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BOOK OF ABSTRACTS

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Identification and functional characterisation of grapevine volatile organic compounds for the sustainable control of downy mildew

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Highlights

- Volatile Organic Compound (VOC) emissions differed between resistant and susceptible grapevine genotypes following *Plasmopara viticola* infection
- VOCs of resistant grapevines significantly inhibit downy mildew severity on leaf disk assays

Introduction

Grapevine (*Vitis vinifera*) is one of the most widely cultivated fruit crops and is susceptible to a large spectrum of pathogens, such as *Plasmopara viticola* that causes downy mildew (Gessler et al., 2011). Wild grapevine species are resistant to *P. viticola* and breeding programs have introduced resistance traits to susceptible cultivars. Plant defence responses are based on different mechanisms and volatile organic compounds (VOCs) play a crucial role in the communication between plants and other organisms. Although the emission of VOCs upon *P. viticola* inoculation was shown in resistant grapevine genotypes (Algarra Alarcon et al., 2015), the functional role of these molecules in the grapevine defence mechanisms was not yet investigated. The aim of this study was to identify and functionally characterise VOCs produced by resistant and susceptible grapevine genotypes in response to *P. viticola* in order to further develop innovative methods for the sustainable control of downy mildew.

Material and methods

The susceptible *V. vinifera* cultivar Pinot noir and four resistant genotypes (Kober 5BB, SO4, BC4 and Solaris) were grown for three months under greenhouse conditions. Plants were inoculated with a suspension of *P. viticola* sporangia as previously described (Perazzoli et al., 2012). Downy mildew severity was assessed at seven days after inoculation according to the OIV-452 descriptor and scores from 1 (the most susceptible) to 9 (the totally resistant) were assigned (Bellin et al., 2009). Leaf samples were collected before (T0) and six days (T1) after *P. viticola* inoculation and five replicates (plants) were analysed for each genotype at each time point and the experiment was carried out twice. Each sample was frozen in liquid nitrogen and ground to a fine powder. Leaf powder was weighed into 20 ml headspace vials and analysed by headspace solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME/GC-MS; Weingart et al., 2012). Eight identified

VOCs were selected according to their emission profiles and pure compounds were tested against *P. viticola* by leaf disks assays. Downy mildew development was assessed on leaf disks at one, two and six days post inoculation (dpi) by aniline blue staining.

Results and discussion

VOC profiles measured by HS-SPME/GC-MS analysis revealed a high reproducibility of the two experiments. Terpenes, isoprenoids, aldehydes, alcohols, esters and heterocyclic compounds were found in both experiments in all the five genotypes tested and their abundance was generally greater in resistant genotypes as compared with Pinot noir at T1. Differences in terms of VOC abundance were found in resistant genotypes at T1 as compared to T0, whereas small changes were found in Pinot noir VOCs between the two time points. The abundance of two sesquiterpenes was higher in all resistant genotypes as compared with Pinot noir at T1. Moreover, other three sesquiterpenes showed a higher abundance in three resistant genotypes (BC4, Kober 5BB and Solaris) as compared with Pinot noir at T1. Kober 5BB and Solaris showed also a higher abundance of one heterocyclic compound and one isoprenoid as compared with Pinot noir at T1. Finally, the abundance of a C5 aldehyde was higher in Kober 5BB as compared with Pinot noir at T1. These eight pure VOCs were tested against *P. viticola* in liquid suspension and in air volume. The eight VOCs impaired the development of downy mildew symptoms at dosages that ranged from 0.1 to 10.0 g/l in liquid suspension. However, five of them caused severe phytotoxic effects on leaf disks at the dosage of 10.0 g/l. Four pure VOCs (one isoprenoid, one alcohol, one C5 aldehyde and one heterocyclic compound) significantly reduced downy mildew symptoms at the dosage of 20.0 mg/l in air volume, when each VOC was applied to a filter paper disk and placed on the lid of the Petri dish.

Microscope observations with aniline blue staining revealed marked differences between control and VOC-treated leaf disks after *P. viticola* inoculation. The number of pathogen structures was reduced in leaf disks treated with one isoprenoid, one alcohol and one heterocyclic compound as compared to control disks at one, two and six dpi. Moreover, no *P. viticola* structures were visible on leaf disks treated with the C5 aldehyde. This aldehyde and one isoprenoid were also able to reduce the diameter of *P. viticola* sporangia.

In conclusion, downy mildew increased the production of VOCs (terpenes, isoprenoid, alcohols, aldehydes and heterocyclic compounds) in resistant and not in susceptible genotypes and these molecules are associated to the activation of grapevine defence mechanisms. Moreover, VOCs of resistant genotypes play a major role in the grapevine resistance and significantly reduced downy mildew symptoms on susceptible leaf disks, indicating that they can be further developed as sustainable control molecules.

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References

Algarra Alarcon A et al. 2015. Emission of volatile sesquiterpenes and monoterpenes in grapevine genotypes following *Plasmopara viticola* inoculation *in vitro*. Journal of Mass Spectrometry 50: 1013-1022.

Bellin D et al. 2009. Resistance traits to *Plasmopara viticola* in grapevine 'Bianca' is controlled by a major dominant gene causing localised necrosis at the infection site. Theoretical and Applied Genetics 120: 163-176.

Gessler C et al. 2011. *Plasmopara viticola*: a review of knowledge on downy mildew of grapevine and effective disease management. Phytopathologia Mediterranea 50: 3-44.

Perazzolli M et al. 2012. Downy mildew resistance induced by *Trichoderma harzianum* T39 in susceptible grapevines partially mimics transcriptional changes of resistant genotypes. BMC Genomics 13: 660.

Weingart G et al. 2012. Establishment and application of a metabolomics workflow for identification and profiling of volatiles from leaves of *Vitis vinifera* by HS-SPME-GC-MS. Phytochemical Analysis 23: 345-358.