OENOBIO

INTERNATIONAL CONFERENCE

Organic vines and wine advancement and challenges

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Alternatives to copper in organic viticulture: can we take advantage of plant defense mechanisms?

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Copper, an effective fungus treatment

- strong biocidal effect and broad spectrum of action

- a major method of crops protection against diseases,
  particularly to fight against downy mildew

- used from the end of 19th century, as Bordeaux mixture

- today always employed
  in conventionnal agriculture, with synthetic pesticides
  in organic systems

but a biggest issue for wine growers this decade

- emergence of copper-resistant strains
  → *doubt on the long-term sustainability*

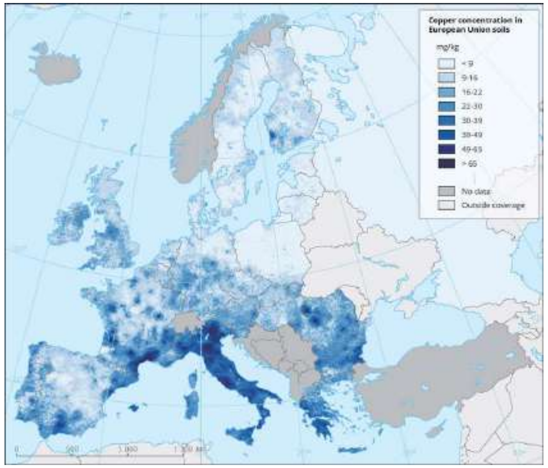
- copper = a non-degradable heavy metal

→ adverse effects on the environment and biodiversity,

such as contamination of soil and groundwater, with significant impact on soil biota (Kandeler et al. 1996; Merrington et al. 2002)

→ affect crop health in the long term due to soil accumulation (Dumestre et al. 1993)

Copper concentration in soil in european union (EEA, Europa)



Controversial use of Cu

- especially in organic farming

- **legislation limiting the use** of Cu compounds
    - European Union (regulation no. 473/2002):
        - 8 kg/ha/year until 2005, now 6 kg/ha/year (possibility to make an average over 5 years in perennial crops)
    - other countries:
        - Australia: use of Cu mixtures allowed by certifying authorities but use of Cu oxychloride prohibited (Van Zwieten et al. 2004)
    - USA: Cu mixtures on the National Organic Program List as synthetics

Controversial use of Cu

- **prediction of the ban of its use** in the near future (Wightwick et al. 2008; Finckh et al. 2015; Tamm et al. 2015)

  - some European countries (Germany, Austria, and Switzerland) already imposed a lower quantitative limit of 3 to 4 kg/ha/year (Wightwick et al. 2008; Finckh et al. 2015; Tamm et al. 2015)

  - banned for organic and conventional farming in some EU countries (ex. the Netherlands, Denmark)

Find effective solutions/alternatives to control diseases

- major challenge due to high complexity of IPM and agro-ecosystem

- solution(s): no only one but a combination of methods

→ integrated management

Find effective solutions to control diseases

Agronomic management

- Decision Support Tools/Systems (to improve decision-making process)
- cultural control (essential vineyard work – prophylactic methods)
- increase biodiversity (mixed cropping…)
- bio-fertilizers/biostimulants (nutritive equilibrium management, particularly nitrogen)

Alternative methods

- resistant varieties
- biocontrol agents
- plant defense stimulators (PDS) / resistance inducers
- biopesticides (e.g. from plants, microorganisms)

Find effective solutions to control diseases

→ Take advantage of plant defense mechanisms

- plant defense stimulators (PDS)/resistance inducers

- use elicitors to enhance plant natural defense responses

- plant biopesticides

- use plant antimicrobial compounds

Can PDS be a solution for grapevine protection?

Plant defense stimulators/resistance inducers

Examples of PDS

Exogenous

- Microbiological structures

(harpins, chitosan, rhamnolipids, yeast cell walls - Cerevisane, *Bacillus* spp., oligosaccharides)

- Synthetic substances

(synthetic analogs of phytohormones: salicylates, jasmonates, ethylene, benzothiadiazole; others: *β*-aminobutyric acid, phosphonates)

- Plant extracts

(laminarin, ulvans, fenugreek extract)

Endogenous

- Components of the cell wall

(oligogalacturonides, peptides)

- Phytohormones

(salicylates, jasmonates)

- Reactive oxygen species

(ROS)

Plant defense stimulators/resistance inducers

Examples of PDS available on the market

Exogenous

- Microbiological structures

COS-OGA

(harpins, **chitosan – Elexa, ChitoPlant, Chitogel, Armour Zen**, rhamnolipids, **yeast cell walls - Cerevisane, *Bacillus* spp.**, oligosaccharides)

- Synthetic substances

(synthetic analogs of phytohormones: salicylates, jasmonates, ethylene, benzothiadiazole; others: β-aminobutyric acid, **phosphonates – LBG, Redeli**)

- Plant extracts

(**laminarin- Iodus40,** ulvans, **fenugreek extract**)

Endogenous

- Components of the cell wall

(**oligogalacturonides,** peptides)

- Phytohormones

(salicylates, jasmonates)

- Reactive oxygen species

(ROS)

Plant defense stimulators/resistance inducers

PDS studied in our lab

Exogenous

- Microbiological structures

(harpins, chitosan, rhamnolipids, yeast cell walls-Cerevisane, *Bacillus* spp., **oligosaccharides of *B. cinerea***)

- Synthetic substances

(synthetic analogs of phytohormones: salicylates, **jasmonates (MeJA), ethylene (Ethephon), benzothiadiazole (BTH)**)

others: β-aminobutyric acid, **phosphonates** – LBG, Redeli)

- Plant extracts

(laminarin, ulvans, fenugreek extract)

Endogenous

- Components of the cell wall

(oligogalacturonides, peptides)

- Phytohormones

(salicylates, jasmonates)

- Reactive oxygen species

(ROS)

Plant and microbe models

Grapevine models

Cell cultures

Foliar cuttings

Vineyard

Studied pathogens

Plasmopara viticola

Erysiphe necator

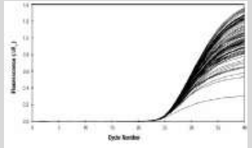
Botrytis cinerea

Grapevine trunk diseases fungi

Used techniques and analytical tools

- **Level of protection**
(Inoculation with *Plasmopara viticola* and visual scale of growth inhibition)

- **Defense-gene expression**
RT-qPCR (Neovigen microarray, Fluidigm[1])



- **Specialized metabolites**
LC coupled with UV-visible, fluorimetry or mass spectrometry
› polyphenols (e.g. anthocyanins, flavanols, stilbenes)

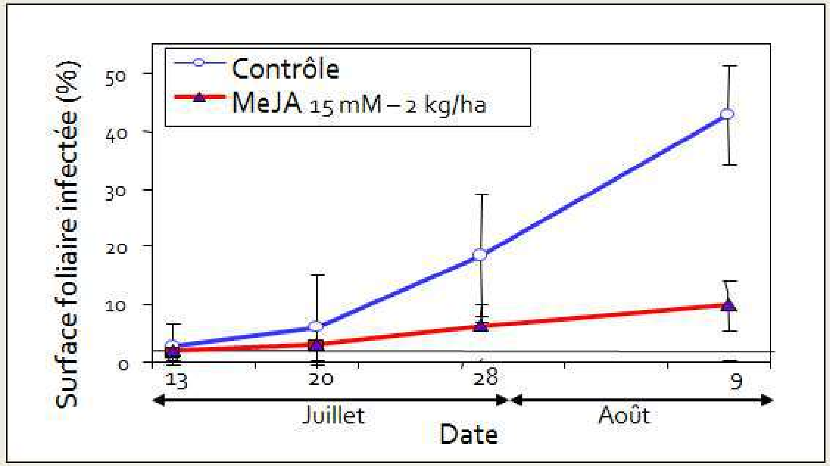
- **Primary metabolites**
Proton nuclear magnetic resonance spectroscopy (1H-NMR)
› amino acids, organic acids, sugars…

[1] In collaboration with M.F. Corio-Costet (UMR SAVE, INRAE, Villenave d'Ornon)

Protection level

Ethephon, MeJA and Botrytis oligosaccharides

	Powdery mildew (*E. necator*)	**Downy mildew** (*P. viticola*)
Ethephon (0.5 g.l-1)	64%	**34%**
MeJA (5 mM)	75%	**14%**
Oligosaccharides of *Botrytis* (2 g eq.Glc.l-1)	63%	**99%**



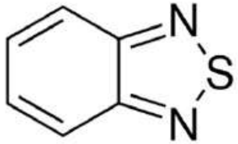
80% of protection against **powdery mildew** (*E. necator*)

➢ Protection against two major pathogens (from 14 to 99 %)

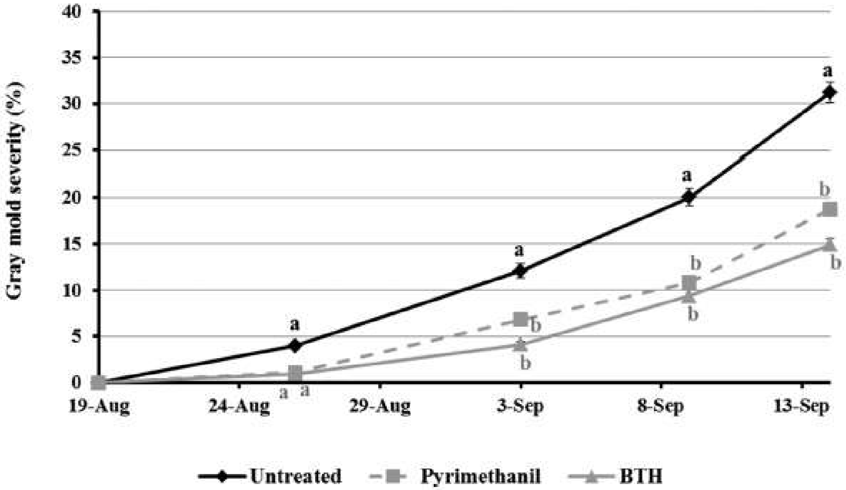
Belhadj *et al.* JAFC, 2006
Belhadj *et al.*, PPB, 2008
Faurie *et al.*, *J Plt Physiol* 2009
Saigne-Soulard *et al.*, *Polysacch*, 2014

Protection level

Benzothiadiazole
(BTH)



Disease
severity

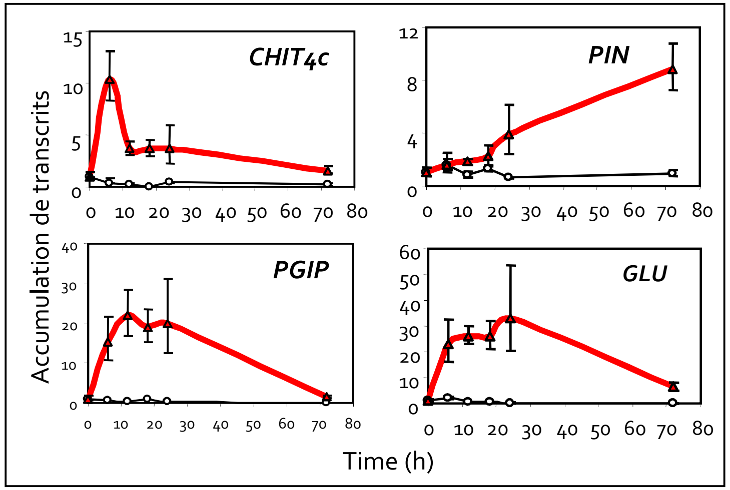


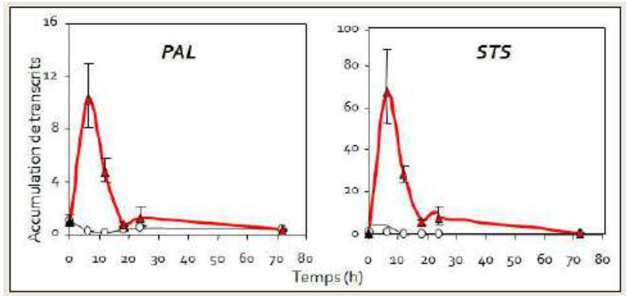
Untreated
BTH (400 g/ha)
Pyrimethanil (1000 g/ha)

Protection against *Botrytis cinerea* (gray mold):
approx. 50% (during 2 vintages: 2014 – 2015)

Modulation of defense-responses

Ethephon, MeJA and Botrytis oligosaccharides





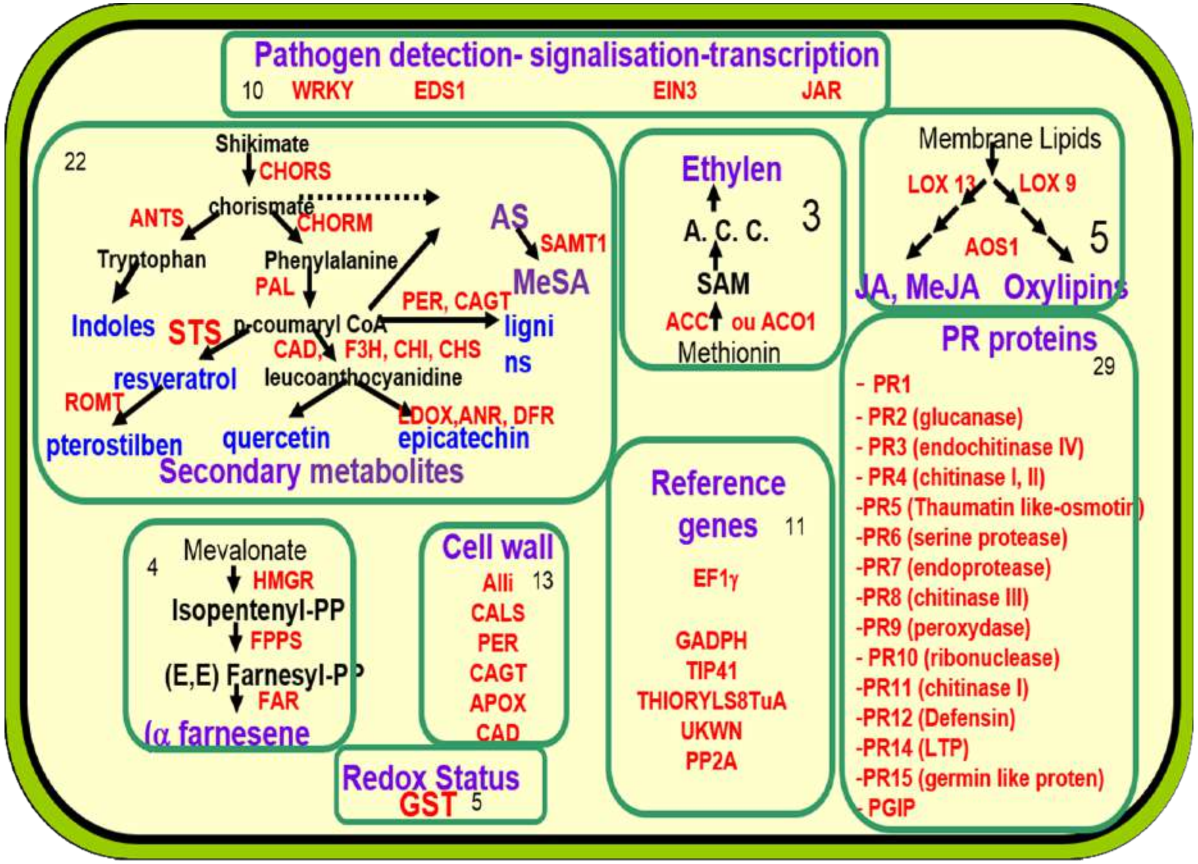
- Induction of the expression of **PR proteins genes**
- Induction of the expression of genes involved in the phenylpropanoid pathway (*PAL* and *STS*)

Belhadj *et al.* JAFC, 2006
Belhadj *et al.*, PPB, 2008
Faurie *et al.*, *JISVV*, 2009
Saigne-Soulard *et al.*, *Polysacch*, 2014

Modulation of defense-responses

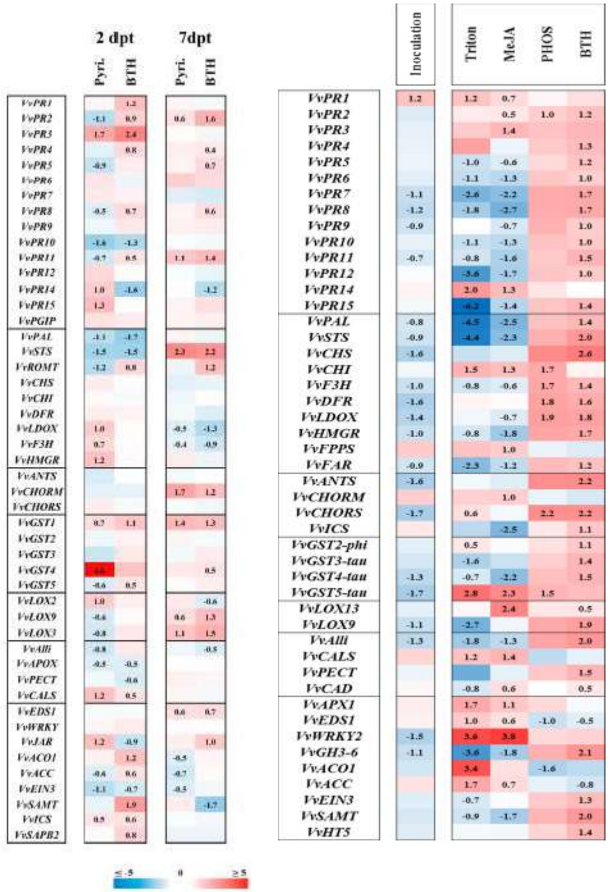
- Neovigen-96 Chip

Expression of 96 genes « defense » (Fluidigm – collab. M.F. Corio-Costet, UMR SAVE, Villenave d'Ornon)



Modulation of defense-responses

BTH, MeJA, PHOS



- Genes differentially modulated

  - up or down-regulation of genes depending of the PDS, the time point

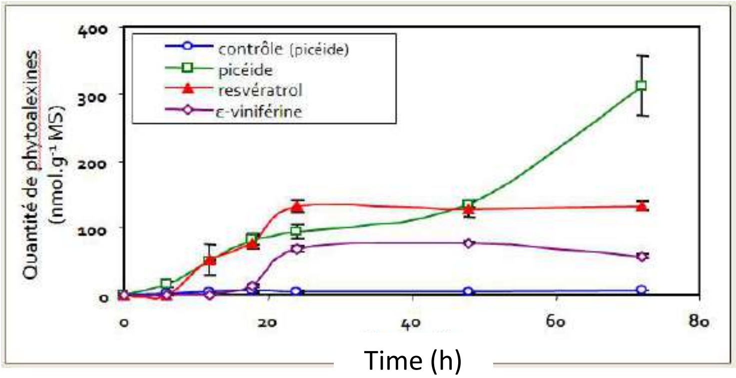
Triton ~ MeJA → pathway JA-ET

BTH ~ PHOS → pathway SA

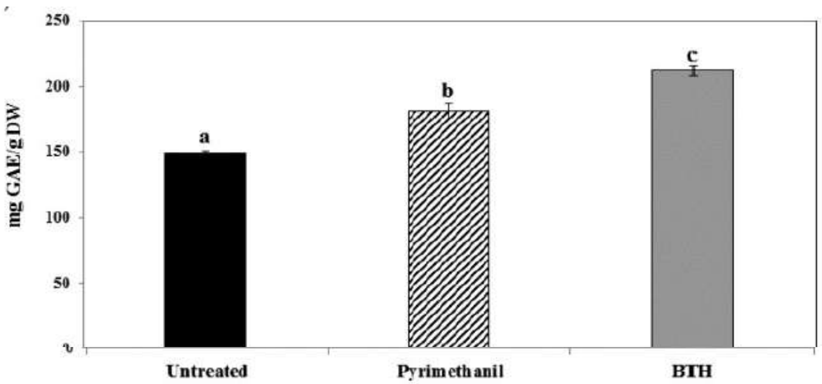
Bellée et al., JAFC, 2018
Burdziej et al., JAFC, 2021

Modulation of defense-responses

Ethephon, MeJA, BTH and Botrytis oligosaccharides



➢ Accumulation of stilbenes (piceids, resveratrol and ε-viniferin)



➢ Increase of the total polyphenol content

Belhadj *et al.* JAFC, 2006
Belhadj *et al.*, PPB, 2008
Faurie *et al.*, *JISVV*, 2009
Saigne-Soulard *et al.*, *Polysacch*, 2014
Bellée et al., JAFC, 2018
Burdziej et al., JAFC, 2021

Transmission of elicitor signals

Determination of early players for **MeJA** responses

Pharmacological approach with specific inhibitors:

Verapamil (calcium fluxes), genistein (kinases), cantharidine (phosphatases), DPI (NADPH oxydase), DETC (SOD)

- Requirement of calcium flux from extracellular medium
- Reversible phosphorylation of proteins
- No effect of H_2O_2
- Inducible role of superoxide ion

Faurie *et al. J Plt Physiol*, 2009

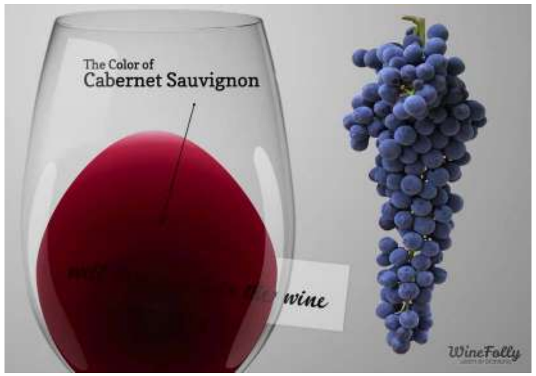
PDSs elicit stilbenes production but…

does it affect other secondary metabolites production and the primary metabolism?

PDSs elicit defense responses but…

does it affect other secondary metabolites production and the primary metabolism?

→ *quality of grapeberries/ red wine:*
*accumulation of **anthocyanins** to be maintained*



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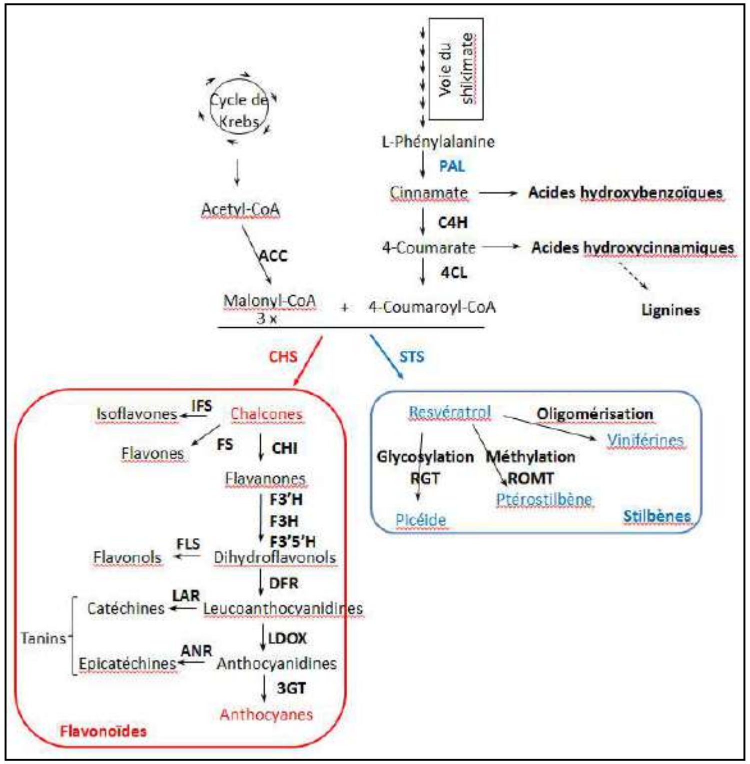
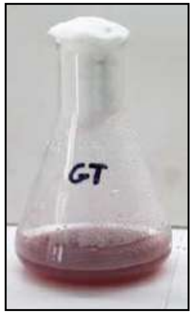
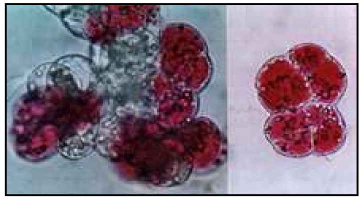


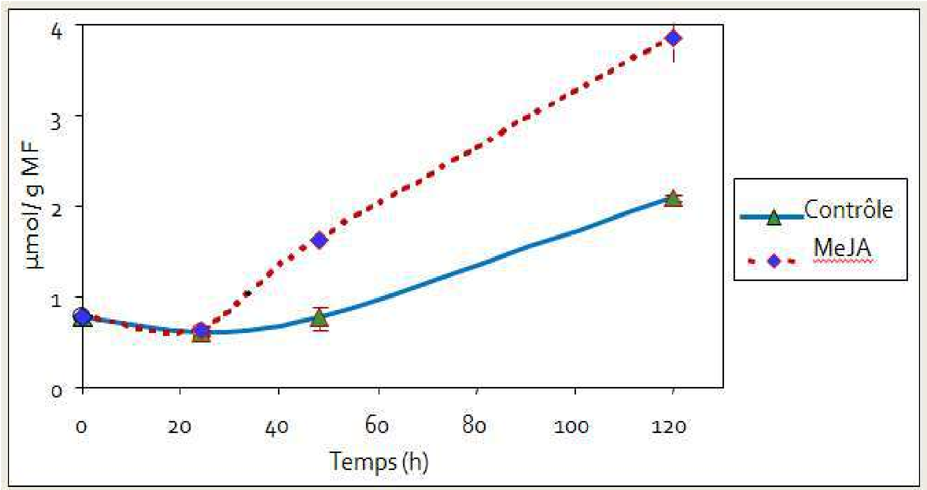
PDS and anthocyanin production





MeJA

Models: cell suspensions producing anthocyanins
(Gamay Teinturier)



➢ Induction of anthocyanins biosynthesis

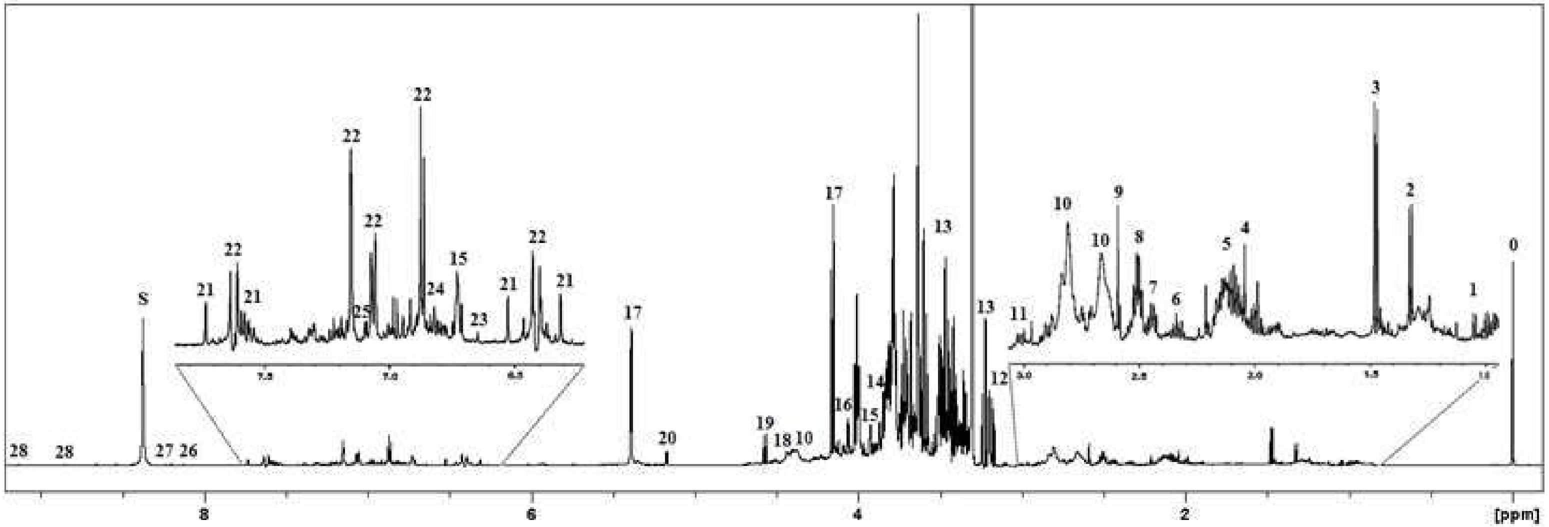
Belhadj *et al.*, *PPB*, 2008

PDS and primary metabolism

MeJA, BTH, PHOS

→ **30 metabolites** followed including
amino acids, organic acids, carbohydrates,
phenolics and amines

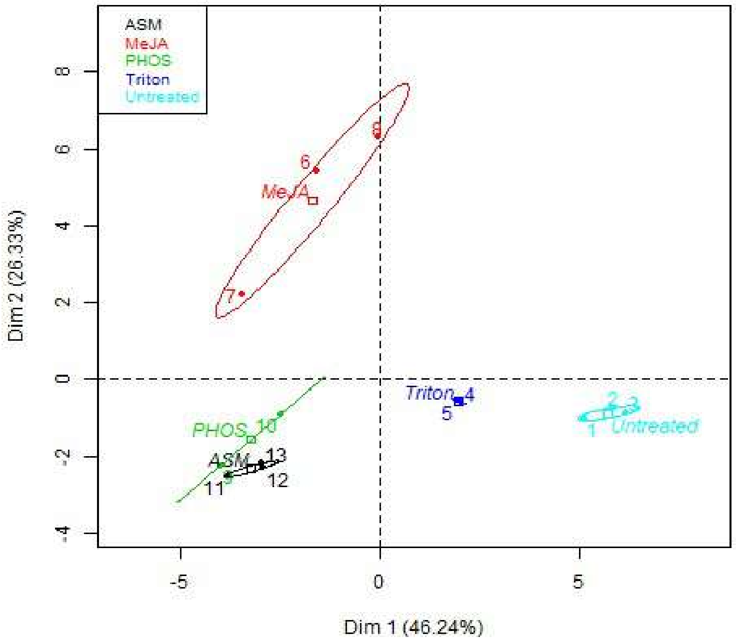
^{1}H NMR experiments

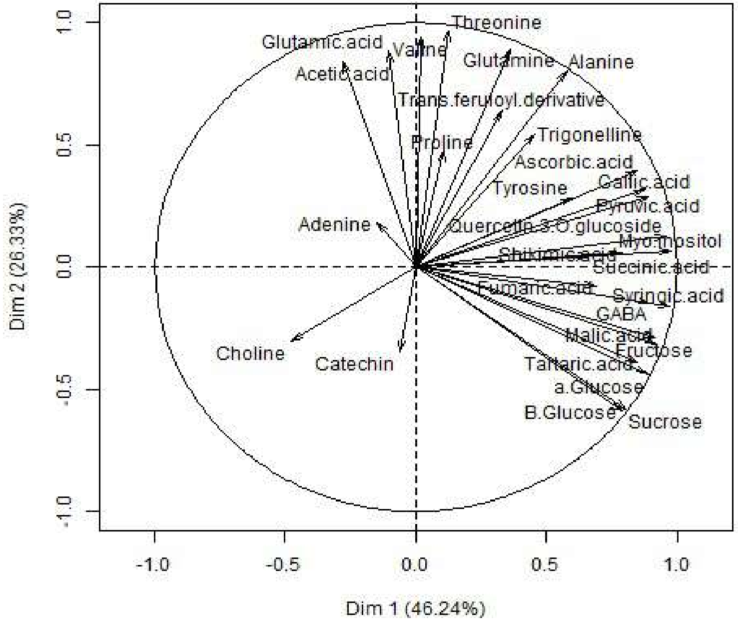


Burdziej *et al.*, *Metabolomics*, 2019

PDS and primary metabolism

MeJA, BTH, PHOS





- negative impact of PDS
- response PDS-specific

Burdziej *et al.*, *Metabolomics*, 2019
Burdziej *et al.*, *JAFC*, 2021

Main conclusions on PDS strategy

- **Effectiveness**

  but can be partial

  - lack of information about PDS mode of action in production conditions
  - formulation issue

  but can be variable

  - biotic and abiotic factors can affect plant response to PDS

    (plant genotype, disease pressure, mineral nutrition, development stage…)

  but can be different *according to the pathosystem*

➡ **PDS: more a complement →** association of strategies

Can biopesticides be a solution for grapevine protection?

Some plant extracts efficient against downy mildew

- **very promising** in controlled conditions and in vineyard
  up to 94% of protection

- essentiel oils
  extracts of clove, cinnamon, tee tree…

- **hydroalcoholic or aqueous extracts** of
  Yucca schidigera (Dagostin et al. 2011)
  Salvia officinalis (Dagostin et al. 2011)
  Trichoderma harzianum (Dagostin et al. 2011)
  Larix decidua bark (James et al. 2016)
  Salix alba, Equisetum arvense,
  Artemisia absinthium, Inula viscosa,
  Frangula alnus, Rheum palmatum (Godard et al. 2009)

Rheum palmatum

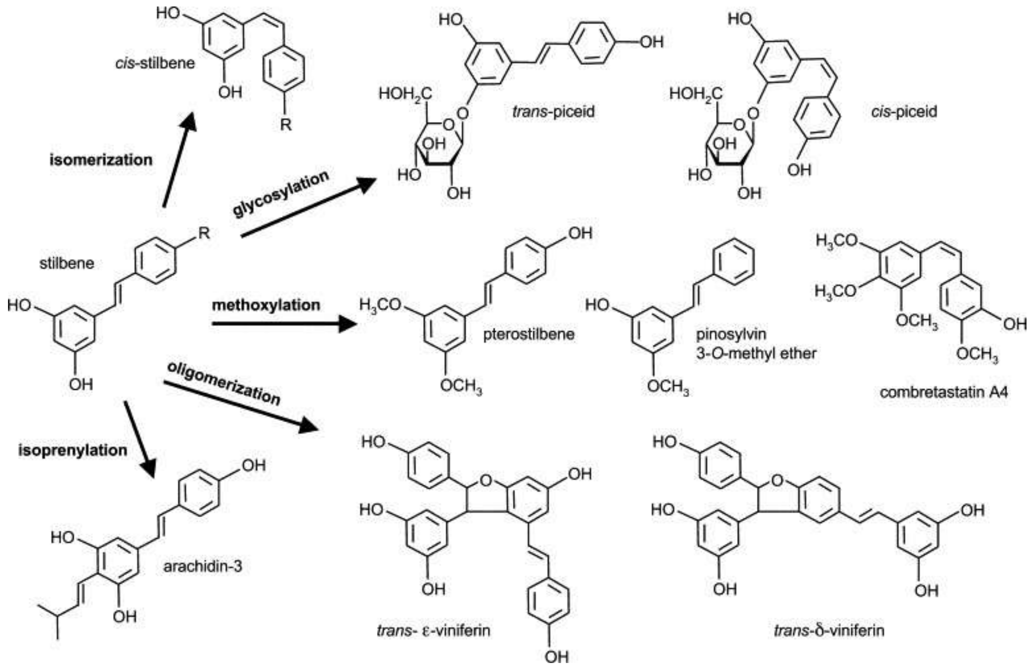
Plant compounds

- 3 main families
  - **phenylpropanoids** (stilbenes)
  - terpenoids and steroids, alcaloids
  - nitrogen compounds

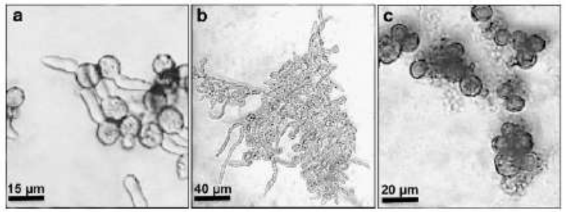
- broad-spectrum of activities
  - against insect pest species
  - antimicrobial (e.g. fungi or bacteria)
  - weeds

Plant extracts enriched in stilbenes

- Stilbenes: secondary metabolites that display **antimicrobial activities**



Chong et al., 2009



Adrian et al., 2012

- Common in diverse plant families
    e.g. grape (Vitaceae), pine (Pinaceae), peanut (Fabaceae) and sorghum (Poaceae)

Rivière *et al.*, *Nat Prod. Rep.*, 2012

Co-products enriched in stilbenes

Valorization of co-products

- from grapevine

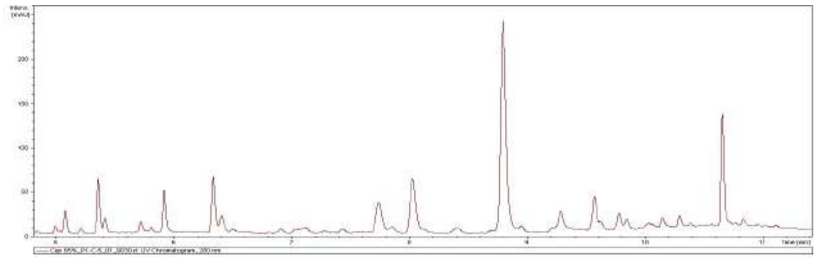
  - canes: pruning → 1-2 tons /ha/an
  - trunks and roots: ~ 2% of annual renewal

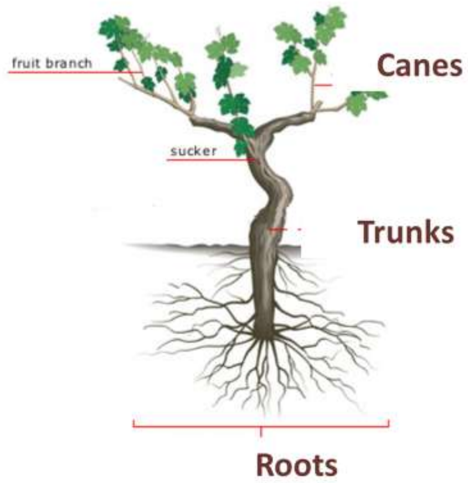
- from pine and picea

  - e.g. generation of 10−40 tons of pine **knots** per day by French paper factory

Grapevine extracts

- Stilbene content





11 stilbenes identified

- **Monomers:** resveratrol, piceatannol
- **Dimers:** ε-viniferin, ω-viniferin, pallidol, ampelopsin A
- **Trimers:** miyabenol C
- **Tetramers:** vitisin A, vitisin B, hopeaphenol, isohopeaphenol

- Stilbene quantity differs according to the organ

Trunks	≥	Canes	> >	Roots	
351.45		339.99		223.72	mg/g extract

Lambert *et al.*, *JAFC*, 2013
Gabaston *et al.*, *JAFC*, 2017

Grapevine extracts

Anti-microbial activity against downy mildew

Foliar disks assays

- **Tetramers**: the most active molecules

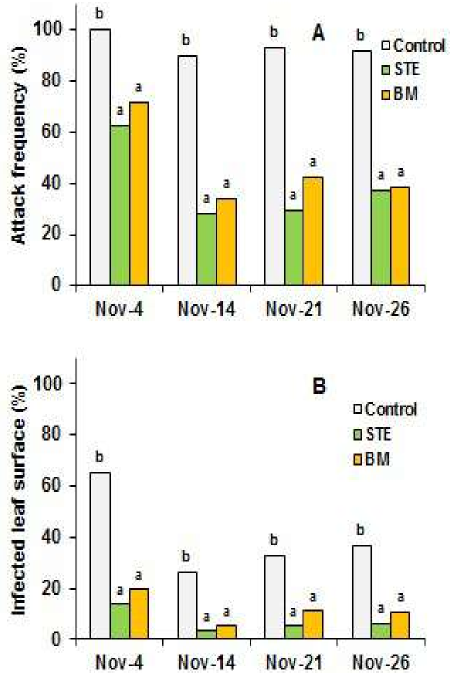
	IC_{50} (mg/L)
Cane extract	210
Root extract	120
Trunk extract	60

Greenhouse

- **Disease reduction**
  - ~ 60% for pathogen attack frequency
  - ~ 85% for infected leaf surface

Vineyard

- **Disease reduction**
  - ~ 25% for pathogen attack frequency
  - ~ 60% for infected leaf surface



Cane extract
Bordeaux mixture

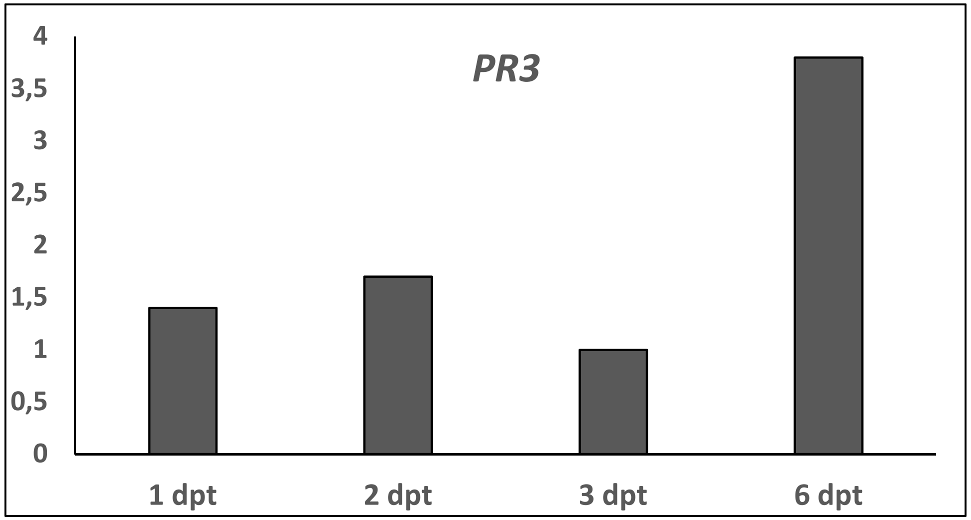
Richard *et al.*, *OenoOne*, 2016
Gabaston *et al.*, *JAFC*, 2017

Grapevine extracts

Protective effects of a grapevine trunk and root extract

→ inhibition on *P. viticola* zoospore mobility and sporulation

→ stimulation of **plant defense responses**
(PR proteins, *PAL* and *STS* genes)

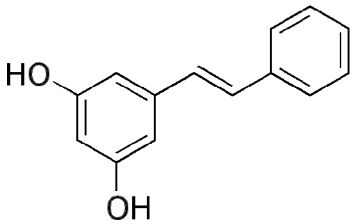


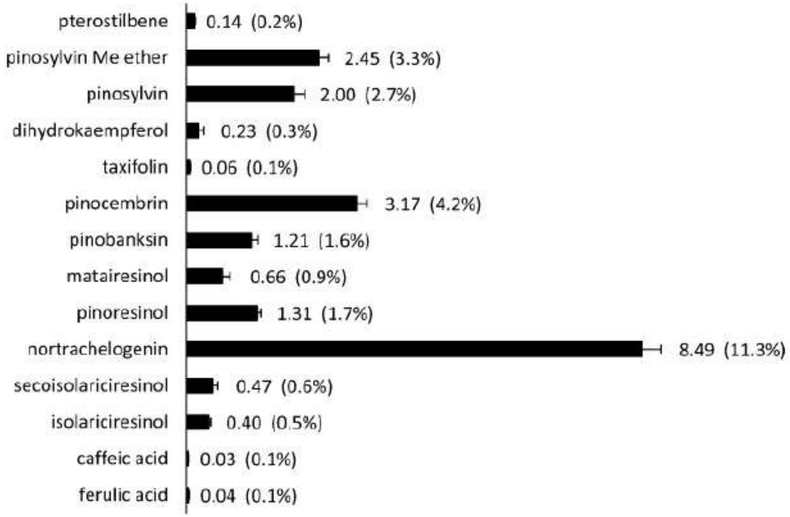
Taillis *et al.* 2021, in preparation

Pine knot extract

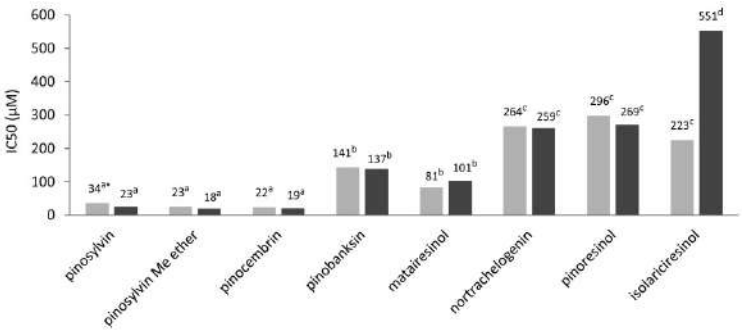
- Polyphenol concentrations (g/kg knot)

Pinosylvin





- Inhibition of downy mildew
  - pine knot: IC_{50} = 76 mg/L
  - pure compounds



Gabaston *et al. JAFC* 2017
Gabaston *et al. Ind Crops Prod* 2017

Eco-friendly extractions

- « Green » solvants

  ethanol
  ethyl acetate

- Subcritical water extraction of stilbenes

  Highest stilbene yield at 160 °C and 5 min
  cane: 3.62 g/kg dry mass
  wood: 9.32 g/kg dry mass
  root: 12.10 g/kg dry mass

Gabaston *et al.*, Ind Crops Prod, 2018
Taillis *et al.* in preparation

Main conclusions on plant biopesticides strategy

- **High level of protection**
  up to 60-95% against downy mildew in the field (e.g. grapevine, pine extracts)

- **Narrow spectrum of activity and different modes of action**
  also PDS properties (e.g. trunk and roots extract)
  → low risks of resistance development in the targeted pathogen populations
  but toxicology studies required

- **Short shelf life**
  sensitivity to fluctuations in temperature and humidity
  but limited field efficacy

Thank You For Your Attention

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