

**PROJECT TITLE**

Functional characterisation of downy mildew control mechanisms mediated by volatile organic compounds in grapevine

**CONSORTIUM**

P 1	Michele Perazzolli		
P 2	Valentina Lazazzara		
P 3	Sara Avesani		
P 4	Giorgio Licciardello		

# SUMMARY OF THE REPORT

The project GrapeVOC (ID: 500) aimed at phenotyping the influence of three different volatile organic compounds (VOCs) on the susceptibility of grapevine (*Vitis vinifera*) cultivar Pinot noir to downy mildew (*Plasmopara viticola*). To this end, Pinot noir plants were transported to the access provider's laboratory. First, the downy mildew inoculation method was optimized in the access provider's laboratory. In August 2021 Dr. Valentina Lazazzara traveled to the access provider's lab to establish and optimize VOC fumigation experiments on whole grapevine plants. After all methods had been optimized, the main experiment was performed between September 20th and October 21st 2021. Two ExpoSCREEN chambers were used to cultivate plants under controlled but near to nature climate conditions (including PAR intensity and solar spectrum) that are typical for the situation in the vineyards in northern Italy during periods with occurrence of downy mildew infection (16 hours day length, temperature 28°C day, 25°C night, relative humidity 65% day, 85% night, PAR 700-800  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). The sub-chambers allowed to separate the VOC-treated plants from each other in a way that no communication between plants of different treatments *via* VOCs can occur. Six replicate experiments were conducted in order to achieve N=6 biologically independent replicate data sets for statistical analysis. In each experiment, 2 plants were fumigated with each of the three VOCs and one control treatment (plants fumigated with distilled water). After one day of treatment, one plant *per* treatment was inoculated with *Plasmopara viticola*, and the second plant was sprayed with distilled water (mock-inoculated). After VOC overnight exposure, plants were transferred in the ExpoSCREEN chambers to allow the infection to commence. Leaf samples were harvested for additional analyses which were conducted in the frame of the accompanying EPPN2020 experiment, DownyGrape (ID: 501), performed in SignalSCREEN. At 1 and 6 days post-inoculation, plants were phenotyped regarding their physiological traits such as capacity of photosystem II (Fv/Fm) using a miniPAM system (Heinz Walz GmbH, D), stomatal conductance with the leaf porometer (Decagon, US), and leaf epidermal pigment accumulation indices (Flavonoids, Antocyanins) with the Multiplex instrument (ForceA, F). Two leaves of each plant were additionally harvested for further transcriptomic and metabolomics analysis. Preliminary data revealed that exposure of Pinot Noir plants to VOCs reduced the susceptibility of the plants to *P. viticola*, and changed leaf physiological parameters. This is the first time, such beneficial effects of VOCs on grapevine downy mildew susceptibility was observed at the whole plant level.

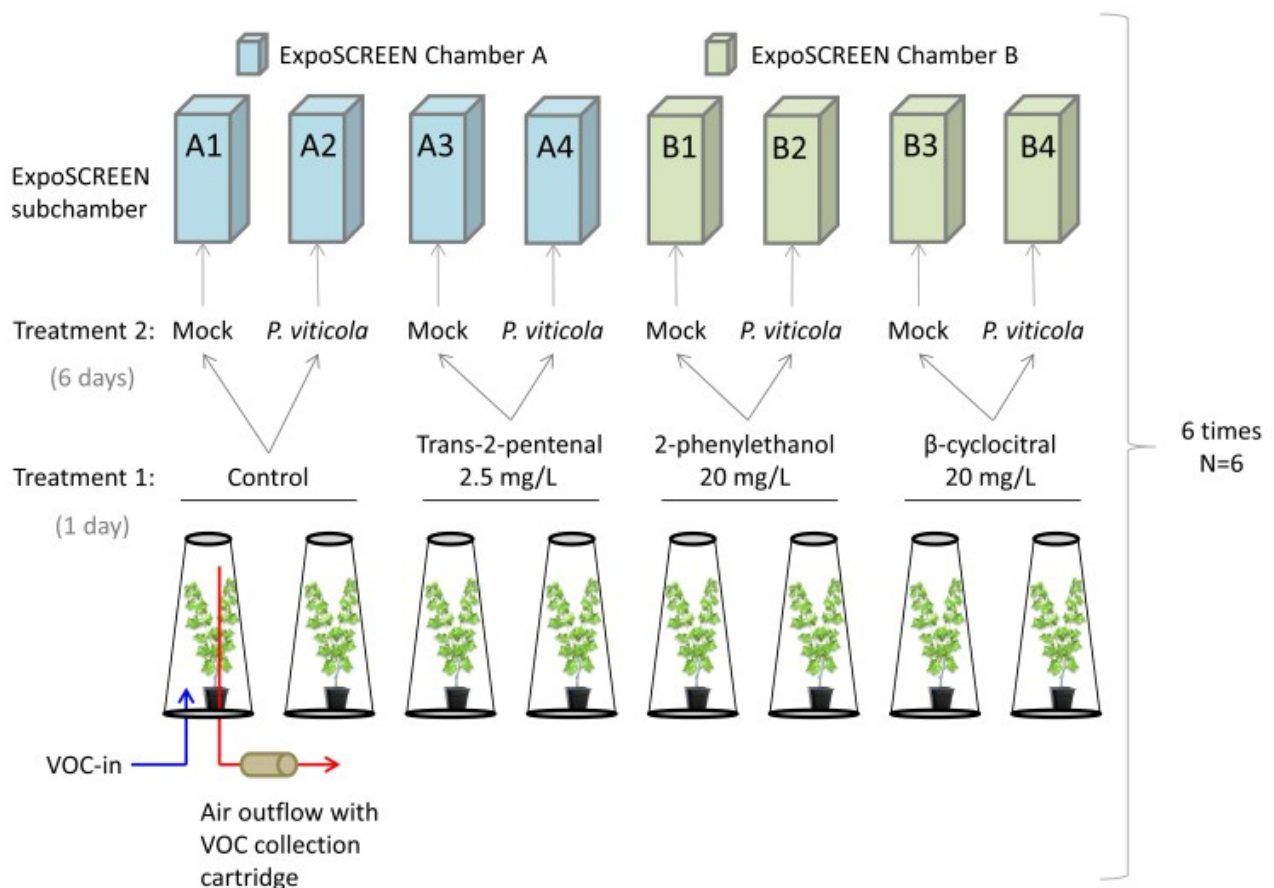


Figure 1. Overview of the experimental design.

Table 1. Timeline of the experiment.

<b>Time</b>	<b>Experiment phase</b>	<b>Installation</b>	<b>Treatment (Pathogen inoculation)</b>
7 days	Grapevine acclimation	ExpoSCREEN	-
1 day	VOC treatments (24 h of VOC application)	Glass cuvette 1	Control
		Glass cuvette 2	Control
		Glass cuvette 3	2.5 mg/L trans-2-pentenal
		Glass cuvette 4	2.5 mg/L trans-2-pentenal
		Glass cuvette 5	20 mg/L 2-phenylethanol
		Glass cuvette 6	2.5 mg/L trans-2-pentenal
		Glass cuvette 7	20 mg/L $\beta$ -cyclocitral
		Glass cuvette 8	20 mg/L $\beta$ -cyclocitral
1 day	Pathogen inoculation (overnight inoculation)	Glass cuvette 1	Control ( <i>P. viticola</i> )
		Glass cuvette 2	Control (Mock)
		Glass cuvette 3	2.5 mg/L trans-2-pentenal ( <i>P. viticola</i> )
		Glass cuvette 4	2.5 mg/L trans-2-pentenal (Mock)
		Glass cuvette 5	20 mg/L 2-phenylethanol ( <i>P. viticola</i> )
		Glass cuvette 6	2.5 mg/L trans-2-pentenal (Mock)
		Glass cuvette 7	20 mg/L $\beta$ -cyclocitral ( <i>P. viticola</i> )
		Glass cuvette 8	20 mg/L $\beta$ -cyclocitral (Mock)
6 days	Phenotyping	ExpoSCREEN	Sample collection at 1, 2 and 6 days after pathogen inoculation