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Role of foliar biostimulants (of plant origin) on grapevine adaptation to climate change

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Abstract. Heat waves and drought stress are typical aspects of current climate change, significantly affecting the grapevine physiology in many world growing areas. Biostimulants can play an important role in reducing the negative effects of climate change; that's why this experiment was set up in order to test two new foliar biostimulants (protein hydrolysates of plant origin). The field experiment was carried out in 2017 and 2018 in Oltrepo pavese area (Lombardia region, northwest Italy, 270 m asl), on a six-year-old vineyard of *V. vinifera* L. cv. Merlot clone 181 grafted on Gravesac, Guyot trellis, 4,000 vines/ha and not irrigated. Two new protein hydrolysates of plant origin were sprayed twice, just after fruit set and 15 days later, by using 2.5 L/ha. Leaf proteomics and metabolomics were studied in 2017, while productive and qualitative data were recorded in both years at harvest (September 1st, 2017 and August 28th 2018). The most significant findings were: (a) the treatments slowed down the grape ripening, by stimulating vegetative activity and reducing sugar accumulation; (b) less heat and drought stress symptoms were observed in the canopies of treated vines, as compared to the control ones.

1 Introduction

Climate change (increasing temperatures, irregular rainfall and extreme meteorological events, since the 80's of the last century) affects all human activities all over the world with mostly negative effects on agricultural and food systems and on health. Temperature rose also in the past due to natural phenomena, when humans just arrived or were not yet present on the earth, but nowadays the humankind is speeding up the climate warming. For instance, according to Schiermeier [1] the Eemian interglacial period (130,000 – 115,000 years ago) begun with a burst of climate warming (reaching +7°C over the mean of the past millennium) due to increases in summer sunshine, resulting from variation in Earth' orbit and axes of rotation. Other causes are related to increasing emission of greenhouse gases (CO_2, methane, halocarbons, tropospheric ozone) and black carbon. From 1860 on there is a positive correlation between the level of CO_2 in the air and its temperature [2]. If we go back 20 million years ago, tectonic degassing of carbon drove global temperature 10°C warmer than at present [3]. While studies on the causes and ways to mitigate global warming belong to climatologists, agronomist and especially grape growers have to rely on some tools, in order to minimize impacts such as too high grape sugar concentration, too low acidity, aggressive tannins, poor color, low terpenes and pyrazines aromas [4] Resilience should be the best way to cope with this aspect, that is breeding (proper rootstocks and scions), but as concerning abiotic stresses such as heat waves no genotypes are available so far. Biostimulants on the other hand are gaining interest as a way to manage the global warming negative effects. Biostimulants are compounds improving: (a) the efficient use of nutrients, (b) the tolerance to abiotic stresses, (c) the quality traits, (d) nutrient availability in the soil or in the rhizosphere. By a commercial point of view they are classified as follows: (a) humic substances; (b) derived protein hydrolysates; (c) seaweed extracts; (d) silicates; (e) mycorrhizal fungi; (f) N-fixer bacteria. In particular plant derived protein hydrolysates (PHs) improve plant functioning, increasing tissue growth and promoting tolerance toward abiotic stresses [5, 6].

The goal of this experiment is to test the efficacy of two new PHs on the vine physiology, grape production and quality under field conditions.

2 Material and methods

The trial was carried out in Casteggio (Pavia province, northwest Italy, 45° 01' N; 9° 08' E) inside the Oltrepò Pavese denomination area, during 2017 and 2018. The vineyard was located at 270 m asl, in a very gentle slope 2.5 x 1 m spacing (4,000 vines/ha). The grape variety was 6-year-old *V. vinifera* L. cv. Merlot clone 181 grafted on Gravesac. The soil was silty-loam, neutral, low organic matter (Table 1), while the meteorological conditions are reported in Table 2.

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Table 1. Physicochemical analysis of the soil.

Physical properties	
Sand (%)	6.6
Silt (%)	73.4
Clay (%)	20.0
Chemical properties	
pH	7.4
Total carbonates (%)	3.6
Active lime (%)	0.9
Organic matter (%)	0.9
Total N (%)	0.07
Available P (ppm)	8.0
CEC (meq/100g)	19.7
Exchang. CaO (ppm)	4238
Exchang. MgO (ppm)	751
Exchang. K_2O (ppm)	288
B (ppm)	0.05

Table 2. Climatic data and bioclimatic indices.

	2017	2018
Annual average mean T (°C)	14.4	14.2
Annual average maximum T (°C)	19.7	19.9
Annual average minimum T (°C)	9.3	9.6
Annual rainfall (mm)	426	509
Rainfall April-October (mm)	254	252
Heat summation* April-September (°C)	1985	2094
Heat summation* April-October (°C)	2143	2253
Selianinov Index**	1.18	1.12

*: ∑ daily average T°-10 °C; ** (∑ rainfall April-October / ∑ daily average T° – 10°C April-October) · 10.

The following treatments were compared:

- Untreated vines (control);
- Treatment with plant derived protein hydrolysates (PHs) Trainer (2.5 L/ha) just after fruit set (27 stage according to Eichhorn- Lorenz stages) and at beginning berry touch (33 stage according Eichhorn-Lorenz);
- Treatment with plant derived protein hydrolysated (PHs) Stimtide (2.5 L/ha) just after fruit set (27 stage according to Eichhorn- Lorenz stages) and at beginning berry touch (33 stage according Eichhorn-Lorenz).

In 2017 only Trainer was utilized, while in 2018 both products (Trainer and Stimtide) were used. Trainer is a mix of plant derived peptides and aminoacids (5% organic N) while Stimtide is a mix of plant derived peptides and aminoacids (7% organic N) + 2% urea + 1% soluble potassium (K_2O), all from Italpollina Hello Nature company (Italy); these protein hydrolysated are obtained from legume biomass according to a patented method (Lisiveg).

Only during 2017 leaf metabolomic and proteomic profiles were detected at the following times: (a) just before the first treatment – T0; (b) 2 days after the first treatment (June 18th, 2017 – T1); (c) 2 days after the second treatment (July 2nd, 2017 – T2). In both year the productive and qualitative parameters were recorded at harvest (September 1st, 2017; August 28th, 2018).

2.1 Leaf metabolomic and proteomic profile

Primary, mature, full exposed leaves were sampled to assess the leaf metabolomic and proteomic profile following biostimulant sprays. Untargeted metabolomic screening was performed via high-resolution mass spectrometry by using a hybrid Q-TOF spectrometer coupled to an UHPLC chromatographic system, as previously reported [7]. Briefly, samples were extracted in 0.1% HCOOH in 70% methanol and then MS acquisition was performed in positive mode, in the range 100–1200 m/z and compounds identification carried out using the software Agilent Profinder B.07, against the online database PlantCyc (pmn.plantcyc.org) and according to the whole isotopic patterns [8].

However, proteomic analysis was carried out according to Salehi et al. [9]. Proteins were extracted from skins using phenol, followed by reduction with DTT, alkylation with iodoacetamide and overnight digestion with trypsin. The following analysis of peptides was performed by data-dependent tandem mass spectrometry on a hybrid quadrupole-time-of-flight (Q-TOF) mass spectrometer coupled to a nano-LC Chip Cube source. Protein inference was done from MS/MS spectra, against the proteome of *V. vinifera* from www.uniprot.org, and using the Spectrum Mill MS Proteomics Workbench (one missing cleavage, false discovery rate 1%) as previously reported [10].

2.2 Productive and qualitative parameters of grapes at harvest

The following parameters were recorded: grape yield/vine (Kg); average cluster weight (g); bud fertility; average berry weight (g); soluble solids (° Brix); pH; titratable acidity (g/L); tartaric acid (g/L); malic acid (g/L); anthocyanins (g/L); polyphenols (g/L).

2.3 Statistical elaboration

Proteomic and metabolomic datasets were aligned, log $_2$ transformed, normalized at 75th percentile and baselined against the median [11]. Thereafter, unsupervised hierarchical cluster analysis was performed using the Euclidean distance (linkage rule: ward's) to describe relatedness of treatments and Volcano Plot analysis was carried out combining analysis of variance (P < 0.05, Bonferroni multiple testing correction) with fold-change analysis. For metabolomic mass and retention time alignment were done in Agilent Profinder B.06, where the following chemometrics and statistics were carried out in Agilent Mass Profiler Professional B.06.

Productive and qualitative data were analysed by a one-way ANOVA using Sigma-Stat 3.5 (Systat Software, San Jose, CA, USA). Means were separated by Student-Newman-Keuls (SNK) test.

3 Results and Discussion

3.1 Metabolomic (Fig. 1)

The data show a clear difference between the 2 sampling dates, while less evident is the difference between the control and the treatment. This means that at the first sampling (T1) the treatment had a significant effect on some metabolites, while at the second sampling (T2) the effect was lower. At the first sampling (T1) the treatment modified 67 compounds; some of those increased (two organic acids, some amides, some aromatic compounds, others), while some others decreased (some aromatic compounds, some fatty acids, many flavonoids, others). At the second sampling (T2) only 8 out of 97 compounds increased, while a lot decreased (especially flavonoids).

3.2 Proteomic (Fig. 2)

The data show a moderate effect of the treatment, since only 13 proteins have been modified and only in T1, while in T2 no modifications occurred. Some proteins increased (ATP synthase, superoxide dismutase, elongation factor Tu) having a positive role in vegetative growth, while the majority decreased (those related to the photosynthesis) most likely responsible for the low sugar accumulation in the grapes.

3.3 Productive and qualitative data at harvest (Tables 3 and 4)

During 2017, Trainer induced a higher yield and titratable acidity and lower sugar and anthocyanin levels than the control (Table 3); the other recorded parameters were not affected in a significant way by the treatment.

Table 3. Productive and qualitative parameters of cv. Merlot at harvest (September 1st, 2017), depending on the treatments.

	Control	Trainer
Grape yield (Kg/vine)	2.2 a	2.8 b
Average cluster wt (g)	82 a	87 a
Bud fertility (n)	2.0 a	2.1 a
Average berry wt (g)	0.9 a	0.9 a
Soluble solids (° Brix)	22.8 a	21.5 b
pH	3.33 a	3.26 a
Titratable acidity (g/L)	6.1 a	7.6 b
Tartaric acid (g/L)	11.5 a	12.4 a
Malic acid (g/L)	0.6 a	0.4 a
Anthocyanins (g/L)	1.40 a	1.19 b
Polyphenols (g/L)	3.62 a	3.31 a

Trainer: 2.5 L/ha at 16 June 2017 + 2.5 L/ha at 30 June 2017.

Table 4. Productive and qualitative parameters of cv. Merlot at harvest (August 28th, 2018), depending on the treatments.

	Control	Trainer	Stimtide
Grape yield (Kg/vine)	4.6 a	4.7 a	4.9 a
Average cluster wt (g)	148 a	146 a	150 a
Bud fertility	1.4 a	1.5 a	1.5 a
Average berry wt (g)	1.2 a	1.2 a	1.2 a
Soluble solids (° Brix)	21.4 a	20.3 a	20.1 a
pH	3.38 a	3.29 a	3.24 a
Titratable acidity (g/L)	6.5 a	6.4 a	6.8 a
Tartaric acid (g/L)	10.0 a	10.1 a	11.1 a
Malic acid (g/L)	0.5 a	0.6 a	0.7 a
Anthocyanins (g/L)	1.18 a	1.00 a	1.00 a
Polyphenols (g/L)	2.51 a	2.32 a	2.37 a

Trainer: 2.5 L/ha at 11 June 2018 + 2.5 L/ha at 25 June 2018.
Stimtide: 2.5 L/ha at 11 June 2018 + 2.5 L/ha at 25 June 2018.

During 2018, no significant effects of the treatments were observed, even though they reduced sugar levels and increased acidity.

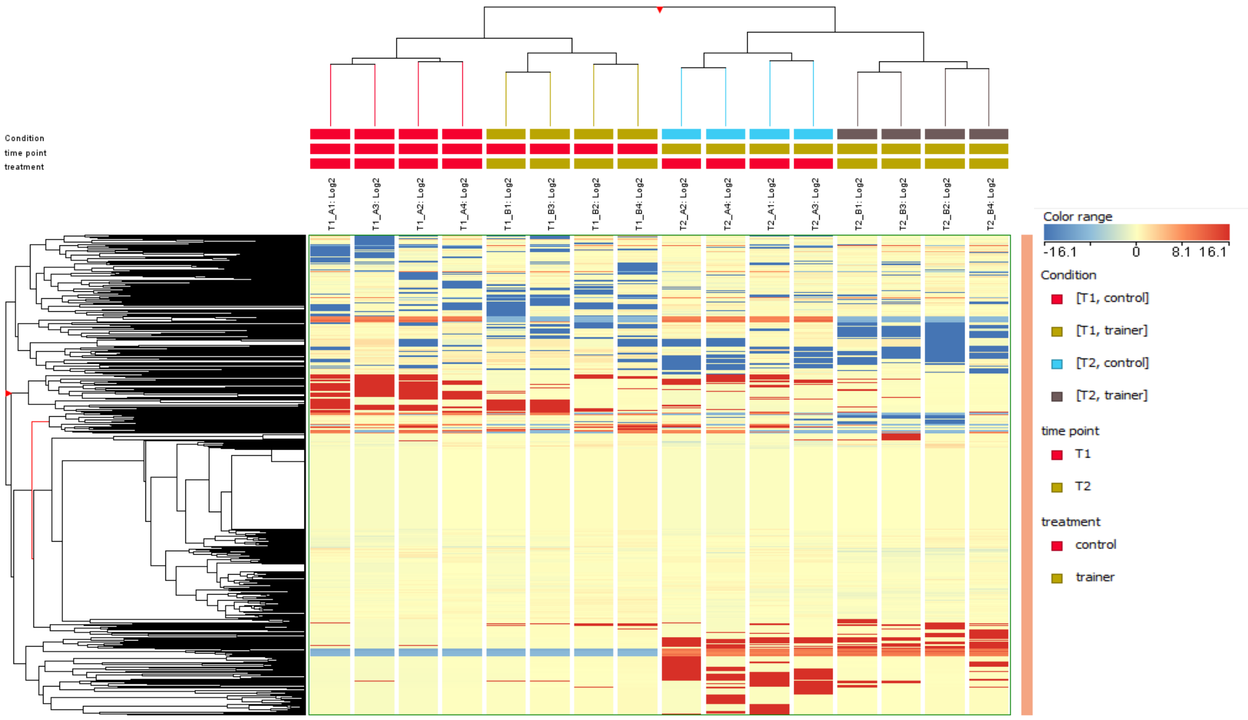

Figure 1. Unsupervised hierarchical cluster analysis of leaf metabolites from control vines and vines treated with Trainer for both the sampling dates. Clustering was carried out on control and treatment and both sampling times.

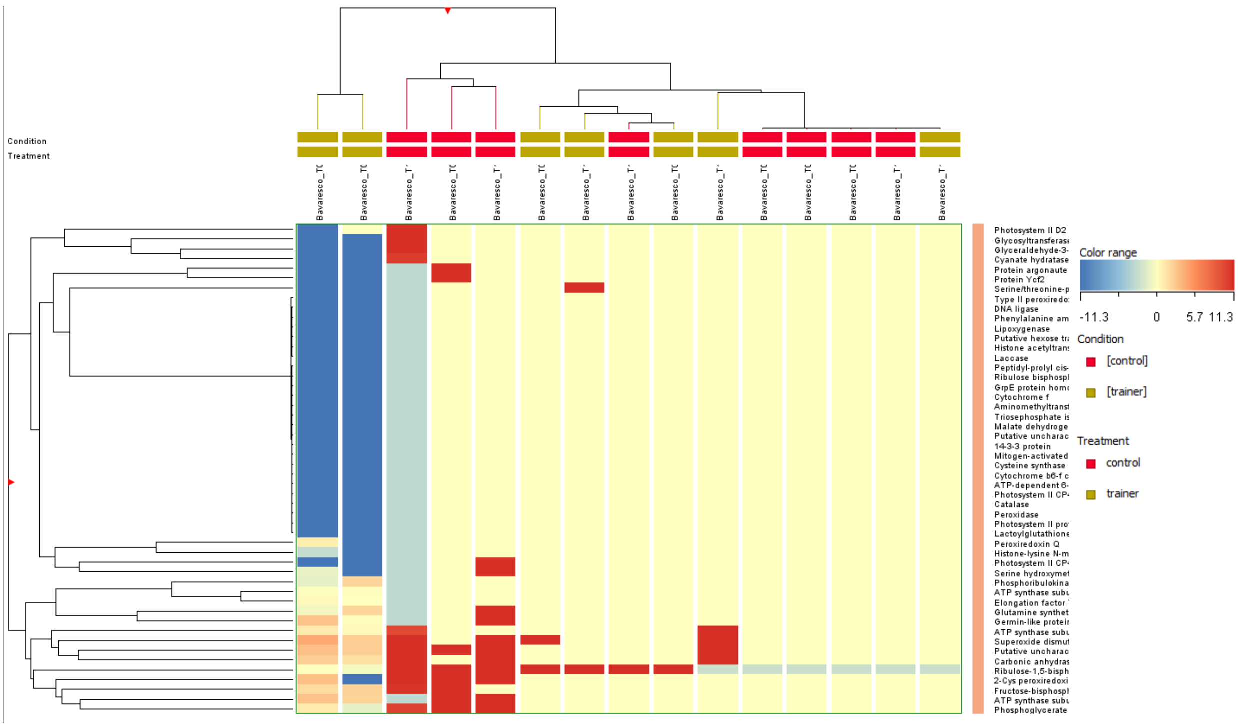

Figure 2. Unsupervised hierarchical cluster analysis of leaf proteins from control vines and vines treated with Trainer for both the sampling dates. Clustering was carried out on control and treatment and both sampling times.

4 Conclusions

The biostimulants modulated leaf metabolomic reducing the levels of the metabolites involved in the flavonoid pathway while leaf proteomics showed an enhancement of proteins responsible of the vegetative growth. The final result was a delay of grape ripening, with lower sugars and higher acids of grapes at harvest.

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