Use of metabolomics to identify bioactive compounds from grapevine eco-extracts that can impair fungal growth and production of mycotoxins by Fusarium graminearum and elucidate their mechanisms of action

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* These authors contributed equally to this work

Problem statement and objectives

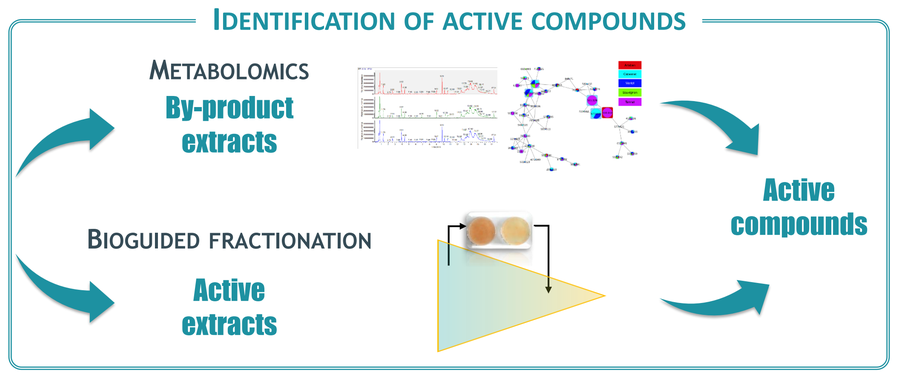
Fusarium Head Blight of small-grain cereals is a devastating fungal disease primarily caused by *Fusarium graminearum* in Europe. Beyond crop losses, *F. graminearum* poses potential health risks due to the production type B trichothecene (TCTB) mycotoxins including deoxynivalenol (DON) and 15-acetyldeoxynivalenol (15-ADON). The development of environmental-friendly strategies guaranteeing the safety of food and feed is a key challenge facing agriculture today.

The goal of the present study is to investigate and exploit the biological activity of grapevine wastes to develop environmental-friendly solutions to counteract the growth of *F. graminearum* and its production of mycotoxins. In the first step, natural extracts from vine by-products were obtained using eco-extraction and were characterized for their antifungal and antimycotoxin activities. Identification of active molecules and their mechanisms of action were investigated using complementary omics approaches.

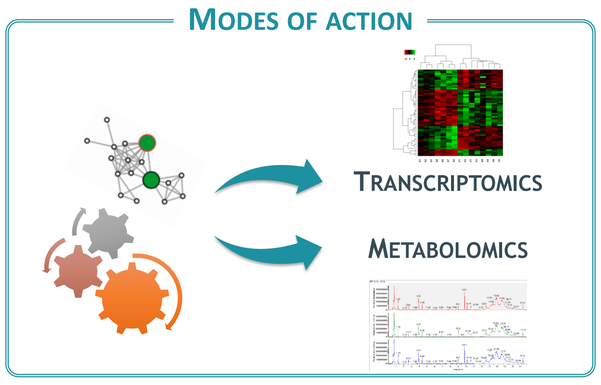
Experimental procedure

Production and screening of by-product extracts

Identification of active compounds



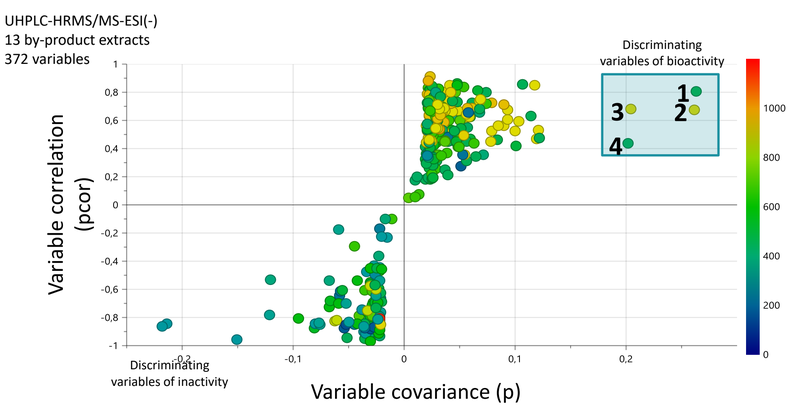
Modes of action



Results

Untargeted metabolomics and molecular network to evidence the active compounds

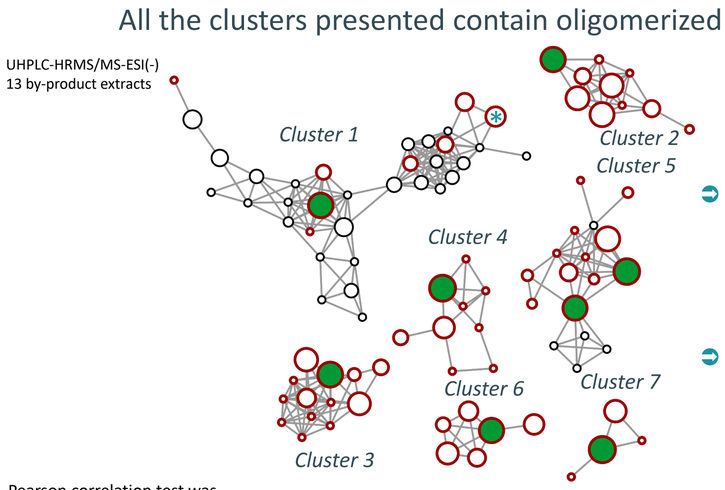
OPLS-DA
S-plot representation of discriminant variables between antifungal and inactive extracts



Number	*m/z* [M-H]⁻	Metabolite	Oligomer	Score (pcor & p)
1	469.1293	Ampelopsin A	Dimer	0.81 & 0.26
2	905.2610	Vitisin B	Tetramer	0.67 & 0.25
3	905.2609	Isohopeaphenol	Tetramer	0.68 & 0.20
4	453.1348	*E*-ε-viniferin	Dimer	0.43 & 0.19

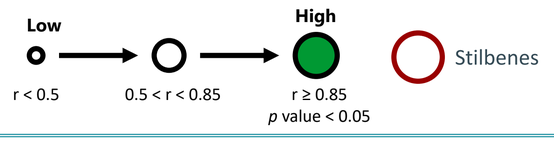
Bioactivity Based Molecular network

All the clusters presented contain oligomerized stilbenes



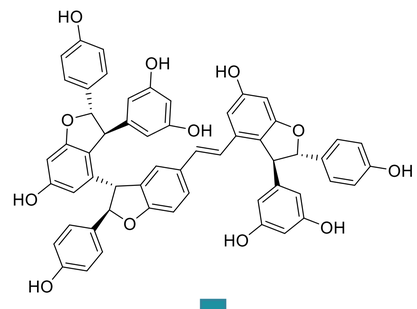
- Clusters 1, 2, 3, 4 and 6 are mainly composed of tetrameric stilbenes, including vitisin B (VIT B) indicated with * in cluster 1
- Other clusters contain dimeric (cluster 7) or trimeric stilbenes (cluster 5)

Pearson correlation test was performed between ion abundance and percentage of fungal growth inhibition at 125 mg/L for each extract to determine bioactivity score



Oligomeric stilbenes appear to be correlated with antifungal activity

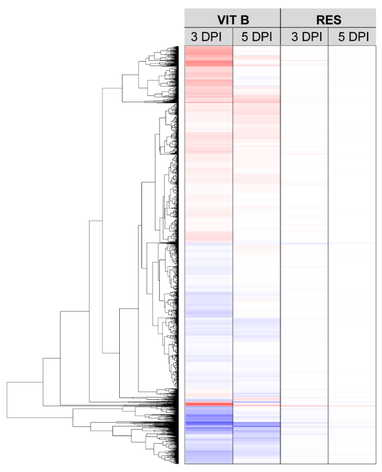
VIT B is the main stilbene quantified by HPLC-UV in most active root extracts



Isolation of VIT B from root extract using preparative HPLC

Multi-omics approach to understand the mode of action of VIT B and *E*-resveratrol (RES)

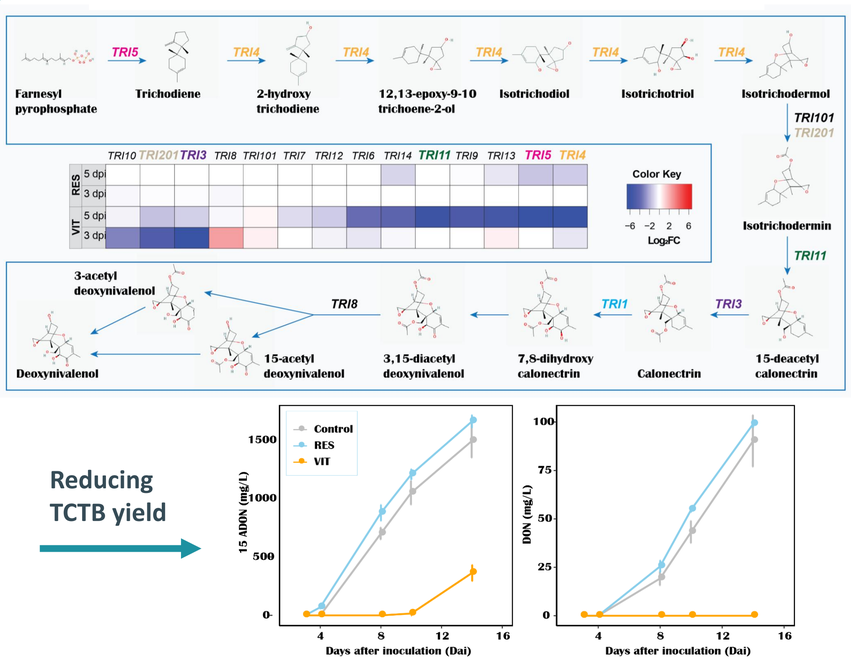
Globally transcriptomic regulation by VIT B and RES



VIT B: main active molecule
RES: most studied vine monomeric stilbene
DPI: day post inoculation

- VIT B induces stronger fungal globally transcriptomic regulation compared to RES
- Approx. 7000 differentially expressed genes are induced by VIT B compared to the Control (C)

Down-regulation of TRI genes by VIT B and RES



Reducing TCTB yield

- VIT B significantly inhibits TCTB yield (by over 70% for 15-ADON, and almost 100% for DON at 14 days), which could be partially ascribed to the downregulation of *TRI* genes

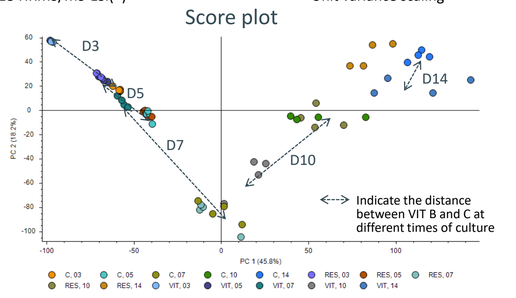
Untargeted metabolomics of fungal supernatants

PCA analysis

Conditions: C , RES 40 µM, VIT B 10 µM
Times of culture, day D3, D5, D7, D10, D14
UHPLC-HRMS/MS-ESI(+)

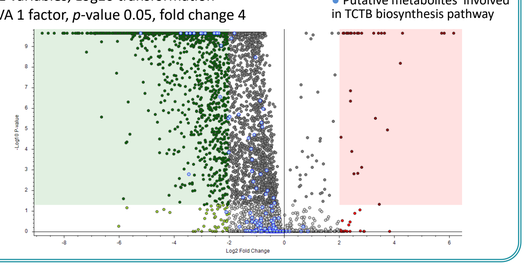
10 232 variables
48 samples
Unit variance scaling

Score plot



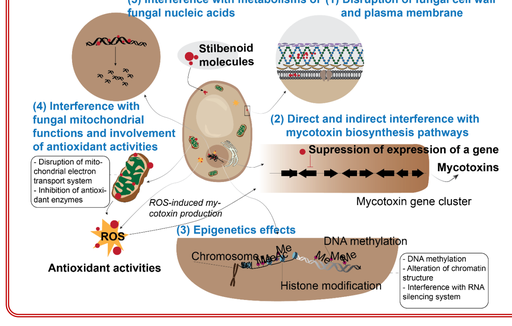
Volcano plot of C vs VIT B at day 7

VIT B 10 µM
10232 variables, Log10 transformation
ANOVA 1 factor, *p*-value 0.05, fold change 4



- At all times of culture, 40 µM RES does not induce significant differences on secondary fungal metabolism
- 10 µM VIT B induces significant delaying effect on secondary fungal metabolism which catches up at D14
- The most important modifications between VIT B treatment and C are observed at D7 with modification of the production of 3778 putative metabolites
- At D7, VIT B induces significant inhibition of the production of 20 putative metabolites involved in the TCTB biosynthesis pathway (*p*-value 0.05, fold change 4)

Modes of action



Conclusions and perspectives

- Untargeted metabolomics combined with molecular network and bioguided fractionation allow us to identify oligomeric stilbenes, in particular VIT B, as predominant active antifungal and antimycotoxin metabolites in vine by-products
- VIT B (at a low concentration of 10 µM) induces significant modifications in secondary fungal metabolism of *F. graminearum*, especially in TCTB biosynthesis pathway
- Omics approaches provide a deeper insight into the mechanism of action of VIT B underlying its antifungal and antimycotoxin activity
- The ongoing *in-planta* assays will study bioactivities of the vine extracts towards developing environmental-friendly solutions

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This work was funded by ANR, France: StilDeTox project, ANR-19-CE21-0007