

# Impact of the application of new biostimulants on the aromatic profile of Vermentino grapes (*V. Vinifera L.*)

Andriani Asproudi<sup>1\*</sup>, Federica Bonello<sup>1</sup>, Loretta Maria Panero<sup>1</sup>, Mauro Eugenio Maria D'Arcangelo<sup>2</sup>, and Maurizio Petrozziello<sup>1</sup>

<sup>1</sup>CREA – Council for Agricultural Research and Economics, Research Centre for Enology and Viticulture, Via P. Micca 35, 14100 Asti, Italy

<sup>2</sup>CREA – Council for Agricultural Research and Economics, Research Centre for Enology and Viticulture, Viale Santa Margherita, 80 - 52100 Arezzo, Italy

**Abstract.** Biostimulants in viticulture, represent a potential sustainable option against abiotic and biotic threats. Moreover, can trigger an innate immune response that leads to the synthesis of secondary metabolites, key compounds for the organoleptic properties of grapes and wines. In this study, the impact of foliar application of biostimulants, prepared with specific fractions of inactivated yeast, on the aromatic profile of Vermentino grapes grown in Tuscany was investigated. Two different products were compared, applied two or three times during veraison, in two years characterized by hot and dry summers. The qualitative and quantitative determination of the aromatic composition of the grapes was carried out using gas chromatography coupled with mass spectrometry (GC-MS). Generally, in treated plants, the sugar content was lower compared to the control. The treatments favoured the content of aromatic precursors in the grapes, particularly terpenoids; in the first year, when applied at three different times during veraison, and in the following year, when applied twice. The effect of biostimulants was positive but varied depending on the frequency of application, the vintage year conditions, and the chemical class of the compounds. Further research is needed to optimize the application of this kind of biostimulants to counteract stress conditions and maintain high grape quality.

## 1 Introduction

In a context of climate change and excessive use of agrochemicals, sustainable approaches in vineyard against abiotic and biotic threats are becoming imperative. The accumulation of sugars is no longer accompanied by optimal polyphenolic and aromatic maturation of the grapes; in fact, the gap between technological maturity (sugar/acid ratio) and aromatic and phenolic maturity is becoming increasingly wider.

Achieving well-balanced grapes at harvest is the primary agronomic and oenological goal for producing high-quality wines, nevertheless, has become an ongoing challenge for European grape growers due to rising temperatures and increasingly extreme drought or precipitation events [1]. In addition to EU policy pressure on European grape growers to use less pesticides and be more sustainable [2], consumers also demanding more sustainability in the production of agricultural products and therefore, from wines.

The use of biostimulants in viticulture represents a potential option, against abiotic and biotic threats [3]. Biostimulants are organic compounds, microbes, or a combination of both, that stimulate plant's vital processes, allowing high yields and good quality products. In vines, may also trigger an innate immune response leading to the synthesis of secondary

metabolites, key compounds for the organoleptic properties of grapes and wines. Yeast extracts, for instance, are known to induce secondary biosynthetic pathways because of plant defence responses stimulated by some yeast components, such as chitin, N-acetylglucosamine oligomers,  $\beta$ -glucan, glycopeptides, and ergosterol [4].

Most studies published nowadays on yeast extracts application in vineyard involved their impact on berry skin phenolic compound [5], whereas there is less knowledge on their effect on grape aroma volatile compounds [6, 7]. Recently a commercial formulation of inactive dry *Saccharomyces cerevisiae* yeast gave some excellent results on both polyphenolic and aroma compounds of some cultivars [7], leading us to further experiment on a white semi aromatic cv Vermentino, widely cultivated in Tuscany, Sardinia and other parts of Italy.

## 2 Materials and methods

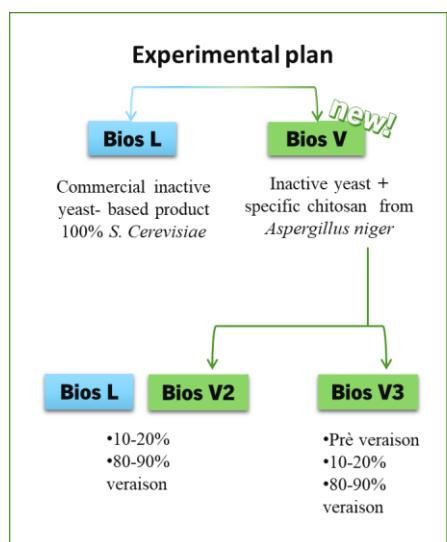
### 2.1 Vineyard sites and Experimental plan

The experiment involved a white semi aromatic cultivar named Vermentino widely cultivated in Tuscany (Italy). More precisely the vineyard was located at 246 m a.m.s.l

\* Corresponding author: [andriani.asproudi@crea.gov.it](mailto:andriani.asproudi@crea.gov.it)

in Cesa, Marciano della Chiana (AR) (43°18'21" N-11°47'15" E). The experimental plots were arranged following a randomized block design. The trials were conducted during two consecutive years 2021 and 2022, on 6 rows treated with two different products; no treated (NT) plants included. Bios L and Bios V, prepared with specific fractions of inactivated yeasts, were compared, and applied in different points during *veraison* with a foliar application protocol (Fig. 1). Bios L is a commercial inactive dry *Saccharomyces cerevisiae* yeast formulation (LalVigne® Aroma, Lallemand Inc., Montreal, Canada), already employed in viticulture with excellent results on other cultivars [7]. The second one (Bios V), a new product generated by a specific process, takes advantage of the synergy of action between an inactivated yeast, characterized by a high content of polysaccharides, mannoproteins, and glutathione, all readily available, and chitosans of fungal origin (*Aspergillus niger*).

Bios L and Bios V were homogeneously applied on the whole canopy of the treated vines, according to the patent foliar application technology WO/2014/024039, at a dose of 3 kg/ha for each treatment in a two time application in the case of Bios L, and at a dose of 0,8 kg/ha for each treatment in two (Bios V2) or three times (Bios V3) foliar application in the case of Bios V. Foliar applications were carried out thus at pre-veraison, 10-20% and 80-90 % of *veraison* according the experimental plan illustrated in Fig. 1. Three biological replicates of each treatment including the control (NT) were collected and analysed for berry composition and berry aroma precursors



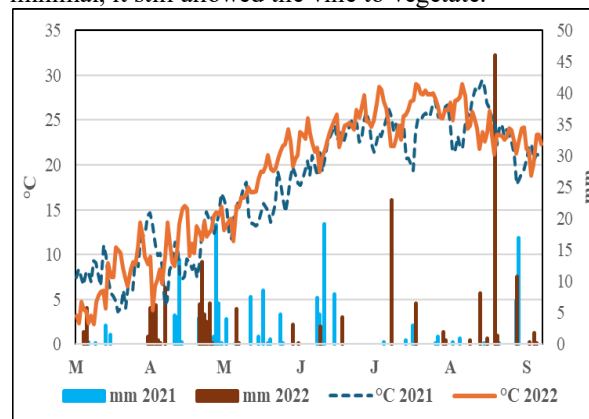
**Fig. 1.** Experimental plan of the treatments with two different products of inactivated yeast-based extracts

## 2.2 Climate and Meteorological assessments

2021 was characterized by low precipitation during spring, late spring frost and a hot and dry summer (Fig.2). The season was particularly unusual, with a long winter followed by a spring marked by sudden decrease of temperature (April 7-8 and 14). These frosts occurred

after the vines had budded, causing significant damage. The dry spring and dry summer brought forward the harvest date.

2022 was characterized by an extremely reduced water supply in the winter season (Fig. 2), through minimal, it still allowed the vine to vegetate.



**Fig. 2.** Average daily temperatures and fallen rainfall during March-September (years 2021-2022)

Moreover, 2022 was also characterized by a relatively warm and dry spring, which likely brought to a shift of the development stages of about a week earlier than in 2021.

Finally, low water availability and a hot and extremely dry summer brought at the interruption of the ripening in 2022. These stoppages are frequent at very high air temperatures, particularly when the soil water availability is already at very low levels [8].

## 2.3 Extraction and determination of aroma precursors from grapes

### 2.3.1 Extraction of glycosylated compounds from grapes

One hundred Vermentino berries, previously weighed and deseeded, were homogenized. The resulting suspension was centrifuged at 4000 rpm for 15 minutes, and the supernatant was transferred into a 300 mL volumetric flask. The residue was washed, and the combined extracts were brought to volume with tartaric acid buffer (pH 3.2).

The isolation of grape heterosides was carried out according to a previously reported method for grape and wine matrices [9,10]. Briefly, 250 mL of extract was passed through a 5 g C18(EC)-RP cartridge (Biotage AB, Uppsala, Sweden) After washing (water and dichloromethane) the glycosides were recovered with 25 mL of methanol (Sigma Aldrich Co., St. Louis, MO, USA).

### 2.3.2 Hydrolysis of glycosides by exogenous enzyme

The hydrolysis of glycosides by exogenous enzyme was carried as previously reported [9, 10]. Briefly the methanolic phase was evaporated to dryness under vacuum and then the enzymatic hydrolysis was carried out with Pectinol (Genencor, Palo Alto, CA, USA) with glycosidase side activity at 40 °C for 24 h. After hydrolysis, 0.25 ml of 2-octanol (50 mg/L), used as internal standard, was added and the hydrolysed extract passed through a 1 g C18 (EC)-RP cartridge previously activated to isolate the aglycons. The free compounds were eluted with dichloromethane, reduced to a small volume under a gentle nitrogen stream (about 200 µL) and stored at -20°C until the analysis by GC-MS.

### 2.3.3 Gas chromatography–mass spectrometry (GC–MS) determinations

GC-MS analysis was carried out by an Agilent 7890A gas chromatograph, equipped with an Agilent 5975C Mass Selective Detector (Agilent Technologies, Palo Alto, CA, USA). The samples (2 µL of concentrated dichloromethane extract) were manually injected into the injector at 250 °C in splitless mode. The separation was achieved using a Zebron ZB-WAX, Column 60 m x 0.25 mm x 0.25 µm (Phenomenex, Torrance, CA, USA).

GC-MS conditions, identification and quantification of volatile compounds are described and carried out according to Asproudi and coauthors [11]. Quantification was performed by comparing the areas of the chromatographic peaks with that of the internal standard (IS), 2-octanol, and compound concentrations were expressed as equivalents of the IS. Attention was paid to the composition of the grapes in terms of terpenoids, C13-norisoprenoids, and benzenoid compounds.

The results were expressed both as µg/kg of berries, as frequently reported in the literature, but also as ng/berry to avoid the influence of the different berry size on the result interpretation.

### 2.3.4 Statistical analysis

All statistical analyses were performed using XLSTAT – Applied Life Sciences (Addinsoft, Paris, France, 2017), a statistical software package for Microsoft Excel. Specifically, to evaluate the overall effects on the chemical classes of volatile compounds released by hydrolysis and quantified in Vermentino grapes, the data were subjected to three two-way analyses of variance (ANOVA). Differences among means were assessed using Tukey’s honestly significant difference (HSD) test at a 95% confidence level ( $P < 0.05$ ).

## 3 Discussion and Results

### 3.1 Grape composition

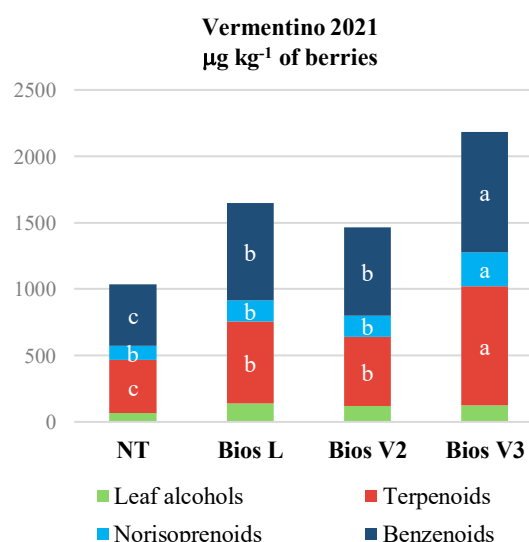
In both years the treatments, generally, did not significantly influenced yield parameters, pH and total acidity (data not shown). In the first year of study, a slightly lower sugar content was recorded in the treated grapes. NT and Bios V3 had values close to 23.4 degrees Brix, slightly higher compared to the Bios V2 trials (22.8 °Brix). For the Bios L grapes, the differences were significant showing at harvest lower sugar levels (22.0 °Brix).

In the second year, at harvest, no significant differences were found but tendentially lower sugars were registered in the grapes treated with Bios L and Bios V2 (22.0 °Brix).

Other previous research in white and red varieties has reveal small significance and not univocal tendency as regards the impact of yeast-based extracts application on technological chemical parameters [5, 6, 12]

### 3.2 Grape aromatic precursors

An important contribution of terpenoids to the total content of aromatic precursors in Vermentino grapes can be noted, which is why it is so called a “semi-aromatic” grape variety (Fig. 3). Among terpenoids the most representative compounds were geraniol, and cis-8-hydroxy linalool. The application of yeast-based extracts brought in significantly higher contents for almost all chemical classes of aromatic precursors in Vermentino grapes, especially in the case of the triple application of Bios V.



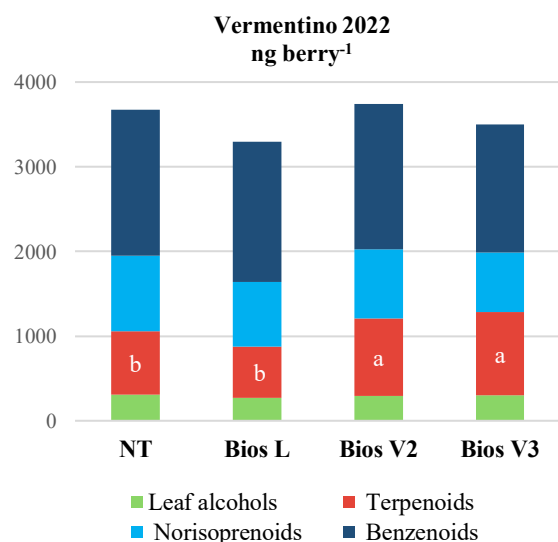
**Fig. 3.** Grape aroma precursors determined during 2021 in Vermentino vines not treated (NT) and treated with yeast-based extracts (Bios L, BiosV). Different letters indicate significant differences among treatments within the same aromatic class (same color) (ANOVA, Tukey’s test,  $p < 0.05$ ).

In the second year the effect of bio stimulants on aroma grape precursors was not as pronounced as in the first year (Fig. 4); some significant differences involved the Bios V2 and V3 treatments, only when results were expressed as ng berry<sup>-1</sup>. The Bios V applications favoured in this case the terpenoids contents in Vermentino grapes.

Although significantly higher amounts were found as concerns the aroma compounds in other cvs, where Bios L was applied in a three-year experiment [7], our results, in Vermentino grapes, confirmed these findings only in the first year and the new product Bios V applied three times gave better results.

A previous study on Sauvignon Blanc grapes added some information on the underpinning molecular mechanisms and revealed that dry yeast extract application (Bios L) may lead to the upregulation of genes encoding enzymes involved in the biosynthesis of berry aromas or of precursors [12]. Furthermore, it may reduce berry oxidative stress through the regulation of specific subsets of metabolic and hormonal pathways. An increased berry skin thickness values in treated grapes with dry yeast-based extracts was found too in literature [5].

The meteorological conditions verified during the second year stopped ripening and the harvest was anticipated in the warmest period, somehow these conditions flattened the differences between treatments. Further investigation is also necessary to assess the efficiency over the years of this type of product.



**Fig. 4.** Grape aroma precursors determined during 2022 in Vermentino vines not treated (NT) and treated with yeast-based extracts (Bios L, Bios V). Different letters indicate significant differences among treatments within the same aromatic class (same color) (ANOVA, Tukey's test,  $p < 0.05$ ).

## 4 Conclusions

Generally, yeast-based extract products didn't affect yield and technological parameters in Vermentino grapes.

In the first year of study all treatments triggered significantly higher contents for almost all chemical classes of aromatic precursors in Vermentino grapes, especially the three-time application of the new Bios V. In the second year the effect of yeast-based products on aroma grape precursors was not as pronounced as in the first year, some differences involved higher terpenoids in the Bios V treatments.

The way vines absorb and metabolize bio stimulants could change over time, potentially due to modifications in vine performance, soil conditions or intensity of extreme conditions and this may result in less pronounced effects in subsequent years. Further future investigation is necessary to optimize bio stimulant modality of application, to contrast stress conditions and improve grape quality.

Andriani Asprudi: Conceptualization, Methodology, Writing - Original Draft, Data Curation, Supervision.

Federica Bonello: Methodology, Data Curation, Supervision.

Loretta Maria Panero: Data curation, Supervision.

Mauro Eugenio Maria D'Arcangelo: Supervision, Original Draft.

Maurizio Petrozziello: Data Curation, Writing - Review & Editing.

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