Technical Note

ARC TRAINING CENTRE FOR INNOVATIVE WINE PRODUCTION

Rapid assessment for prediction and quantification of *B. cinerea* off-flavours in grapes using mass spectrometry

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Background and Aims

Infection of grapes (*Vitis vinifera*) by *Botrytis cinerea* is a frequent occurrence in vineyards during prolonged wet and humid conditions, and it can lead to a significant detrimental impact on grape yield and overall quality (Hua et al., 2018). Growth of *B. cinerea* causes the oxidisation of phenolic compounds resulting in a loss of colour and the formation of a suite of off-flavours and odours in wine made from excessively infected fruit and may therefore lead to huge economic losses. (Ky et al., 2012). Grapes with 1 to 3% of *B. cinerea* infection, based on visual observation, can result in negative impacts on wine quality (Steel et al., 2020). Early detection and prediction of such infection could therefore aid early harvest decision-making and eliminate potential economic loss. Ideally, a rapid quality assessment of grapes must be done to establish an objective measurement of the phytosanitary aspects of the crops. However, with the techniques currently available, including visual inspection of grapes, ergosterol measurement, detection of *B. cinerea* antigens, or molecular diagnostic techniques such as qPCR, rapid identification and quantification of *B. cinerea* infection in grape berries remains a challenge due to the time frames for analysis, low accuracy, and lack of fungal species specificity.

Mass spectrometry (MS) is widely used in chemical and biological analysis owing to its ability to rapidly and sensitively detect compounds at low concentrations. MS analysis can be coupled with both targeted and untargeted analysis, which would be beneficial for the detection of key aroma volatiles, potential markers for the *B. cinerea* infection, and the discrimination of healthy and infected grapes.

The overarching aim of this work was to develop a more rapid method for early detection of *B. cinerea* grape infection than is currently available using conventional methods. Rapid detection and quantification of *B. cinerea* infection can ultimately lead to the development of improved methods for near real-time fungal infection monitoring on grapes as well as other agricultural products.

Key outcomes

- Volatile organic compounds (VOCs) were detected and quantified from naturally infected or lab-inoculated wine grapes of different *B. cinerea* infection severities using headspace solid phase microextraction (SPME) gas chromatography (GC) mass spectrometry (MS). The correlation between the detection of volatile compounds and the degree of *B. cinerea* infection as assessed by ergosterol and *B. cinerea* antigen analysis was investigated. Partial Least Squares (PLS) predictive models were established with detected VOCs for *B. cinerea* infection severities from different wine grape cultivars.
- A time course experiment was conducted to confirm and monitor the presence of key markers such as 1,5-dimethylnaphthalene, 1,5-dimethyltetralin, 3-octanol, and phenylethyl alcohol, which were accumulated in the inoculated grapes and were detectable around six days post *B. cinerea* infection. One potential early marker for *B. cinerea* infection, trans-2-octen-1-ol, was detected as early as two days post inoculation (Jiang et al., 2023a).

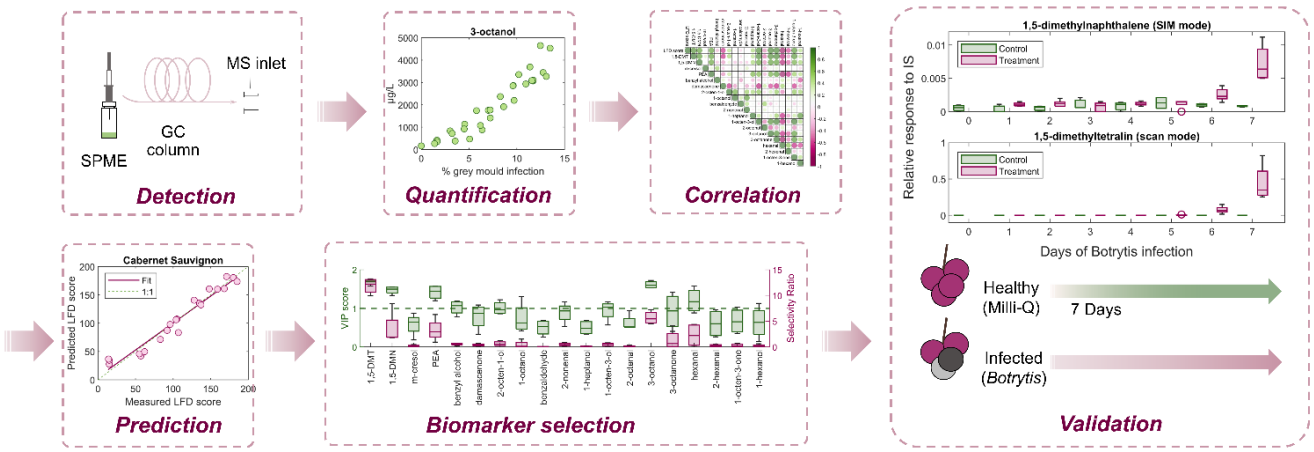

Figure 1. Volatiles generated from grapes of different *B. cinerea* infection severities were detected and quantified using SPME GC-MS. The infection severities were assessed using ergosterol measurement and *B. cinerea* antigen detection. The correlation coefficients of the detected volatile compounds and the *B. cinerea* infection severities in grapes were investigated and the predictive models were establish using the volatile markers. The importance of the volatiles in such PLS predictive models were assessed using variable importance to projection (VIP) and selectivity ratios to select key markers related to *B. cinerea* infection in grapes. Finally, a time-course experiment was conducted to confirm the formation of selected volatile compounds and monitor potential early markers for *B. cinerea* infection.

- A new metal-organic solid phase material compromising zeolitic imidazolate framework-8 (ZIF-8) was utilised as the absorbent material for volatile collection coupled with thermal desorption GC-MS. In the field sampling experiment, ZIF-8 was proved to be more sensitive and efficient for the absorption and detection of *B. cinerea*-related volatile compounds such as 3-octanone, 1-octen-3-one, 3-octanol, and 1-octen-3-ol, than a commercially available material, Tenax®-TA (Jiang et al., 2023b).

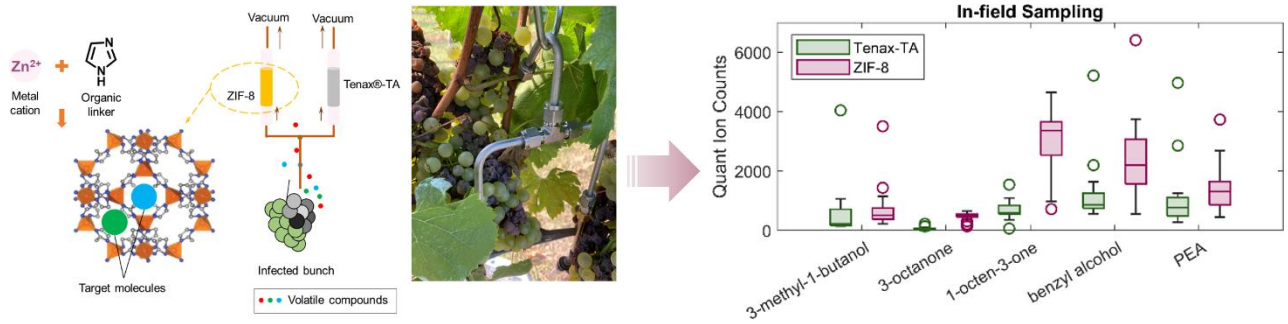

Figure 2. A schematic diagram of in-filed sampling. In this experiment, ZIF-8 was used as absorbent material for a rapid volatile extraction of 15 mins from an open air in the vineyard. Two sampling tubes containing ZIF-8 and a commercially available material, Tenax®-TA were used simultaneously as comparison. During the sampling, one end of each tube was connected to low flow-rate vacuum pump to create the air flow and the other end was pointed to a bunch of grapes. The extracted volatiles were then analysed and quantified using thermal desorption GC-MS.

- Direct electrospray ionisation (ESI) MS was applied for the metabolomic analysis to discriminate healthy and *B. cinerea* infected wine grapes, with the detection of approximately two minute per sample. A robust predictive model was acheived with an overall predictive error of less than 10%. It is a rapid, highthroughput detection method important for the application of vineyard samples on an industrial scale.
- The important molecular features were annotated with a separate experiment using a high-resolution quadrupole time-of-flight (qTOF) ultra-high performance liquid chromatography (UHPLC)-MS. Grape derived metabolites such as linoleic acid, oleic acid, and succinic acid were identified with high relevance for *B. cinerea* infection.

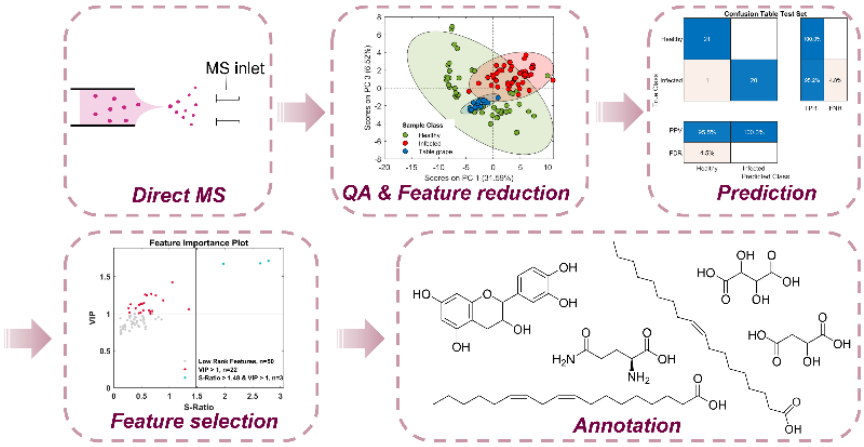

Figure 3. A schematic showing the workflow of untargeted metabolomic analysis for the discrimination of healthy and *B. cinerea* infected grapes. In this experiment, grape homogenates were quickly extracted with acetonitrile and analysed using direct mass spectrometry (MS). A total of 70 healthy grapes and 70 *B. cinerea* infected grapes were randomly divided into a 70% calibration group for quality assurance and feature reduction, and a 30% test group for prediction. A combined variable importance to projection and selectivity ratio plot was used for the assessment and selection of features and an independent experiment with high resolution qToF MS was conducted for the feature annotation.

Recommendations

This work investigated an innovative approach for rapid detection and prediction of *B. cinerea* infection in grapes with high sensitivity and reproducibility, that could potentially be suitable for non-destructive in-field detection prior to harvest. This analytical and data processing method can then be applied in other biological and environmental samples and would be beneficial for rapid quality assessment, disease control, and food security studies.

What's next?

Additional research could apply these methods of detection and quantification to a broader range of important commercial plants and horticultural produce susceptible to fungal pathogens. Rapid quantification of volatiles associated with fungal infection would enable real-time or at-sample monitoring, which could be an effective means of monitoring fresh produce in storage for the growth of fungal contaminants post harvest. Alternative GC-MS devices such as selected ion flow tube (SIFT) MS (Zhao et al., 2023), may also prove to be suitable for the detection of fungal biomarkers. Finally robust models of infection using homogenised samples and MS detection without a requirement for chromatographic separation during analysis demonstrates the potential for metabolic workflows to be utilised within an analytical context for rapid sample assessment.

Acknowledgements

We would like to acknowledge Dr QiuYu, Prof Leslie A Weston, Dr Paul A Weston, Dr Nhat Le-Minh and Prof Richard M Stuetz for laboratory arrangements and technical assistance. We would like to acknowledge the generous donation of grapes from commercial vineyards in the Riverina and Global Access Diagnostics, UK for generously providing the *B. cinerea* antigen test kit and strips.

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Australian Government
Australian Research Council

The ARC Training Centre for Innovative Wine Production is funded by the Australian Government (IC170100008) with additional support from Wine Australia and industry partners.

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