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

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“SafeGrape”

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



On the Vitis side:



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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

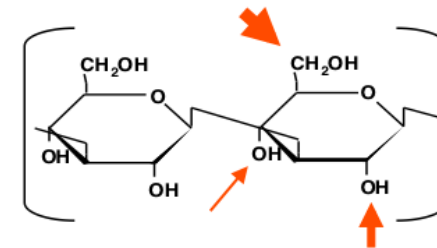
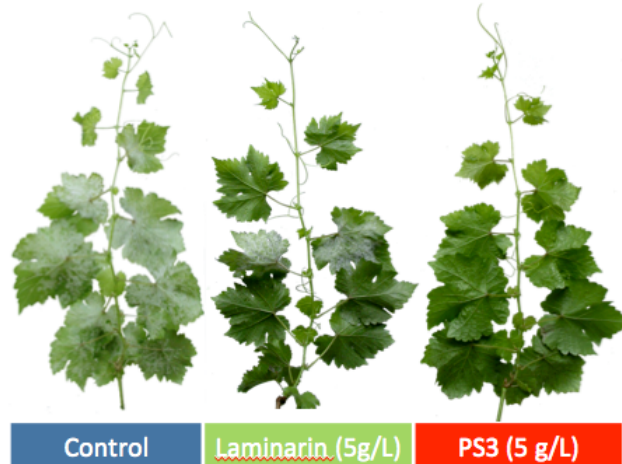
- **Half of the fungicides used in France sprayed in vineyards** but they should be reduced by 50% by 2018 (Grenelle de l'environnement).

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.



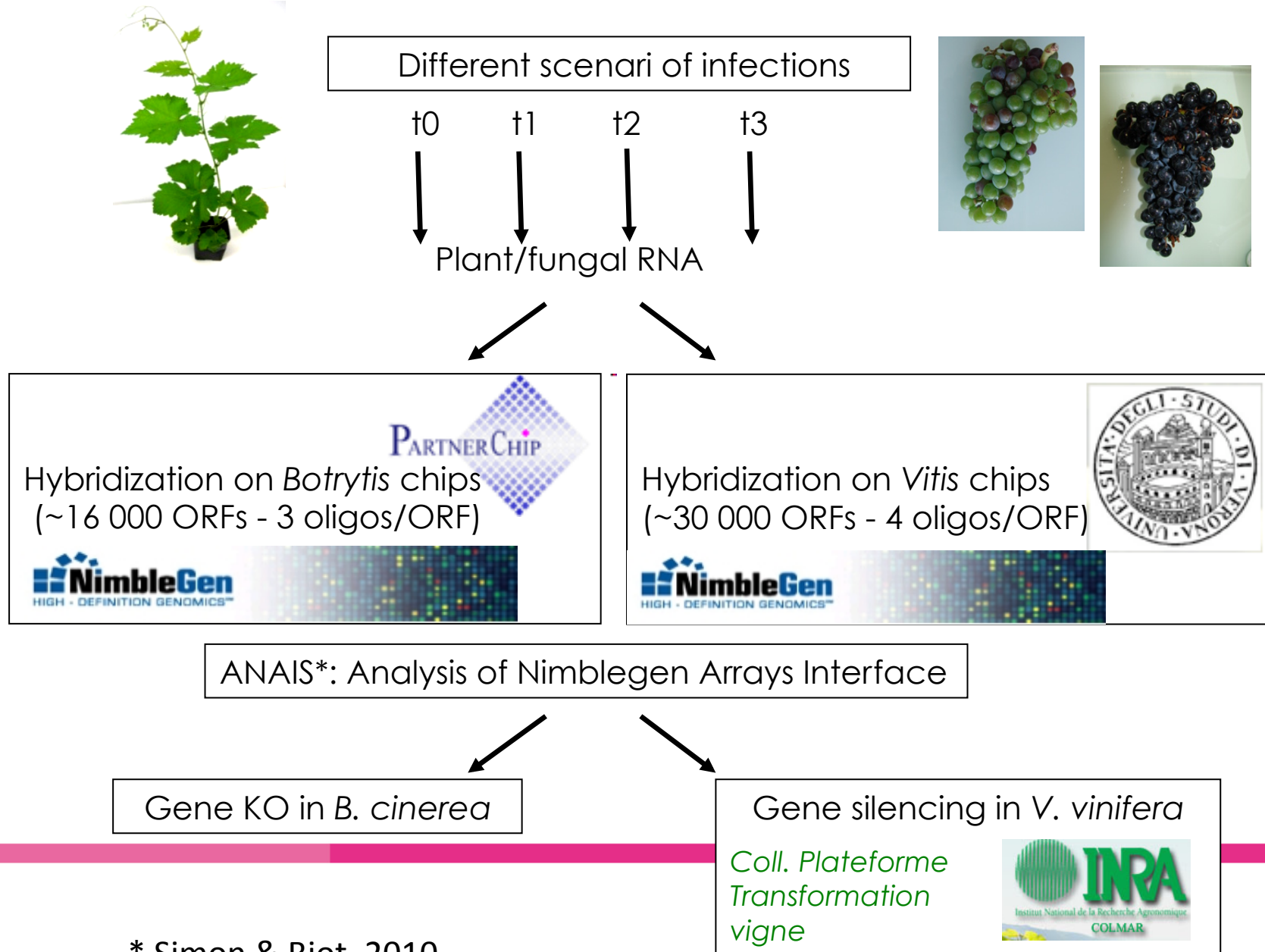
What are the mechanisms involved?

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:

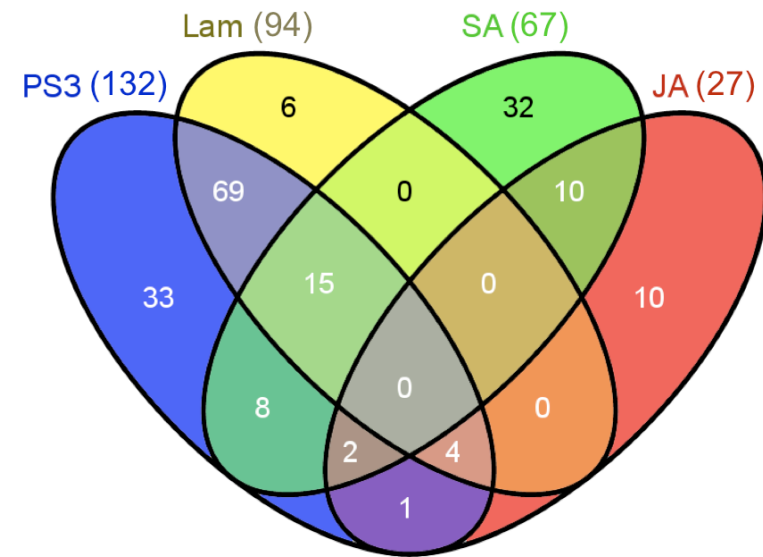
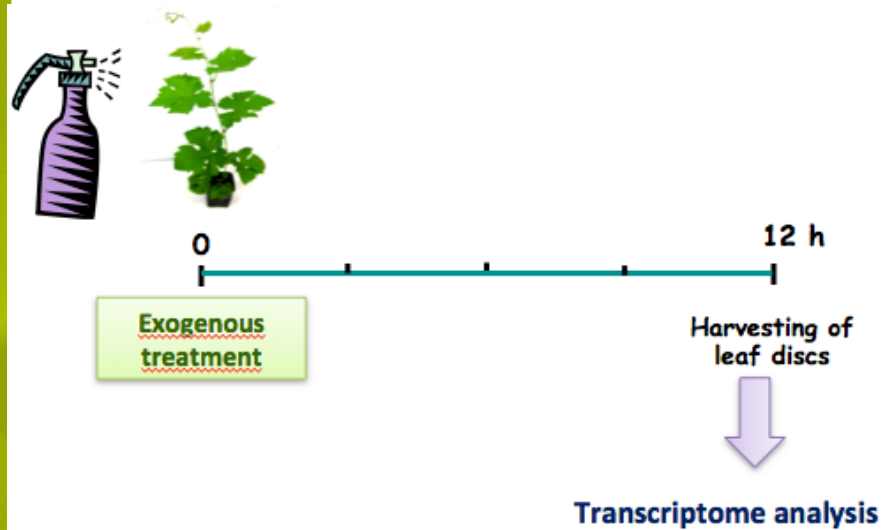
- **To identify the pathways involved in induced resistance ...**
 - against different pathogens (*B. cinerea* versus *P. viticola*)
 - in different organs (leaves, veraison and mature berries)
- **To elucidate the mode of action of sulfated laminarin (PS3)**
- **To characterise *B. cinerea* genes involved ...**
 - in infection structures development
 - in the successful infection of berries

Transcriptomic view of the interaction



* Simon & Biot, 2010

PS3 induced grape genes

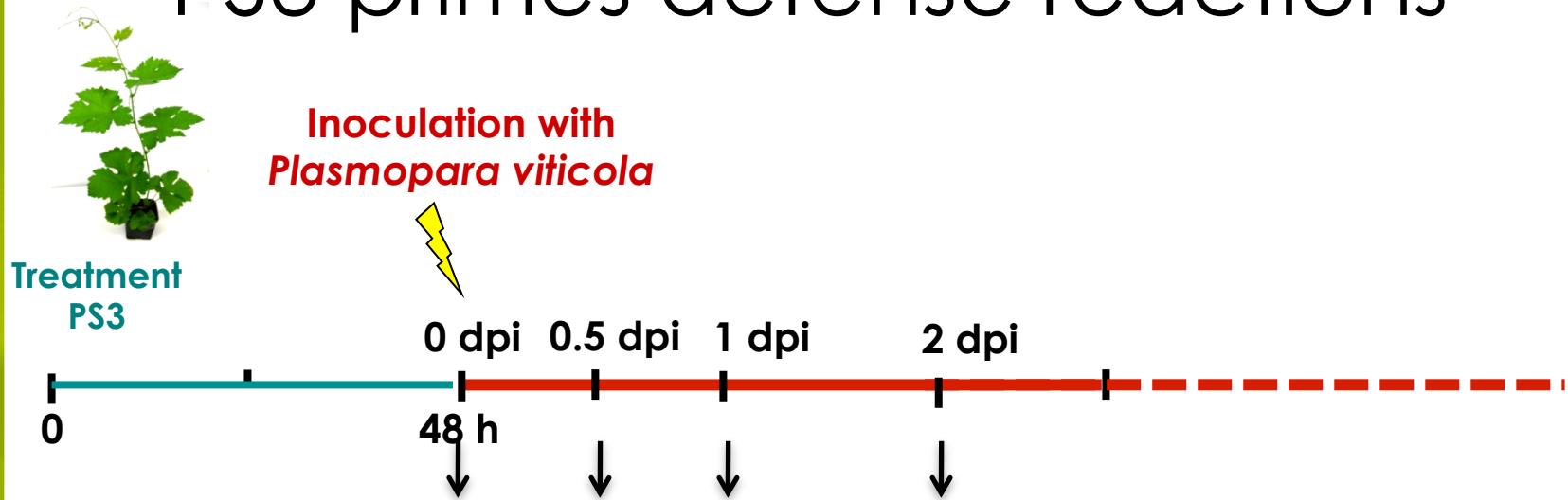


Up-regulated transcripts at 12hpt
(FC > 2 with p < 0.05)

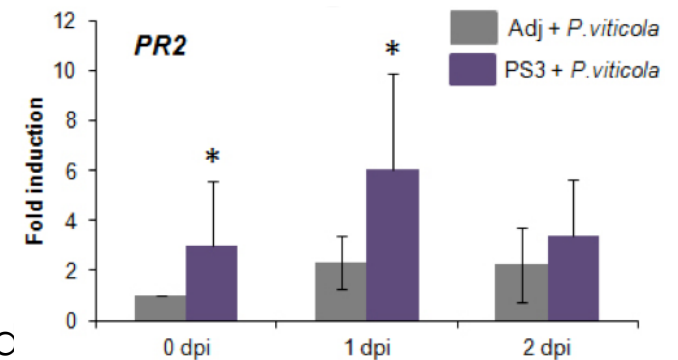
- The two β -glucans (PS3 and Lam) share only a part of their transcriptome
- **PS3 does not up-regulate SA-marker genes** (NRX1, WRKY40, PR-2) **or JA-marker genes** (13-LOXA, FAH Lyase, JAZ1, AOC1)
- **PS3 up-regulates genes involved in response to biotic and abiotic stresses, in glycolysis ...**

Does PS3 prime grapevine defense reactions during the infection?

PS3 primes defense reactions



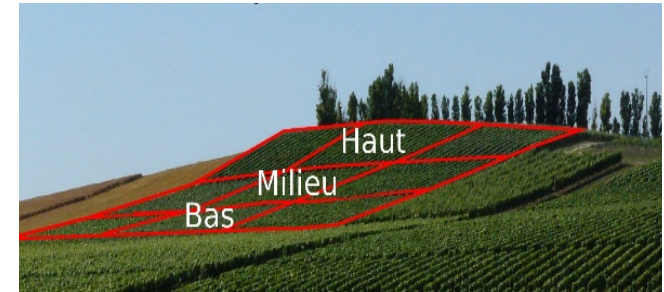
- Arrays data showed that **PS3 primes a faster induction of SA-marker genes (PR1, PR2, PR5, NRX1...)** and **PRR genes (PAMP perception)**
- Faster production of SA confirmed by metabolomics
- Origin of SA currently tested (phenylpropanoic versus isochorismate pathway)
- NB: **PS3 also induce a faster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



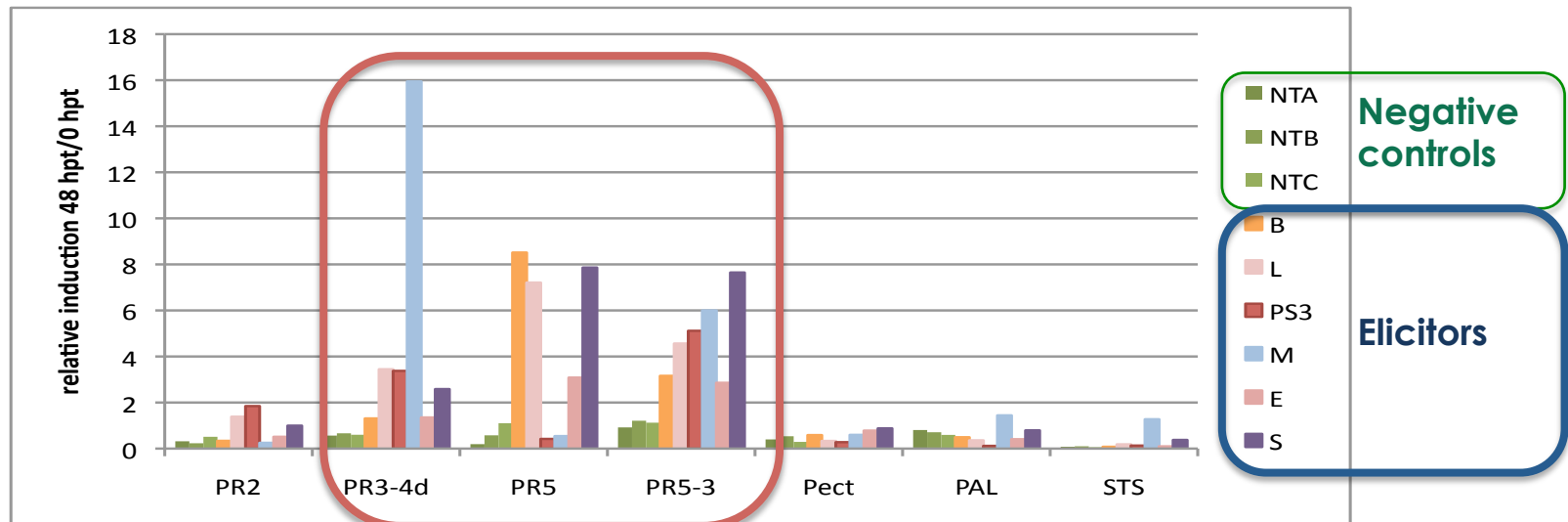
And in vineyards ?



- **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)

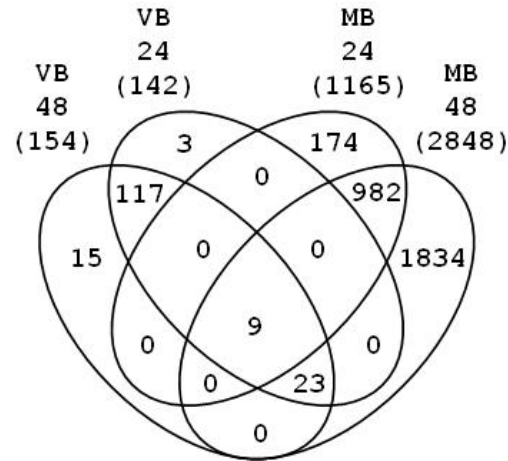
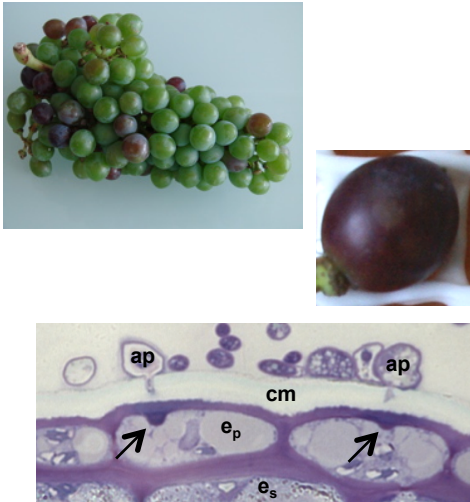


Possible “decision tools” for chemical treatments ?



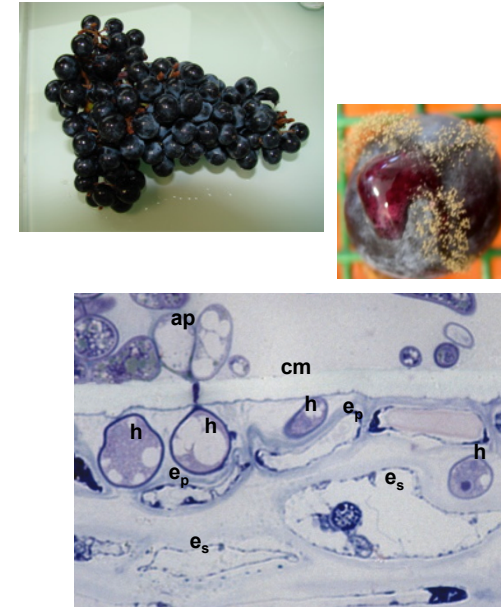
What happens when *Botrytis* infects berries?

Veraison berries (VB)



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

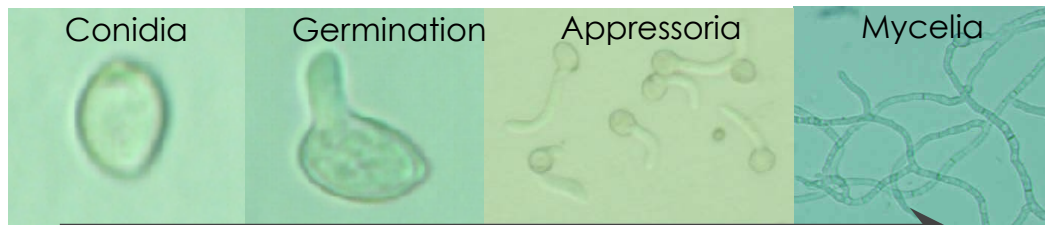
Harvest berries (HB)



- *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

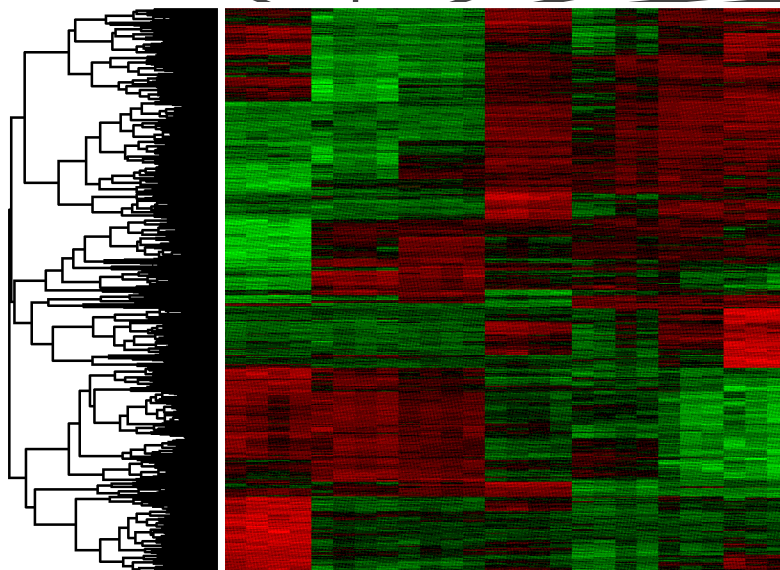
Infection of mature berries



16h

24h

48h



- 1036 gènes ANOVA $p\text{-val-fdr} < 10^{-6}$
- Identification of clusters of **genes specific for the different stages of infection structure development**
- Identification of **genes that are up-regulated *in planta***

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:**

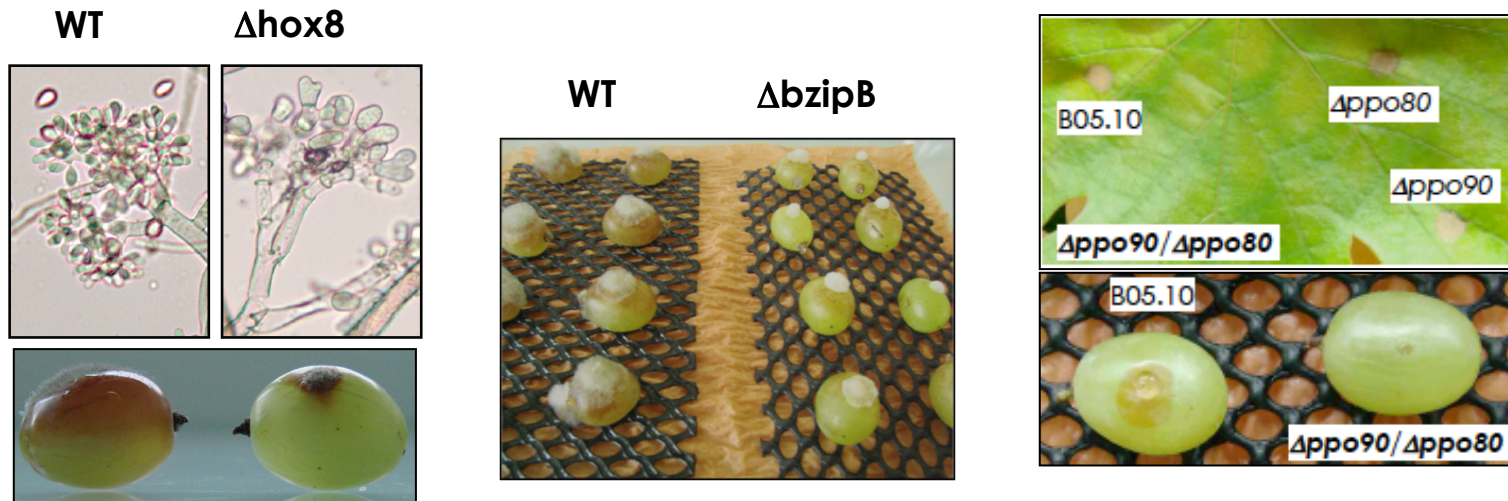
- **Cell wall degrading enzymes**
- **Production of secondary metabolites including phytotoxins** (botrydial and botcinic acid), unknown terpenes and polyketides, and **ABA!**
- **Transmembrane transport:** ABC, MFS-type sugar and amino acids transporters
- **Oxydative stress response**

Additionally, 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** synthesized 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.

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?



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A.-F.; Adam-Blondon



Thank you!



Meeting in Champagne, July 2009