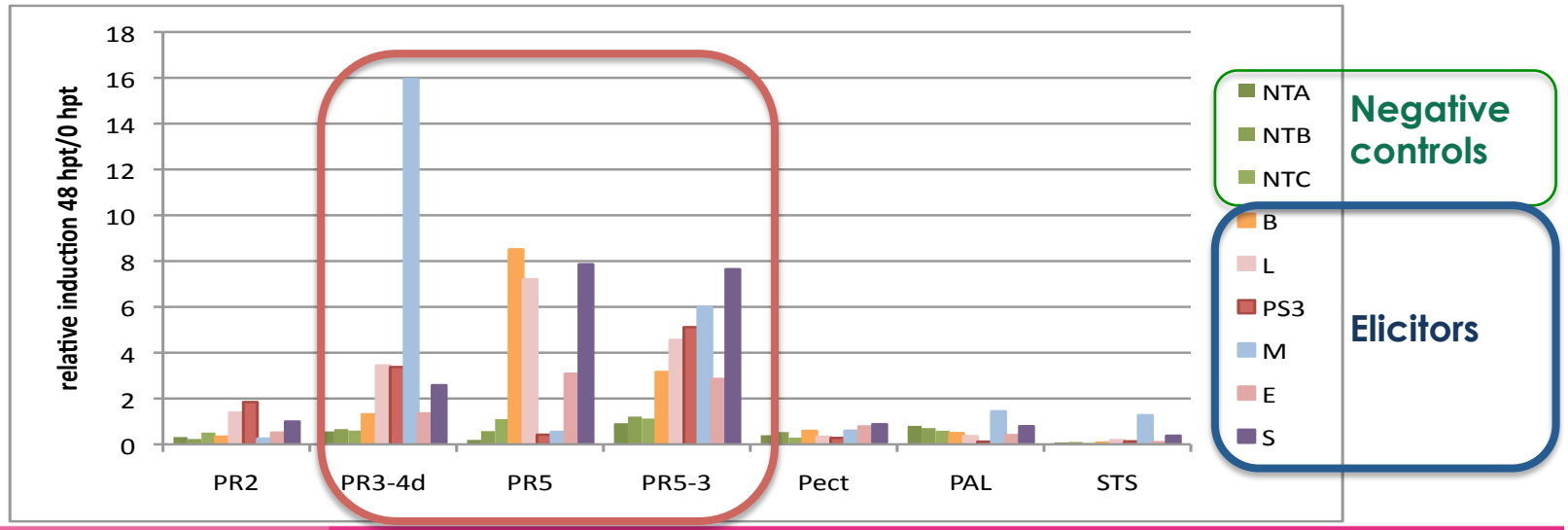
And in vineyards ?

- **7 PS3-induced genes were tested in vineyards (2011):**
- Green berries treated with different elicitors including PS3 (and S: non protective)
- Q-RT-PCR 0 and 48hpt (preliminary results):

3 genes seem to be good indicators of the berry elicitation state (no indicator of the resistance state : induction by S)

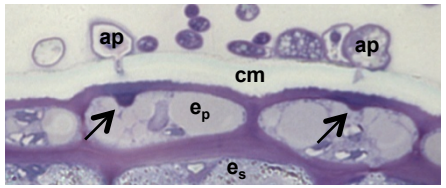


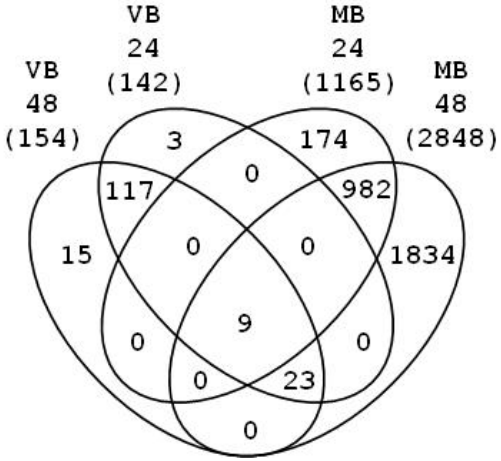
Possible "decision tools" for chemical treatments ?



What happens when *Botrytis* infects berries?

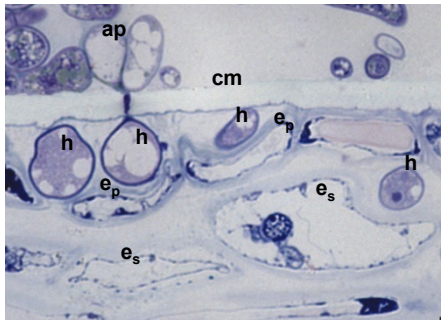
Veraison berries (VB)





Up–regulated genes
at 24 and 48 hpi
(FC > 2 with $p < 0.05$)

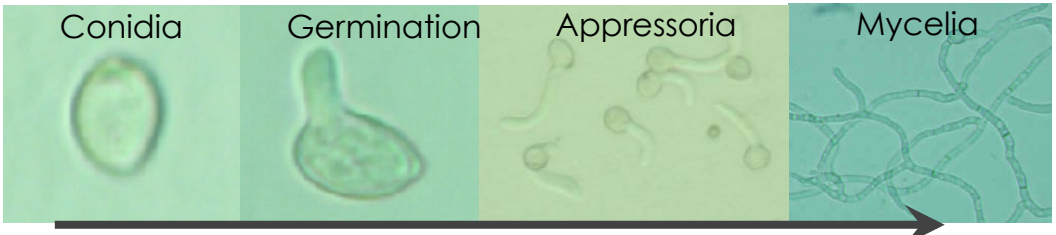
Harvest berries (HB)



- ***B. cinerea* infection is successful on mature berries but not at the veraison stage**
- **Transcriptomics revealed very different reactions from the host.**

What happens on the grey side?

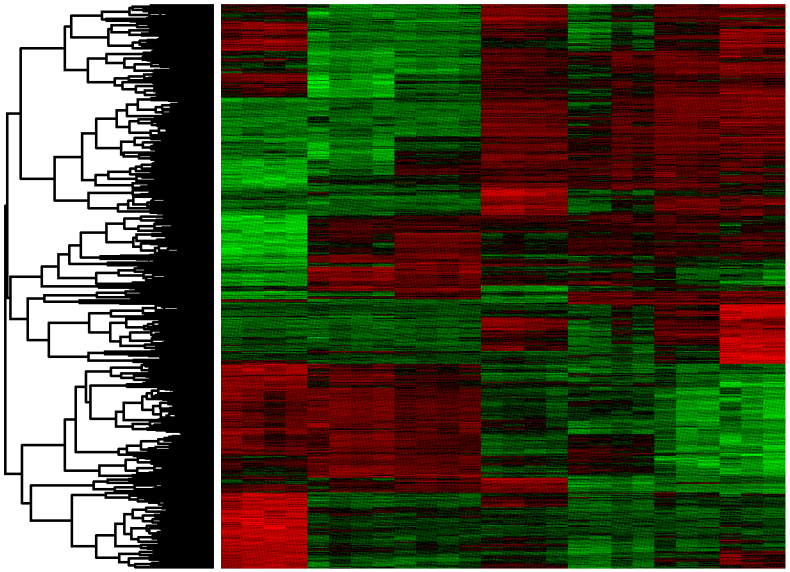
Early developmental stages on Teflon



0h 3h 7h 24h

Infection of mature berries

16h 24h 48h



- 1036 gènes ANOVA pval-fdr < 10-6
- Identification of clusters of **genes specific for the different stages of infection structure development**
- Identification of **genes that are up-regulated *in planta***

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Botrytis genes up-regulated in berries

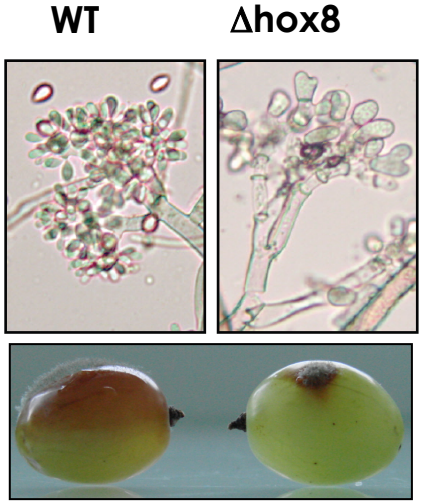
Fungal genes up-regulated during the early stages of berries infection showed **an enrichment in biological processes related to necrotrophy:**

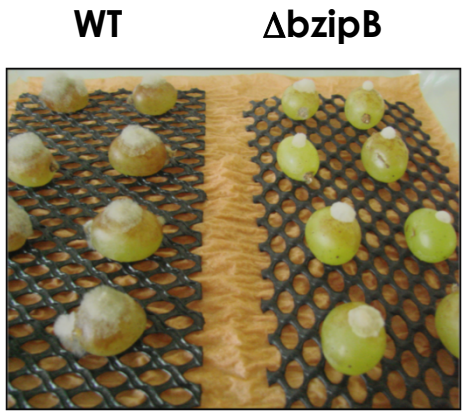
- **Cell wall degrading enzymes**
- **Production of secondary metabolites including phytotoxins** (botrydial and botcinic acid), unknown terpens and polyketides, and **ABA!**
- **Transmembrane transport:** ABC, MFS-type sugar and amino acids transporters
- **Oxydative stress response**

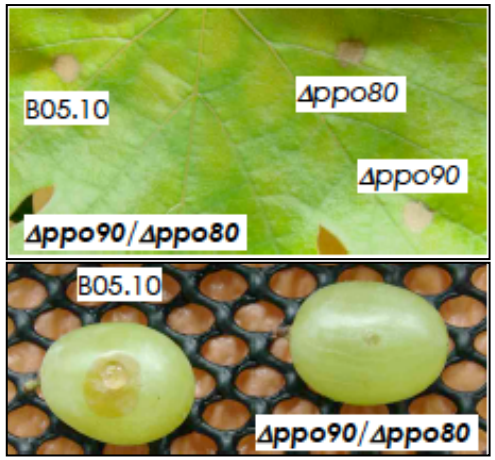
Additionnaly, some genes coding for components of signalisation (e.g. transcription factors) were highly up-regulated during the infection.

About 20 KO mutants constructed so far to validate these functions …

Regulation of fungal development and necrotrophy







- The **homeodomain HOX8 (*Arabesque*) and BzipB transcription factors** are both involved in conidiation and necrotrophic growth.
- **Fungal oxylipins** synthetized by *PPO80 and PPO90* dioxygenases are also necessary (NB: fungal AND plant oxylipins regulate conidiation and mycotoxins production in *Aspergillus* …)
- Further transcriptomics analyses are planned to identify the **downstream target genes** … and investigate the regulation of phytotoxic secondary metabolites.

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SafeGrape - CONCLUSIONS

Main inputs

Large set of grape and *B. cinerea* transcriptomics data : 1st complete view of the interaction

MAMP- and priming-induced resistances:

- **Induced pathway depends on the pathogen but also on the grape organ**
- **PS3 allows a faster activation of these pathways ("priming")**
- **During berries maturation, there is a shift in the response pathway.**
- Silencing of candidate *PRR* genes … (Coll. INRA Colmar)
- Validation of markers of the elicitation state in vineyards … (Coll. Comité Champagne)

Fungal virulence factors: mutagenesis of about 20 genes highlights the important roles of

- **Secondary metabolism: phytotoxins, ABA**
- **Key regulators of fungal development and virulence: transcription factors, kinase, oxylipins**

Possible crosstalks ? Oxylipins? ABA?

SafeGrape - CONTRIBUTORS & SUPPORTS

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CNIV comité national des interprofessions des vins d'appellation d'origine

AGENCE NATIONALE DE LA RECHERCHE ANR

UMR pme

Partner 3
UMR 1088, Dijon

Jani Kelloniemi, post-doc ANR
Lucie Trda
Xavier Daire
Marie Claire Heloir
Sophie Trouvelot
Marielle Adrian
Benoît Poinssot

M. Delledone
A. Ferrarini

PartnerChip

S. Baulande
P. Soularue
L. Couvelard
M. Duthieuw

Partner 1
UR INRA 1290, Grignon

Agnès Cimerman, post-doc ANR
Adeline Simon
Bérengère Dalmais
Jean-Marc Pradier
Sabine Fillinger
Pascal Le Pêcheur
Guillaume Morgant
Muriel Viaud

BIOGER

BOURGOGNES
Bureau Interprofessionnel des Vins de Bourgogne

J-P. Gervais

URGI Génomique-info

J. Amselem

Partner 2
UMR 5240, Lyon

Microbiologie MAP Adaptation et Pathogénie

Patrick Frettinger, post-doc ANR
Zsuzsanna Antal
Christine Rascle
Marie-Josephe Gagey
Christophe Bruel,
Nathalie Poussereau
Mathias Choquer

Partner 4
UR VVC-SE, Reims

Université de Reims Champagne-Ardenne

Christophe Clément
Fabienne Baillieul

Comité Champagne

A. Bonomelli
D. Moncomble

Colmar
Jean Masson

INRA

Bordeaux
Marc Fermaud
Jean Roudet

Evry
A.-F; Adam-Blondon

Thank you!

Meeting in Champagne, July 2009