Forest/wood and vine by-products as sources of new drugs for sustainable strategies to control fungal growth and type B trichothecene production by *Fusarium graminearum*

Vessela Atanasova,

Charles Tardif, Laetitia Pinson-Gadais, Pierre Waffo-Téguo, Caroline Rouger, Florence Richard-Forget

**UR1264 Mycology and Food Safety (MycSA), INRAE, France**
**FACTORS INFLUENCING *FUSARIUM* INFECTION AND MYCOTOXIN ACCUMULATION: CONTROL STRATEGIES**

**The use of synthetic fungicides is one of the strategies to control *Fusarium* disease and mycotoxins**

But their repeated use:
- increases the cost of production
- leads to disruption in natural biological systems
- fosters environmental and human health concerns
- results in the development of fungal resistance

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**To decrease the use of synthetic fungicides**

**Development of bio-based and eco-friendly alternatives**

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*Weather conditions*  
*Inoculum* (Life cycle, Toxigenic potential)  
*Agronomic practices*  
- Previous crop  
- Fungicide  
- Tillage  
*Varieties*  

Factors from most (****) to least (*) important (Arvalis)
PHENOLIC COMPOUNDS, A POSSIBLE ALTERNATIVE OF SYNTHETIC FUNGICIDES: HYPOTHESES ON THE MECHANISMS OF ACTION

1. Antifungal activity of phenolic compounds is related to their lipophilic properties
   - Montibus et al., 2021, Molecules
   - Ponts et al., 2011, Phytopathol.

2. Anti-mycotoxin activity of phenolic compounds is related to their antioxidant properties
   - Ponts et al., 2007, FEBS Lett.
   - Montibus et al., 2013, PLOS ONE

3. Phenolic compounds inhibit the expression of the key genes involved in the mycotoxin biosynthesis
   - Gautier et al., 2020, Toxins
   - Ferruz et al., 2011, Molecules
   - Boutigny et al., 2009, Mycol. Res.

4. Phenolic compounds affect primary and secondary metabolism of *F. graminearum*
**OBJECTIVE AND EXPERIMENTAL DESIGN**

**Objective:** investigate the biological activity of natural extracts obtained from wood/forest and vine by-products to develop environmental-friendly alternatives of chemical fungicides

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**Maritime pine**  
**Vine**  
**Chestnut**  
**Locust**  
**Oak**

- **VINEYARD MANAGEMENT:** 5 TONS/YEAR OF SOLID WASTE  
- **WOOD/FOREST SECTOR IN THE EUROPEAN UNION:** 53 MILLION TONS/YEAR OF WOOD WASTE

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**GREEN EXTRACTION PROCEDURE**  
**PRODUCTION OF BY-PRODUCT EXTRACTS**

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**By-product extracts**  
**Active by-product extracts**  
**Active compounds**

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**BIOGUIDED FRACTIONATION**

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**F. graminearum**

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**GREEN EXTRACTION PROCEDURE**

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**SCREENING OF BY-PRODUCT EXTRACTS & IDENTIFICATION OF ACTIVE COMPOUNDS**

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**METABOLOMICS ANALYSIS AND MOLECULAR NETWORK**

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**STUDY OF THE MECHANISM OF ACTION**

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**MECHANISM OF ACTION**

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**ADAPTED FORMULATIONS**  
**BIO-FUNGICIDES**

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Montibus et al, 2021, *Molecules*
**SCREENING OF NATURAL EXTRACTS OBTAINED FROM BY-PRODUCTS FOR THEIR ANTIFUNGAL AND ANTI-MYCOTOXIN ACTIVITIES**

- Four extracts inhibit significantly the fungal growth of *F. graminearum* CBS 185.32 (35-89%)

- The maritime pine sawdust extract $175^\circ C$ is the most active to inhibit TCTB production by *F. graminearum* CBS 185.32 (> 50%)

- The activities of maritime pine sawdust extract are confirmed on a panel of *F. graminearum* strains

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Montibus et al, 2021, *Molecules*
Antioxidant activity & Total phenolic content of the by-product extracts

- Active extract is not characterized by the highest antioxidant activity
- Active extract is not the richest in total polyphenols

Other specific characteristics contribute to the activity?
CHARACTERIZATION OF THE PHENOLIC COMPOSITION OF THE ACTIVE EXTRACT BY LC-DAD/MS

Three main groups of phenolic compounds: phenolic acids/aldehydes/alcohols, lignans, and flavonoids

More than half of the compounds are mono- or di-methylated

Lignans, phenolic acids and/or flavonoid activity?

Role of methylation (increased hydrophobicity of the molecules) in antifungal and anti-mycotoxin activity?

Synergistic effect between the compounds?

Montibus et al, 2021, Molecules
SCREENING OF NATURAL EXTRACTS FOR THEIR ANTIFUNGAL AND ANTI-MYCOTOXIN ACTIVITIES
EX: VINE BY-PRODUCTS

Vine by-product extracts

PhD Charles Tardif
StilDeTox ANR project

Percentage of inhibition/activation of the fungal biomass
- *F. graminearum* CBS 185.32 - 125 mg/L - Gardiner liquid medium - 6 days of culture

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</table>

- Ten extracts significantly inhibit fungal growth
- Four extracts with strong antifungal activity over than 90%
- All extracts significantly inhibit TCTB production by *F. graminearum* CBS 185.32
**ISOLATION AND IDENTIFICATION OF ACTIVE COMPOUNDS BY BIO-GUIDED FRACTIONATION**

- Six fractions were obtained by centrifugal partition chromatography
- Active compounds were eluted in the F6 fraction which reduced mycotoxin production by 85%
- Two major pics identified as phenolic oligomers which represent 60% of the total dry weight of the F6 fraction

> Studies on the activity and the mechanism of action of these compounds are underway
Data obtained from LC-MS/MS untargeted metabolomics study of 13 extracts with known bioactivity.

- GNPS molecular network based on MS/MS spectra similarity
- Cytoscape mapping of bioactivity to identify bioactive clusters

Four ions potentially linked to the biological activity of the extract against *F. graminearum*

These ions were attributed to the phenolic oligomeric compounds

- PhD Charles Tardif (charles.tardif@u-bordeaux.fr)
- StiDeTox project
CONCLUSION AND PERSPECTIVES

PRODUCTION OF BY-PRODUCT EXTRACTS

- Maritime pine and vine by-products are sources of bioactive compounds against *F. graminearum* and its mycotoxin production
- Development of eco-extraction method that permits the obtention of active extracts

SCREENING OF BY-PRODUCT EXTRACTS & IDENTIFICATION OF ACTIVE COMPounds

- Extracts with strong antifungal and anti-mycotoxin activities
- The two strategies used for isolation and identification of the active compounds in the extracts of interest are complementary
- Active molecules: - oligomeric compounds - methylated compounds

MECHANISMS OF ACTION

- Microscopy analysis to investigate the morphological disorders induced by the active molecules
- Transcriptomic approach to investigate the effect of active molecules on gene expression linked to the oxidative stress and toxin biosynthesis
- Combination of $^{1}H$ NMR and LC-MS/MS analyses to investigate the effect of active molecules on primary and secondary metabolic pathways

ADAPTED FORMULATION

Bio-fungicides

Collaborations with private and academic partners

- Development of adapted formulations
- Tests in real conditions

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