Metabolomic and cytological approaches to better understand grapevine trunk diseases disorders

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❶ Context

Grapevine trunk diseases (GTDs) are devastating diseases involving xylem-inhabiting fungi. Among them, **Esca** and **Botryosphaeria dieback** affect vineyards in major worldwide grape-producing areas. The causal fungi **alter the woody tissues** and could **produce toxins** that **disturb the plant physiology**, leading to chronic or apolectic leaf symptoms. The unique effective fungicide against these diseases, sodium arsenite, is now prohibited because of toxicity. As GTDs are complex and still partly unknown, the identification of protection strategies remain a deadlock. **Global and targeted approaches** were therefore conducted **to get new information on the impact of the diseases and sodium arsenite treatment on grapevine metabolism, development and physiology**.

❷ Methodology

Metabolomics (GC-MS and **FT-ICR-MS)** was used to highlight the impact of the disease / sodium arsenite treatment on the plant metabolic pathways and to obtain the associated metabolite signatures.

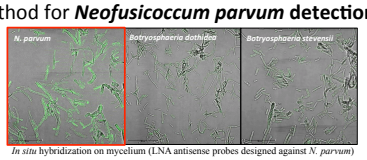
Imaging approaches including **histological observations** by **epifluorescence**, **scanning-** and **transmission-electron microscopy** were used to observe grapevine responses to disease / treatment, and pathogen localization.

❸ Main results

A better knowledge of plant / pathogen interaction (France Agrimer & BIVB-CR BFC projects)

(1) Development of a method for ***Neofusicoccum parvum*** **detection**

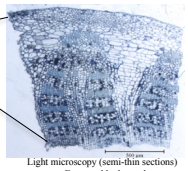
N. Parvum mycelium on PDA



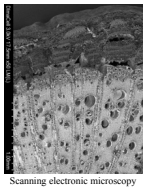
In situ hybridization on mycelium (LNA antisense probes designed against *N. parvum*)

(2) **Evidence of alteration of secondary histogenesis** in canes affected by Botryosphaeria dieback

Cane harvested at the beginning of September on a grapevine affected by Botryosphaeria dieback

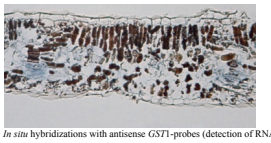


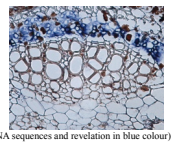
Light microscopy (semi-thin sections) on Epon-embbed samples



Scanning electronic microscopy

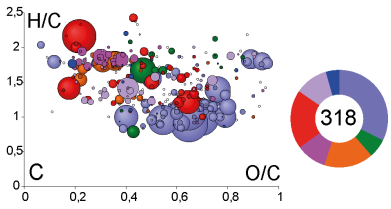
(3) Specific localization of the ***GST1*** (glutathione-S-transferase; role in detoxication process) expression in **the phloem** of esca-symptomatic leaves

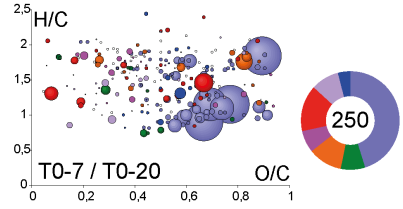


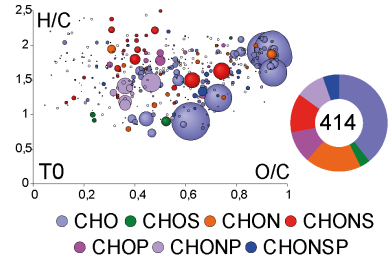


In situ hybridizations with antisense *GST1*-probes (detection of RNA sequences and revelation in blue colour)

(4) **Specific metabolic signatures** of leaves from asymptomatic and symptomatic **Esca**-grapevines (before and at time of apoplexy occurrence)

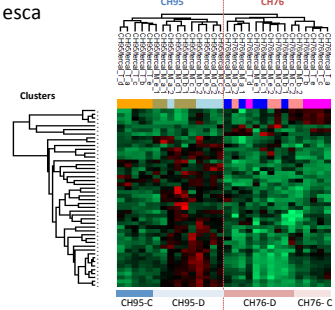






FT-ICR-MS analysis. Van Krevelen diagrams obtained for the different leaf extracts (C: asymptomatic; T0-20 / T0-7: 20 and 7 days before apoplexy, respectively; 0: apoplexy) and the corresponding chemical diagrams (number of chemical formula and their distribution according to their elemental composition)

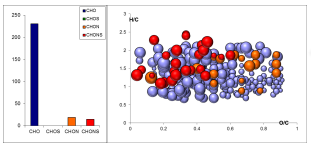
(5) **Clone-dependent** metabolic **response** to esca



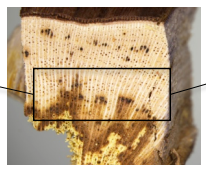
Hierarchical clustering analysis of metabolic data (GC-MS analysis) obtained from leaves of chardonnay clones 76 and 95 and collected on vines expressing Esca disease (D) or not (C)

Impact of sodium arsenite (Asn) treatment (Casdar V1301 Project)

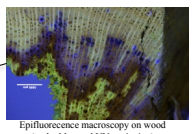
(1) Determination of a **trunk metabolic signature of Asn-treated grapevines**



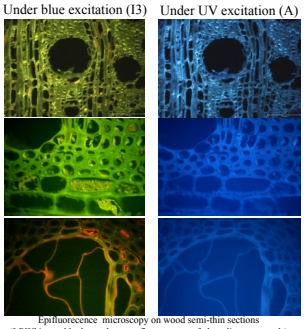
FT-ICR-MS analysis. Chemical histogram and Van Krevelen diagram corresponding to the arsenite treatment signature in the interaction area of grapevine trunks.



(3) **Plant defense reactions** in trunk of Asn-treated grapevines

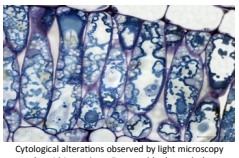


Epifluorescence macroscopy on wood (under blue and UV excitation)

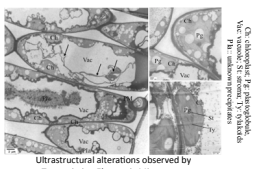


Epifluorescence microscopy on wood semi-thin sections (LRWhite-embbed samples; autofluorescence of phenolic compounds)

(2) Highlighting of **vacuolar** and **chloroplastic alterations** in leaves of **Asn-treated grapevines**



Cytological alterations observed by light microscopy (semithin sections; Epon-embbed samples)



Ultrastructural alterations observed by Transmission Electronic Microscopy

❹ Conclusion

All these experiments were integrated into research projects combining **multidisciplinary approaches**. They allowed us to **shape tools** for further experiments and to **progress in the knowledge of the impact of GTDs and Asn treatment** on grapevine. All these results constitute precious information to define further experiments with the objective to find solutions to prevent - otherwise to restrain- such diseases in order to ensure the vineyard sustainability.

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