## Pre- and post-harvest ozone fumigation of Sangiovese vines and grapes to reduce the use of chemicals in the wine production chain

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European Community imposed a considerable restriction of the use of pesticides establishing a regulatory framework for the sustainable use of agro-chemicals. Considering the huge use of pesticides used for wine production, the concerns regarding the environmental impact of viticulture have grown in the recent years. In addition to the environmental issues, consumers are increasingly oriented towards the purchase of safer food and beverage, which is pushing the wine sector to reduce the use of chemicals both in vineyards and wineries. Alternative protocols must be efficient, economically affordable and also harmless for the environment and human health. Our goal was to clarify the effects of different pre and postharvest ozone  $(O_2)$  application to reduce the use of chemicals in the wine production chain.  $O_2$ is already applied in the wine industry (as post-harvest applications) to produce sulphur dioxide-free wines. On the other hand, much less is known on the possible effects of O3 in controlling pathogens and/or inducing resistance in the field. Pre-harvest treatments have been performed spraying potted vines (Vitis vinifera cv Sangiovese) with gaseous O<sub>2</sub>. Different O<sub>2</sub> doses and lengths of exposition have been tested. The possible activation of the systemic acquired resistance (SAR) has been analysed through SAR-related gene-expression. Furthermore, plants structural changes induced by the treatment have been monitored with non- destructive approach (NIR). Post-harvest treatments have been carried out on bunches of wine grapes cv Sangiovese with gaseous O<sub>3</sub> overnight. The post-harvest trial was performed for three consecutive years (2017, 2018 and 2019). Technological parameters, free and glycoconjugate volatile organic compounds (VOCs) and gene expression analysis of selected genes involved in polyphenols and VOCs biosynthesis, have been analysed in grapes. Pre-harvest O<sub>3</sub> treatment on vines leaded to a transient up-regulation of SAR-related genes. NIR spectrum analysis revealed structural changes in O<sub>3</sub> treated plants, especially in leaves exposed to higher doses of O<sub>3</sub>. In harvested grapes, O<sub>3</sub> treatment increased polyphenols and flavonoids content. Consistently, key genes involved in phenols biosynthesis were up-regulated in treated bunches. Lastly, O<sub>2</sub> slightly affected aromatic profile, especially considering LOX-HPL related VOCs and increased the content of terpenes oxides, important compounds for the wine aromatic traits. Taken together, these data suggest that O<sub>3</sub> application, by stimulating the expression of SAR-related genes, could represent a promising substitute to the use of chemicals in viticulture. On the other hand, when applied in post-harvest O<sub>3</sub> induces limited changes in grape composition and wine aroma, and it can be safely used to avoid the use of sulphur dioxide, while preserving the final quality of the product.

Keywords: wine grapes, Vitis vinifera, VOCs, polyphenols, systemic acquired resistance.