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Research article

Effects of *Ascophyllum nodosum* extract on *Vitis vinifera*: Consequences on plant physiology, grape quality and secondary metabolism

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ABSTRACT

Seaweed-based extracts have been recently employed as sustainable tools to improve abiotic stress tolerance and increase grape quality. However, the effect of these extracts on secondary metabolism compounds, that are fundamental for grape and wine quality, is still scarce. In the present study, the effects of foliar treatments with an *Ascophyllum nodosum* extract on physiological and biochemical parameters of *Vitis vinifera* (cv. Sangiovese) were investigated. We hypothesized an enhancement in the biosynthesis of secondary metabolites in berry skins and in leaves in response to these treatments, effective in improve grape quality and help vines to cope with abiotic stresses. Gas exchanges, chlorophyll fluorescence and midday stem water potential on leaves treated with *A. nodosum* extract and non-treated control leaves, were monitored over two growing seasons at three phenological stages: full *véraison*, mid maturation and full maturation. In addition, anthocyanins, flavonols and hydroxycinnamic acids were quantified both in berry skins and in leaves. The foliar treatments with *A. nodosum* increased photosynthesis and stomatal conductance in treated compared to control plants. Furthermore, extract-treated vines were able to maintain the potential efficiency of photosystem II close to the optimal value even during the hottest periods. No effect of *A. nodosum* extract treatments was observed on stem water potential. *A. nodosum* applications delayed berry ripening, leading to a lower sugar content and a higher anthocyanin content in treated berry skins. Interestingly, treatments also affected the content and the partitioning of secondary metabolites in berry skins, as anthocyanins and flavonols contents were higher in treated compared to control plants, while the ratio of methoxylated to non-methoxylated anthocyanins was lower in treated than in control vines. Furthermore, *A. nodosum* extract-treated plants also had higher content of flavonols and hydroxycinnamic acids both in berry skins and in leaves and showed a reduction in the biosynthesis of methoxylated anthocyanins, which are usually accumulated in grapes under environmental constraints. Considering the challenges posed by climate change in the Mediterranean basin, the use of seaweed extracts might represent a sustainable tool to mitigate the increasing severity of drought, often associated to heat-waves, on the viticulture sector.

1. Introduction

Climate change constitutes a serious challenge for both viticulture and wine making. These two key economic sectors will have to cope with increasing environmental stresses in the upcoming decades in many regions worldwide. Climate change can strongly affect the development of grapevine and grape quality (Jones and Webb, 2010; Fraga et al., 2012), because high temperatures, combined with high radiation intensities and water deficit, influence vine water status, inhibits photosynthesis, and promote photo-oxidative stress (Bertamini and Nedunchezhian, 2003; Cifre et al., 2005; Hernández et al., 2012; Das and Roychoudhury, 2014; Hossain et al., 2015; Lovisolo et al., 2016). Moreover, environmental consequences related to climate change will accelerate berry ripening processes, that, especially in red varieties, can result in unbalanced wines, with high alcoholic content and low polyphenolic contents (Mosedale et al., 2016).

The decisive role of phenolic compounds in red grape is due to their influence on important properties of berries and wines, such as flavor, color and stability against oxidation processes (Waterhouse, 2002; Mattivi et al., 2006; Silva and Queiroz, 2016). Anthocyanins and flavonols are among the most abundant polyphenol subclasses detected in grape berries (Adams, 2006). The more representative flavonols are

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glucosides, galactosides and glucuronides of quercetin, myricetin and kaempferol (Makris et al., 2006; Braidot et al., 2008). Each grape cultivar has a unique set of anthocyanins, which include delphinidin-, cyanidin-, petunidin-, peonidin- and malvidin-3-*O*-glucoside, eventually acylated as esters of acetic acid, caffeic acid or p-coumaric acid on the molecule of glucose (Boss et al., 1996; Mattivi et al., 2006; Koyama et al., 2012). It is commonly accepted that anthocyanin profile in grape berry is greatly dependent on genotype, but differences have been found as a result of environmental factors (Guidoni et al., 2002; Downey et al., 2004; Ortega-Regules et al., 2006). In particular, it is well known that water deficit from the onset of ripening until full maturation raises the content of anthocyanins in berry skin (Castellarin et al., 2007a; Zarrouk et al., 2012). In addition, both water deficit and radiation load consistently stimulate methoxylation of B-ring-substituted flavonoids (Castellarin et al., 2007b), incrementing the ratio of methoxylated to non-methoxylated anthocyanins, thus enhancing the stability of these compounds (Castellarin and Di Gaspero, 2007; Tarara et al., 2008). Zarrouk et al. (2016) have recently proposed that increasing anthocyanins stability through methoxylation may constitute an acclimation response to severe environmental constraints, such as the combined effect of drought and heat stress.

It is well known that agronomical practices, such as the use of fertilizers, greatly affect the biosynthesis of flavonoids (Downey et al., 2006; Teixeira et al., 2013). *Ascophyllum nodosum* L. is a brown seaweed, traditionally applied as biofertilizer in agriculture (Chouliaras et al., 2009; Jannin et al., 2013; Hernández-Herrera et al., 2014; Sabir et al., 2014; du Jardin, 2015). Seaweed extract products have been also recently employed as biostimulants to promote short-term plant acclimation to climate constraints, without adversely impacting on the environment (Craigie, 2011; Sharma et al., 2014; Tanou et al., 2017; Yakhin et al., 2017). *A. nodosum* extracts may stimulate numerous metabolic pathways, eliciting plant physiological and biochemical responses to abiotic stress and enhancing plant performance (Khan et al., 2009; Zhang and Ervin, 2008; Rayirath et al., 2009; Paradikovic et al., 2011; Nair et al., 2012). As an example, *Citrus* spp. plants exposed to treatments with *A. nodosum* commercial extract displayed higher drought tolerance (Spann and Little, 2011) by increasing photosynthesis and stomatal conductance (Little and Spann, 2010). Furthermore, recent evidences suggest that the beneficial effects of *A. nodosum* treatments on plant acclimation to stressful condition involve the activation of antioxidant enzymes and secondary metabolic pathways (Calvo et al., 2014; Santaniello et al., 2017), in particular the biosynthesis of flavonoids (Fan et al., 2011; Cai et al., 2012; Santaniello et al., 2017).

In grapevine, foliar application of seaweed extracts enhances root development (Mancuso et al., 2006; Mugnai et al., 2008), mineral nutrient uptake (Turan and Köse, 2004; Mancuso et al., 2006; Khan et al., 2012; Sabir et al., 2014) and growth (Mugnai et al., 2008; Khan et al., 2012; Popescu and Popescu, 2014; Sabir et al., 2014). There are numerous reports also on the positive effects of these extracts on yield and grape quality (Norrie et al., 2002; Colapietra and Alexander, 2006; Norrie and Keathley, 2006; Kok et al., 2010; Sabir et al., 2014), but their activity on secondary metabolism is scarcely investigated (Mancuso et al., 2006; Cai et al., 2012). Thus, we conducted an experiment with Sangiovese variety over two consecutive growing seasons, hypothesizing that treatments with seaweed extracts could promote an enhancement in the biosynthesis of secondary metabolites in grape berry skins and leaves, thus improving grape quality and helping vines to cope with abiotic stresses. The aims of our study were to 1) evaluate how foliar treatments with an *A. nodosum* extract affect physiological performances of *Vitis vinifera* L., and 2) investigate the biochemical adjustments induced in grape berry skins by these treatments under field conditions.

2. Materials and methods

2.1. Grapevine field conditions and experimental design

Experiments were conducted during the 2016 and 2017 growing-seasons in a commercial vineyard in the Chianti Classico area (Lat. 43.668°N, Long. 11.145°E), Tuscany, Italy, located at an elevation of 250 m a.s.l. facing South-West exposure. The climate is typically Mediterranean, characterized by rainy winters and dry, warm to hot summers. Soil horizons present a clay loam texture with the following average characteristics: clay 38.8%; silt 37.8%; sand 23.4%; organic matter 2.0%; pH (H_2O) 7.8.

The 18-year-old vineyard of the red cv. Sangiovese (*V. vinifera*), clonal selection R 24, grafted on 420 A rootstock, was planted with a spacing of 1.2 m × 3 m (~2778 vines/ha). Vines were trained on a vertical shoot positioning and spur-pruned single cordon system, at 80 cm above ground with a load of 12 buds per vine distributed over 6 spurs. Vines were rain fed-cultivated and grown using standard cultural practices as applied by commercial farmers.

The experimental plots arranged in a randomized complete block design, consisting in four blocks (3 rows each, approximately half a hectare) and one factor (*A. nodosum* extract foliar application). Seaweed extract-treated vines (SWE) were sprayed with 3 g of a non-commercial *A. nodosum* extract, diluted in 1 l of water (Santaniello et al., 2017; Frioni et al., 2018). The first application was performed one week after full *véraison* (23 August 2016; 7 August 2017; modified Eichorn and Lorenz (E-L) 36 stage) (Coombe, 1995) and the second one was repeated, on the same vines, after 15 days (6 September 2016; 21 August 2017; E-L 37 stage), according to Salvi et al. (2016). On the same days, untreated control vines (CTRL) were sprayed with the same amount of water.

From the central row of each block, 10 homogeneous vines, for both SWE and CTRL vines, were randomly tagged and used for eco-physiological measurements, yield and biochemical samplings, at three phenological stages: full *véraison* (100% of the berries presented full color change; pre-treatment; 16 August 2016; 31 July 2017; E-L 36 stage), mid maturation (one week after the first treatment; 30 August 2016; 14 August 2017; E-L 37 stage), and full maturation (one week after the second treatment; 13 September 2016; 28 August 2017; E-L 38 stage).

2.2. Chemical characterization of the A. nodosum extract

The *A. nodosum* extract was manufactured using a proprietary process at alkaline pH. Briefly, 25 g of dry *A. nodosum* biomass were treated with 1 N NaOH (100 ml) for 24 h at 150 rpm and 55 °C. After base treatment, the mixture was centrifuged at 8000 rpm for 10 min and the supernatant was stored at 4 °C. Then, the extract was characterized by quantifying proteins, carbohydrates, lipids, ashes, total phenolics, total nitrogen and total organic carbon contents, antioxidant capacity, macro-/micro-nutrients and amino acids. For the analyses of proteins, carbohydrates, lipids and ashes, the *A. nodosum* extract was dried at 80 °C for 5 days, and finely ground (< 1 mm), with a mortar.

Protein content was determined following Lowry et al. (1951); carbohydrates were determined following Dubois et al. (1956) and lipids following Marsh and Weinstein (1966). Ashes were analysed following ISTISAN protocols (ISTISAN Report, 1996/34).

The total phenolic content in the dried *A. nodosum* extract was determined according to Ganesan et al. (2008), using the Folin Ciocalteu assay. Results were expressed in gallic acid equivalents (mg GAE g^{-1}) of dry powder, using a calibration curve of authentic gallic acid (0–500 μg mL^{-1}) (Sigma Aldrich, Italy).

The DPPH (1,1-diphenyl-2-picrylhydrazyl) Radical-Scavenging assay was carried out according to Rajauria et al. (2013). Briefly, the assay was performed in cuvettes with 1:1 (v/v) ratio of 1000 μL of freshly prepared DPPH radical solution (165 mM) and 1000 μL of sample, solubilised in methanol. The reaction mixtures were incubated

for 30 min at 25 °C in dark condition, and absorbance measured at 517 nm.

Total organic carbon (TOC) was determined with a TOC analyzer (Shimadzu, USA). Macro- and micro-nutrients were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES) using a Perkin Elmer-Optima 2000 DV sequential multi-element instrument (Perkin Elmer, USA). Total nitrogen content was determined by the improved Kjeldahl method for nitrate-containing samples (AOAC, 1990).

Amino acid composition was determined as described by Mustafa et al. (2007). The analysis was performed using a protocol based on the EZ:faast kit (Phenomenex, USA) and the derivatized amino acids were quantified by gas chromatography/mass spectrometry (GC–MS) (Agilent Technologies, USA) using Ala, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val as external standards and I-norvaline (Sigma Aldrich, Italy) as internal standard.

2.3. Vineyard microclimate and eco-physiological traits

Monthly total precipitations (mm) and mean daily values of maximum and minimum air temperatures (°C) were collected by an automatic meteorological station (Ecotech, Germany) located close to the vineyard. Growing degree days (GDD) or Amerine and Winkler (1944) index was also calculated on a 10 °C base temperature. Briefly, GDD expresses the sum of all daily temperatures for the active growth in an area during the vine growing season between the 1st of April and the 31st of October. Net CO_2 assimilation rate (P_n, $\mu molm^{-2}s^{-1}$) and stomatal conductance (g_s, $mmolm^{-2}s^{-1}$) were measured with a Ciras 3 (PP Systems, USA) on 10 fully expanded leaves (one leaf from each tagged vine) from each block, for both SWE and CTRL vines. Measurements were performed between 10 and 12 a.m., setting the leaf chamber flow at ambient temperature, at ambient CO_2 concentration (400 $\mu mol\,mol^{-1}$) and at saturating photosynthetic photon flux of 1300 $\mu mol\,m^{-2}s^{-1}$ during the three phenological stages of full *véraison*, mid maturation and full maturation. Chlorophyll *a* fluorescence transients of dark-adapted leaves were recorded using a saturating flash of actinic light at 3000 $\mu molm^{-2}s^{-1}$ for 1s (Handy-PEA®, Hansatech Instruments, UK). Briefly, the maximum quantum yield of photosystem II (PSII) was calculated as $F_v/F_m = (F_m - F_0)/F_m$ where F_v is the variable fluorescence and F_m is the maximal fluorescence of dark-adapted (over a 30-min period) leaves (Maxwell and Johnson, 2000).

Midday stem water potential (Ψ_w, MPa) of dark-adapted leaves (over a 60-min period) was measured by a pressure chamber (PMS Instrument Co, USA) following Scholander et al. (1965). Chlorophyll *a* fluorescence and midday stem water potential were taken on the same leaves used for leaf gas exchanges measurements at the same three phenological stages.

2.4. Berry composition and productivity parameters

A 100-berry sample was collected randomly from the tagged vines of each block, of both SWE and CTRL vines, at full *véraison*, mid maturation and full maturation, taking care to remove berries from all the positions within the cluster. The berry sample was weighed with a digital scale (PCE Italia s.r.l, Italy) and immediately juiced. Soluble solids concentration (°Brix) was measured using a refractometer (ATAGO, USA); must pH was measured using a portable pH meter (Hanna instrument, USA) and titratable acidity (gL^{-1} tartaric acid) was determined on a 10 mL sample by manual glass burette using 0.1 M NaOH to an endpoint of pH 7.0. At full maturation, the tagged vines were harvested, and their production was weighed with a portable electronic scale (Bonso Advanced Technology Ltd., Hong Kong) to obtain yield per vine (kg) and cluster weight (g).

2.5. Phenylpropanoids in berry skin and in leaf

Berry phenylpropanoids content was measured on four replicates, each constituted by a pool of 30 berries (3 berries for each tagged vine), collected from the tagged vines of each block, of both SWE and CTRL vines, at full *véraison*, mid maturation and full maturation. Berry were immediately frozen in liquid nitrogen, then skins were removed and lyophilized (Lio-5P, Cik solution, Germany).

Lyophilized berry skins (0.7 g) were ground in a mortar under liquid nitrogen and the obtained powder was extracted with 75% of aqueous ethanol acidified to pH 2 by HCOOH (3 × 5 mL) and sonicated for 30 min. The supernatant was partitioned with 3 × 5 mL of *n*-hexane, the extracts were added together and reduced to dryness under vacuum, then, rinsed with MeOH/H_2O (50/50, pH 2). Aliquots of 5 µL were injected into a liquid chromatograph equipped with a quaternary 200Q/410 pump and an LC 200 diode array detector (DAD) (all from Perkin Elmer, USA). Anthocyanins, flavonols and hydroxycinnamic acids were separated in a 250 × 4.6 mm Agilent Zorbax SB-C18 (5 µm) column operating at 30 °C, at a flow rate of 0.6 ml min^{-1}, using a gradient solvent system consisting of H_2O (plus 5% HCOOH) (A), MeOH (plus 5% HCOOH) (B), CH_3CN (plus 5% HCOOH (C), during a 25 min run: 0–2 min 90% A, 5% B, 5% C; 2–7 min to 80% A, 10% B, 10% C; 7–12 min to 70% A, 15% B, 15% C; 12–17 min to 60% A, 20% B, 20% C; 17–20 min to 56% A, 22% B, 22% C; 20–24 min to 10% A, 45% B, 45% C; 24–25 min to 90% A, 5% B, 5% C. Quantification of anthocyanins was performed at 530 nm using calibration curves of cyanidin 3-*O*-glucoside, delphinidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, peonidin 3-*O*-glucoside and malvidin 3-*O*-glucoside (Extrasynthese, France). Quantification of flavonols and hydroxycinnamic acids were performed at 330 nm using the calibration curve of quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, myricetin 3-*O*-glucoside, *trans*-caftaric acid, *trans*- and *cis*-coutaric acid and kaempferol 3-*O*-glucoside (Extrasynthese, France). The quantification of anthocyanins was used to calculate the proportion of methoxylated/non-methoxylated derivatives and the percentage of methoxylated anthocyanins among 3-glucoside anthocyanins.

Leaf phenylpropanoids content was measured on four replicates, each constituted by a pool of 10 leaves (one leaf each tagged vine) from each block, of both SWE and CTRL vines, collected at full *véraison*, mid maturation and full maturation. The harvested leaves were immediately frozen in liquid nitrogen, stored at −80 °C and then lyophilized.

Lyophilized leaf samples (0.3 g) were ground in a mortar under liquid nitrogen and the obtained powder was extracted and quantified with the same procedure of phenylpropanoids in berry skin, as reported above. The gradient solvent system consisted of 90% H_2O (plus 1% HCOOH) + 10% CH_3CN/MeOH 25/75 (plus 1% HCOOH) (A), 10% H_2O (plus 1% HCOOH) + 90% CH_3CN/MeOH 25/75 (plus 1% HCOOH) (B), during a 40 min run: 0–3 min 90% A, 10% B; 3–33 min to 50% A, 50% B; 33–38 min to 10% A, 90% B; 38–40 min 90% A, 10% B.

2.6. Statistical analysis

Year (2016 and 2017), phenological stage (full *véraison*, mid maturation and full maturation) and treatment (SWE and CTRL) were considered as factors. A three-way ANOVA ($P \leq 0.05$) was used to compare SWE and CTRL in different years and phenological stages, and factors interactions.

Then, significant interactions among factors were investigated with one-way ANOVA ($P \leq 0.05$). Mean values were separated by Fisher's least significant difference (LSD) post-hoc test ($P \leq 0.05$). Where appropriate, prior to analysis, the original percentage data were transformed by arcsine function. All statistical analyses were performed using SPSS Statistic 25 (IBM, USA).

Table 1
Main macro/micronutrients and free amino acids quantified in the *A. nodosum* extract.

Macro and Micronutrients	Unit	Amount	Amino Acids	Unit	Amount
Total N	%	4.2	Alanine	ppm	12695.8
Organic N	%	4.1	Phenylalanine	ppm	7172.3
Total C	%	17	Proline	ppm	6561.1
Organic C	%	15.3	Methionine	ppm	3796.9
K	ppm	102.6	Glutamic acid	ppm	2679.2
P	ppm	31.8	Alloisoleucine	ppm	1869.8
Mn	ppm	2.87	Leucine	ppm	1489.8
Mg	ppm	0.4	Tryptophan	ppm	52.5

3. Results

3.1. Chemical properties of the A. nodosum extract

A. nodosum extract was composed by ~39.8% in proteins, ~8.0% in carbohydrates, ~2.1% in lipids and ~50.1% in ashes. The DPPH radical-scavenging capacity and total phenolic content were 82.5% ± 3.3 and 74.7 mg GAE g^{-1} of dry weight (DW) ± 5.0, respectively. The *A. nodosum* extract contains mainly organic nitrogen (~4%), organic carbon (~15%), potassium (~103 ppm), phosphorous (~32 ppm), sulfur (~6 ppm) and manganese (~3 ppm) (Table 1). The most abundant free amino acids in the *A. nodosum* extract were alanine, phenylalanine, proline and methionine.

3.2. Vineyard microclimate and eco-physiological traits

Vineyard microclimate conditions of 2016 and 2017 are reported in Fig. 1. Mean air temperature measured from bud break to leaf fall (April–October) was, on average, 1 °C higher in 2017 than in 2016. Whereas global radiation was ~15 Wm^{-2} higher in 2017 than in 2016. Annual cumulative precipitations were around 690 mm in 2016 and 492 mm in 2017. Accumulated heat expressed as GDD was lower in 2016 than in 2017 (1987 vs. 2137 GDD, respectively). The hottest and driest period of these two seasons was mid maturation 2017 (August), when minimum and maximum air temperatures were, respectively, ~1° and ~4° higher than in 2016, and precipitations were mostly absent.

To investigate the role of *A. nodosum* treatments on eco-physiological traits of vines, leaf gas exchanges (P_n and g_s), maximum efficiency of PSII photochemistry (F_v/F_m) and midday stem water potential (Ψ_w) were measured (Table 2 and Supplementary Table 1). The results of the three-way ANOVA show that gas exchanges and F_v/F_m were significantly affected by year, phenological stage and treatment whereas differences in Ψ_w depended mostly on phenological stage and year (Supplementary Table 1).

There were no significant differences in all physiological parameters observed at full *véraison* (pre-treatment stage) between SWE and CTRL plants (Table 2). In 2016, SWE and CTRL showed similar values of P_n and g_s at mid maturation. By contrast, higher P_n (+59%) and g_s (+57%) values were observed in SWE at full maturation compared to CTRL. The same trend was monitored in 2017, when SWE plants displayed significantly higher P_n and g_s than CTRL plants in the hottest period, at mid maturation (Table 2). Moreover, SWE vines were able to maintain significantly higher F_v/F_m than CTRL at mid maturation in 2016 and at full maturation in 2017; Ψ_w was not significantly affected by *A. nodosum* treatments.

3.3. Berry composition and productivity parameters

Year significantly affected ($P \leq 0.05$) berry weight, cluster weight and yield/vine, whereas *A. nodosum* treatments affected significantly only total sugar content (Supplementary Table 1). Berry weight was more than two times higher in 2016 (2.4 g) than in 2017 (1 g), and almost the same difference was also reported for cluster weight (362.3 g in 2016 and 151.5 g in 2017) and yield/vine (2.7 Kg in 2016 and 1.0 Kg in 2017).

The 2016 and 2017 values of total sugar and total acidity contents, pH and berry weight are presented in Table 3. Both in 2016 and in 2017, there were no significant differences in all parameters at full *véraison* (pre-treatment stage), whereas significant differences in total sugar content were observed at full maturation between SWE and CTRL berries. At full maturation SWE presented 1°Brix lower total sugar content as compared with CTRL. There were no statistical differences between the two treatments in yield/vine and cluster weight (data not shown).

3.4. Phenylpropanoids in berry skin and in leaf

Five anthocyanins (i. e. delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside and malvidin-3-*O*-glucoside) were identified in the chromatogram of the hydro alcoholic extracts of the berry skins (Fig. 2A).

Statistical differences related to year and treatment were recorded in all anthocyanin, whereas, on the contrary, their contents were not significantly affected by the phenological stage (Supplementary

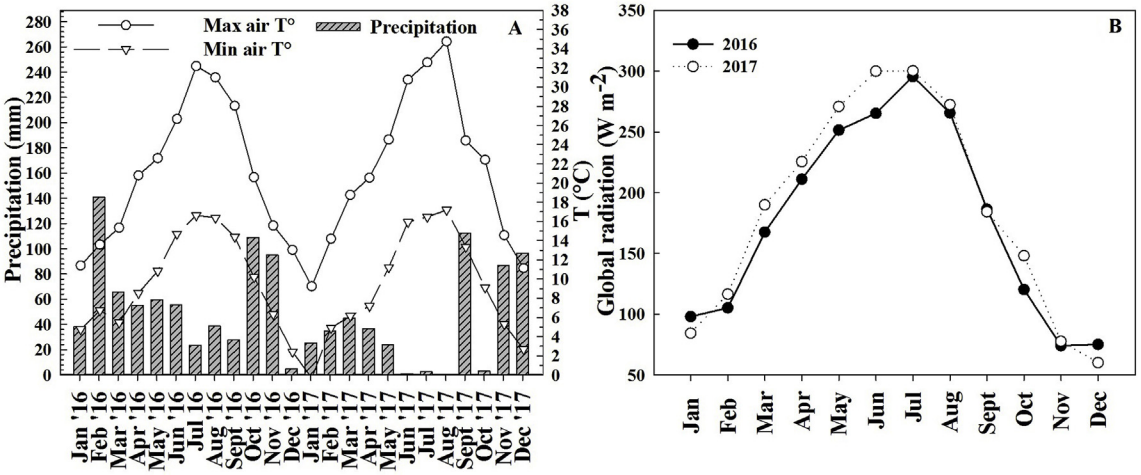

Fig. 1. Vineyard microclimate conditions: monthly total precipitation (mm) and mean daily values of maximum and minimum air temperature (°C) for 2016 and 2017 (A); monthly means of global radiation (W m^{-2}) in 2016 and 2017 (B).

Table 2
Net assimilation rate (P_n), stomatal conductance (g_s), maximum quantum yield of PSII (F_v/F_m) and midday stem water potential (Ψ_w) in leaves of *V. vinifera* treated with *A. nodosum* extract (SWE) and under control conditions (CTRL, untreated plants). Measurements were conducted at the stages of full *véraison* (Full *vérais.*), mid maturation (Mid matur.) and full maturation (Full matur.). Data (mean ± SE, n = 10) were subjected to one-way ANOVA. Different letters within the same parameter and row indicate significant differences among treatments (Fisher's LSD test, $P \leq 0.05$).

Year	Phenology	P_n (μmol $m^{-2}s^{-1}$)		g_s (mmol $m^{-2}s^{-1}$)		F_v/F_m		Ψ_w (MPa)	
		CTRL	SWE	CTRL	SWE	CTRL	SWE	CTRL	SWE
2016	Full *vérais.*	8.6 ± 1.2 a	10.4 ± 1.2 a	138 ± 20 a	179 ± 23 a	0.78 ± 0.01 a	0.79 ± 0.01 a	−1.11 ± 0.08 a	−1.11 ± 0.10 a
	Mid matur.	12.3 ± 0.7 a	11.9 ± 1.0 a	249 ± 18 a	264 ± 22 a	0.75 ± 0.01 b	0.79 ± 0.01 a	−1.27 ± 0.07 a	−1.15 ± 0.10 a
	Full matur.	6.9 ± 0.5 b	11.0 ± 0.6 a	205 ± 14 b	322 ± 11 a	0.79 ± 0.01 a	0.79 ± 0.01 a	−1.19 ± 0.08 a	−1.12 ± 0.10 a
2017	Full *vérais.*	15.0 ± 1.5 a	14.0 ± 1.5 a	166 ± 20 a	164 ± 20 a	0.77 ± 0.01 a	0.76 ± 0.01 a	−1.06 ± 0.03 a	−1.01 ± 0.04 a
	Mid matur.	6.6 ± 0.8 b	9.1 ± 0.7 a	46 ± 10 b	87 ± 8 a	0.76 ± 0.01 a	0.78 ± 0.01 a	−1.33 ± 0.02 a	−1.26 ± 0.08 a
	Full matur.	10.5 ± 1.2 b	13.9 ± 1.1 a	164 ± 10 a	179 ± 5 a	0.61 ± 0.08 b	0.77 ± 0.03 a	−1.17 ± 0.04 a	−1.08 ± 0.08 a

Table 2). Petunidin and peonidin were 80% and 36% lower in 2017 than in 2016, whereas the opposite was observed for delphinidin, cyanidin and malvidin, significantly more abundant in 2017 compared to 2016 (Supplementary Table 2).

Fig. 2B reports the chromatogram of the hydro alcoholic extracts recorded at 330 nm from SWE and CTRL berry skins. Different derivatives of hydroxycinnamic acids (*trans*-caftaric and *trans-/cis*-coutaric acid, ferulic acid derivatives) and flavonols (glucosides, galactosides and glucuronides derivatives of quercetin and kaempferol) were identified (Fig. 2B). Among flavonols, quercetin derivatives were the most represented. It is important to note that myricetin derivatives abundance was strongly and significantly affected by the year (Supplementary Table 2), indeed myricetin-3-*O*-glucoside was detected and quantified in 2017 but not in 2016.

Anthocyanins (Fig. 3) were not significantly affected by the *A. nodosum* extract treatments at full *véraison* (pre-treatment stage). In 2016, petunidin (Fig. 3B) and cyanidin (Fig. 3D) contents were higher in SWE than CTRL berry skins at mid maturation. Nonetheless, at full maturation, SWE berry skins showed significantly higher total anthocyanin contents than CTRL (Fig. 3F). In detail, at full maturation, the *A. nodosum* extract treatments increased the abundance of cyanidin (Fig. 3D) and peonidin (Fig. 3E) of about 129% and 167% respectively, and of delphinidin (Fig. 3A) and petunidin (Fig. 3B) of about 46% and 35%, respectively, compared to control. At the same stage, malvidin contents (Fig. 3C) remained almost unchanged between SWE and CTRL. In 2017, the contents of all anthocyanins, except malvidin, were higher in SWE than in CTRL, both at mid maturation and at full maturation (Fig. 3). At full maturation, the *A. nodosum* treatments raised the amounts of cyanidin (117%) (Fig. 3D), peonidin (107%) (Fig. 3E), delphinidin (67%) (Fig. 3A) and petunidin (73%) (Fig. 3B) in SWE compared to CTRL; on the contrary, malvidin (Fig. 3C) was 26% higher in CTRL than in SWE.

The methoxylated (peonidin, petunidin and malvidin) to non-methoxylated (cyanidin and delphinidin) anthocyanins ratio (Fig. 4A), and the percentage of methoxylated anthocyanins on total anthocyanins (Fig. 4B) were calculated in order to investigate how SWE treatments affected anthocyanin composition. There was significant year effect ($P \leq 0.05$) on both parameters (Supplementary Table 2). Irrespective of the treatment, in 2016, the percentage of methoxylated anthocyanins was about 19% less abundant respect to 2017, resulting in a 54% reduction of the methoxylated to non-methoxylated ratio in 2016 (1.6 μmol g^{-1}DW), than in 2017 (3.5 μmol g^{-1}DW). Both in 2016 and 2017, as a consequence of treatments with *A. nodosum*, the ratio of methoxylated to non-methoxylated anthocyanins was lower in SWE than in CTRL at full maturation (Fig. 4A). Similarly, the *A. nodosum* treatments induced a strong reduction in the percentage of methoxylated anthocyanins (Fig. 4B).

In 2016, hydroxycinnamic acids content was higher in CTRL than in SWE berry skins at mid maturation, whereas the opposite was found at full maturation (Fig. 5A). Quercetin derivatives content (Fig. 5B) decreased with berry development and was higher in SWE compared to CTRL berry skins at mid maturation and at full maturation. By contrast, kaempferol derivatives (Fig. 5C) remained almost unchanged among phenological stages and were only marginally affected by the *A. nodosum* treatments. In 2017, the contents of hydroxycinnamic acids and quercetin derivatives increased both in SWE and CTRL berry skins at mid maturation and reached the maximum levels at full maturation (Fig. 5). Hydroxycinnamic acids increased more than twice in SWE compared to CTRL berry skins at mid maturation and at full maturation (Fig. 5A), and the same trend was observed for quercetin derivatives at full maturation (Fig. 5B). Myricetin-3-*O*-glucoside was slightly higher in SWE (3.27 nmol g^{-1}DW) than in CTRL (2.39 nmol g^{-1}DW) (data not shown). The phenolic total contents (Fig. 5D) were higher in SWE than in CTRL berry skins at mid maturation and at full maturation, in both years.

The same hydroxycinnamic acids and flavonols contained in berry skins were also identified in leaves, with the exception of myricetin derivatives and anthocyanins that were not detected. Among flavonols, quercetin derivatives represented approximately the 80–85% of the total phenolic content in both years (Fig. 6). Year significantly affected ($P < 0.05$) quercetin and kaempferol derivatives contents

Table 3
Total sugars, titratable acidity, pH and berry weight in berry of *V. vinifera* treated with *A. nodosum* extract (SWE) and under control conditions (CTRL, untreated plants). Measurements were conducted at the stages of full *véraison* (Full *vérais.*), mid maturation (Mid matur.) and full maturation (Full matur.). Data (mean ± SE, n = 4) were subjected to one-way ANOVA. Different letters within the same year and phenological stage, indicate significant differences among treatments (Fisher's LSD test, $P \leq 0.05$).

Year	Phenology	Total sugars (°Brix)		Titratable acidity (gL^{-1})		pH		Berry weight (g)	
		CTRL	SWE	CTRL	SWE	CTRL	SWE	CTRL	SWE
2016	Full *vérais.*	20.3 ± 0.2 a	20.6 ± 0.2 a	6.7 ± 0.3 a	6.9 ± 0.2 a	3.08 ± 0.04 a	3.03 ± 0.03 a	2.1 ± 0.1 a	2.4 ± 0.2 a
	Mid matur.	23.8 ± 0.1 a	23.6 ± 0.4 a	5.5 ± 0.1 a	5.7 ± 0.1 a	3.16 ± 0.02 a	3.13 ± 0.01 a	2.5 ± 0.1 a	2.7 ± 0.1 a
	Full matur.	23.9 ± 0.1 a	22.9 ± 0.1 b	5.0 ± 0.1 a	5.2 ± 0.1 a	3.25 ± 0.03 a	3.25 ± 0.01 a	2.4 ± 0.1 a	2.5 ± 0.1 a
2017	Full *vérais.*	18.9 ± 0.2 a	18.6 ± 0.2 a	13.2 ± 0.2 a	13.5 ± 0.2 a	2.80 ± 0.01 a	2.82 ± 0.01 a	0.9 ± 0.0 a	0.9 ± 0.1 a
	Mid matur.	20.7 ± 0.5 a	19.6 ± 0.1 b	10.1 ± 0.2 a	9.7 ± 0.6 a	2.99 ± 0.02 a	3.00 ± 0.04 a	1.2 ± 0.2 a	1.1 ± 0.1 a
	Full matur.	25.3 ± 0.4 a	24.5 ± 0.3 b	7.4 ± 0.3 a	8.2 ± 0.6 a	2.98 ± 0.02 a	3.03 ± 0.04 a	1.0 ± 0.1 a	1.1 ± 0.1 a

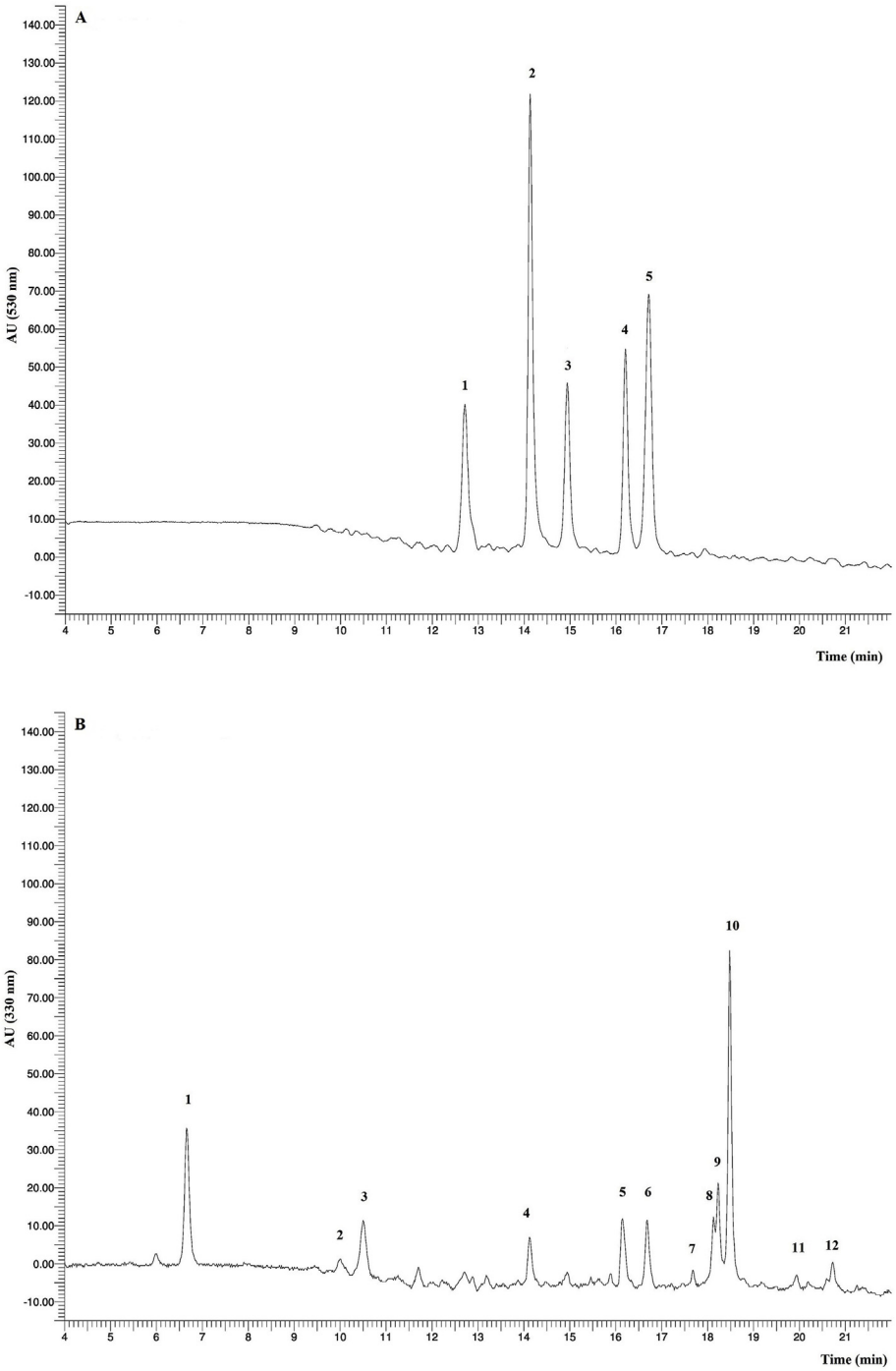

Fig. 2. HPLC-DAD chromatograms of hydroalcholic extract of CTRL berry skins, recorded at 530 nm for anthocyanins (A), and recorded at 330 nm for hydroxycinnamic acid and flavonols (B). Peak numbering in (A): (1) Delphinidin-3-*O*-glucoside; (2) Cyanidin-3-*O*-glucoside; (3) Petunidin-3-*O*-glucoside; (4) Peonidin-3-*O*-glucoside; (5) Malvidin-3-*O*-glucoside. Peak numbering in (B): (1) *trans*-caftaric acid; (2) *cis*-coutaric acid; (3) *trans*-coutaric acid; (4) Ferulic acid; (5), (6) Ferulic acid derivatives; (7) Myricetin-3-*O*-glucoside; (8) Quercetin-3-*O*-galactoside; (9) Quercetin-3-*O*-glucuronide; (10) Quercetin-3-*O*-glucoside; (11) Kaempferol-3-*O*-glucuronide; (12) Kaempferol-3-*O*-glucoside.

(Supplementary Table 2). Quercetin derivatives were significantly more abundant in 2017 (37.5 μmol g^{-1}DW) than in 2016 (33.7 μmol g^{-1}DW), while kaempferol derivatives were higher in 2016 (3.2 μmolg^{-1} DW), than in 2017 (2.2 μmol g^{-1}DW). Despite significant differences were not recorded at full *véraison* and mid maturation, *A. nodosum* treatments improved hydroxycinnamic acids accumulation in leaves (Fig. 6A), in both years at full maturation. Quercetin derivatives (Fig. 6B) were always higher in SWE than in CTRL, irrespective of the year and phenological stage. In particular, quercetin derivatives were +13% (2016) and +85% (2017) in SWE than in CTRL, at full maturation (Fig. 6B). As in berry skins, kaempferol derivatives (Fig. 6C), were not affected by *A. nodosum* in 2016, but significant differences occurred in 2017, with a relevant increase in SWE leaves at mid maturation (+90%) and at full maturation (+40%). Consequently, the total phenolic contents (Fig. 6D) were higher in SWE than in CTRL at full maturation, both in 2016 (+17%) and in 2017 (+43%).

4. Discussion

Climate change is speeding up the expected frequency and severity of drought periods often associated to heat-waves. The risks deriving from climate change include the impact on primary physiological processes, the anticipation of phenological stages and the uncoupling of technological and phenolic maturity in red grape cultivars, thus inducing a depletion in grape composition at full maturation (Jones and Webb, 2010; Palliotti et al., 2014). In this context, the use of biostimulants, such as seaweed extracts, might represent a sustainable tool to enhance plant physiological responses under severe stress conditions to

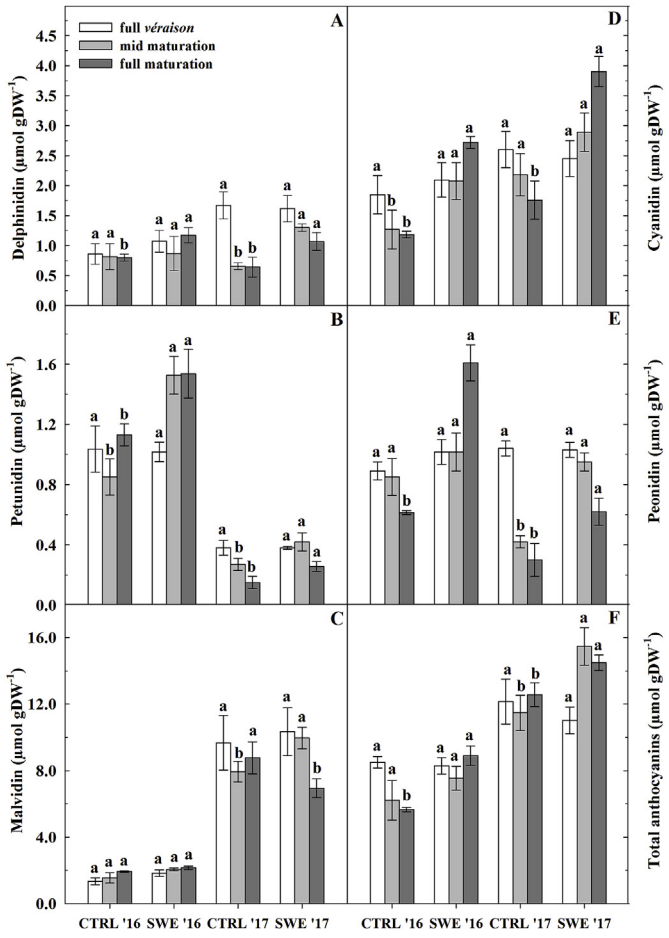

Fig. 3. Delphinidin-3-*O*-glucoside (A), Petunidin-3-*O*-glucoside (B), Malvidin-3-*O*-glucoside (C), Cyanidin-3-*O*-glucoside (D), Peonidin-3-*O*-glucoside (E), and Total anthocyanin content (F) in berry skins (µmol gDW^{-1}) of *V. vinifera* plants treated with *A. nodosum* extract (SWE) and untreated (CTRL) in 2016 and 2017. Analyses were conducted at the stages of full *véraison* (white bars), mid maturation (grey bars) and full maturation (dark grey bars). Data are means ± SE (n = 4). Different letters within the same year and phenological stage indicate significant differences between SWE and CTRL, assessed by one-way ANOVA (Fisher's LSD test, $P \leq 0.05$).

achieve an optimal ripening (Khan et al., 2009; Craigie, 2011). The positive activity of biostimulants on plant physiology, shown by an increasing number of studies (Khan et al., 2009; Fan et al., 2011; Sharma et al., 2014), is elicited by the presence of "bioactive" compounds, such as small peptides, organic constituents, phenolics. However, the mode of action of bio-stimulatory molecules contained in seaweed extracts remains still largely unknown. To our knowledge, this is the first report analyzing *A. nodosum* effects on eco-physiology and partitioning of secondary metabolites in *V. vinifera*.

4.1. *A. nodosum* extract improved leaf gas exchanges and protected photosystem II from photo-inhibition during microclimate limiting conditions

Stem water potential is commonly used as a main indicator of water stress in grapevine (Lovisolo et al., 2010), ranging from −1.0 to −1.2 MPa and from −1.2 to −1.5 MPa under moderate and severe water stress, respectively (Acevedo-Opazo et al., 2010). However, several studies have clearly shown that in field-grown grapevines g_s is more sensitive to severe water stress, than stem water potential (Dry and Loveys, 1999; Medrano et al., 2002; Flexas and Medrano, 2002; Cifre et al., 2005). In particular, mid-morning g_s is commonly accepted as an integrative parameter reflecting the intensity of water stress in grapevine (Chaves et al., 2010; Tombesi et al., 2015). In our study, we did not record significant differences in Ψ_w between SWE and CTRL vines, in both growing seasons (Table 2). By contrast g_s fell in CTRL vines below 50 mmol H_2O m^{-2} s^{-1}, that is considered the threshold indicating the occurrence of severe water stress in grapevine (Medrano et al., 2002; Cifre et al., 2005). *A. nodosum* treatments had a significant effect in reducing stomatal closure and increasing photosynthesis, even under severe water stress (mid maturation 2017, Table 2). Diffusional and non-diffusional factors interact to limit down-regulation of the photosynthetic systems (Lawlor and Cornic, 2002; Centritto et al., 2003) to avoid irreversible damage during periods characterized by intense temperature and high radiation load (Palliotti et al., 2009). In 2016 at full maturation and in 2017 at mid maturation, despite lower values of P_n and g_s in CTRL than SWE, untreated plants were able to maintain F_v/F_m values similar to treated vines and close to the optimum (~0.80) (Table 2). Hence, at these stages, diffusional limitations were likely the only limitation in photosynthesis (Lawlor and Cornic, 2002; Centritto et al., 2003) in untreated vines. On the contrary, in 2017 at full maturation, CTRL vines showed significantly lower F_v/F_m values than SWE. Therefore, at this phenological stage, initial signs of metabolic limitations to photosynthesis were observed.

The positive effects on plant physiological functions induced by the *A. nodosum* treatments have been previously reported also in *Citrus* spp. (Little and Spann, 2010; Spann and Little, 2011), and other species (Xu and Leskovar, 2015; Elansary et al., 2016). On the basis of the chemical characterization (Table 1), we hypothesize that the enhancement of physiological performances promoted by *A. nodosum* treatments may be related to the presence of amino acids and phenolics, which probably confers high DPPH Radical-Scavenging Capacity to the extract. In

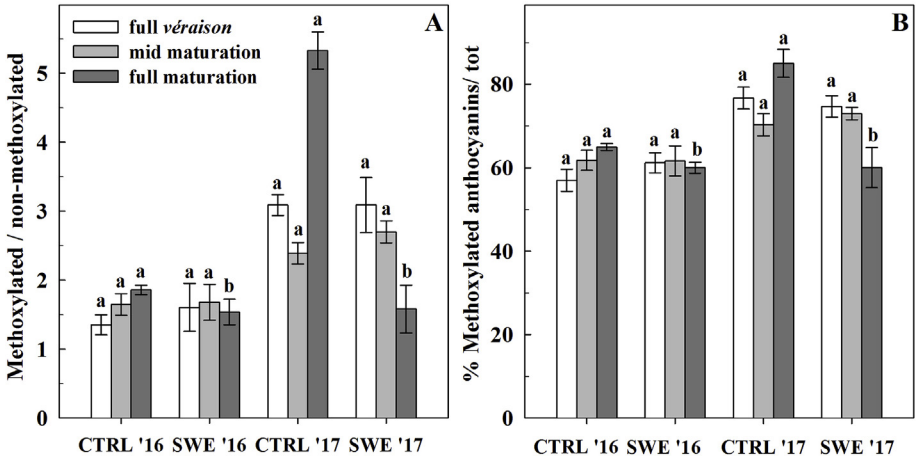

Fig. 4. Ratio of methoxylated to non-methoxylated anthocyanins (A) and percentage of methoxylated anthocyanins on the total amount of anthocyanins (B) in berry skins of *V. vinifera* plants treated with *A. nodosum* extract (SWE) and untreated (CTRL) in 2016 and 2017. Analyses were conducted at the stages of full *véraison* (white bars), mid maturation (grey bars) and full maturation (dark grey bars). Data are the mean ± SE (n = 4). Different letters within the same year and phenological stage, indicate significant differences between SWE and CTRL, assessed by one-way ANOVA (Fisher's LSD test, $P \leq 0.05$).

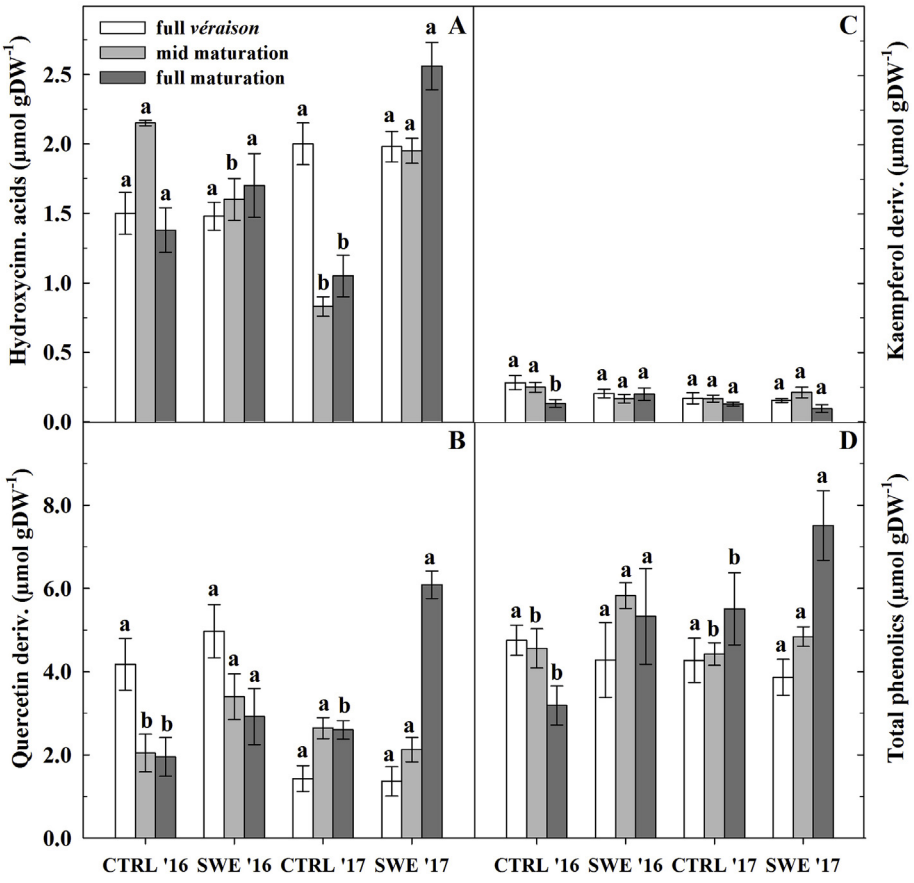

Fig. 5. Hydroxycinnamic acids (A), Quercetin derivatives (B), Kaempferol derivatives (C), and Total phenolic content (D) in berry skins (µmol gDW^{-1}) of *V. vinifera* plants treated with *A. nodosum* extract (SWE) and untreated (CTRL) in 2016 and 2017. Analyses were conducted at the stages of full *véraison* (white bars), mid maturation (grey bars) and full maturation (dark grey bars). Data are means ± SE (n = 4). Different letters within the same year and phenological stage, indicate significant differences between SWE and CTRL, assessed by one-way ANOVA (Fisher's LSD test, $P \leq 0.05$).

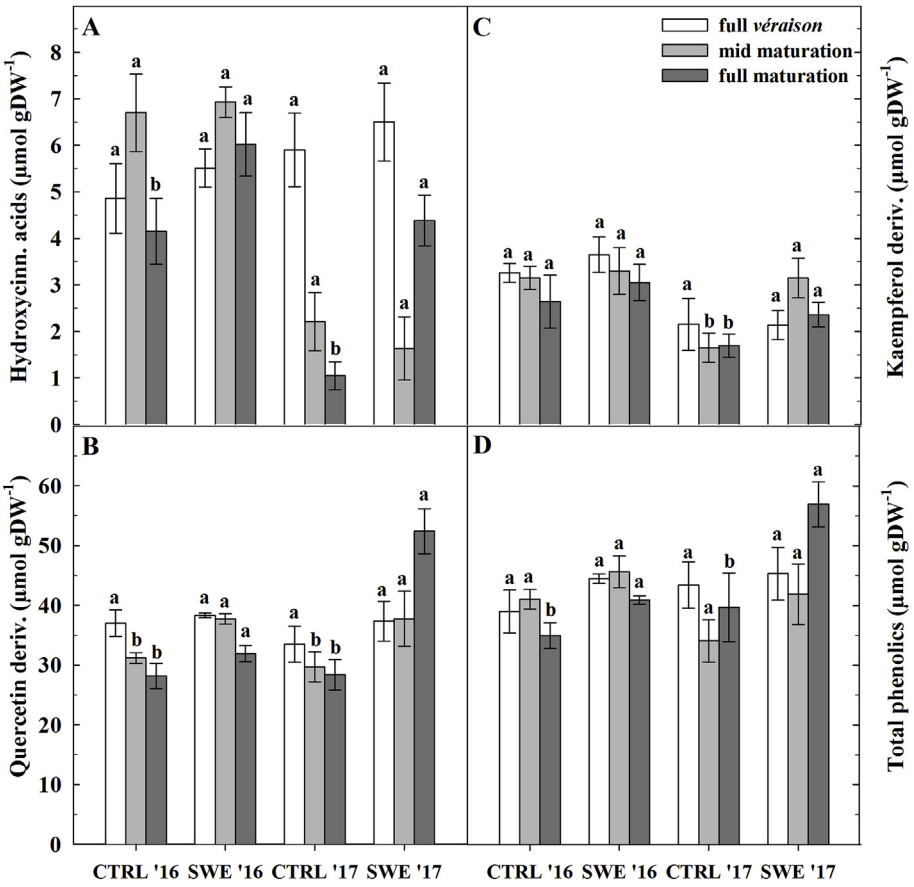

Fig. 6. Hydroxycinnamic acids (A), Quercetin derivatives (B), Kaempferol derivatives (C), and Total phenolic content (D) in leaves (µmol gDW^{-1}) of *V. vinifera* plants treated with *A. nodosum* extract (SWE) and untreated (CTRL) in 2016 and 2017. Analyses were conducted at the stages of full *véraison* (white bars), mid maturation (grey bars) and full maturation (dark grey bars). Data are means ± SE (n = 4). Different letters within the same year and phenological stage, indicate significant differences between SWE and CTRL, assessed by one-way ANOVA (Fisher's LSD test, $P \leq 0.05$).

particular, proline is involved in drought tolerance, offering protection against extreme osmotic stress (Ashraf and Foolad, 2007; Hayat et al., 2012; Sharma et al., 2014), acting both as cytoplasmic osmolyte and as ROS-scavenging compound (Matysik et al., 2002; Cuin and Shabala, 2007).

4.2. A. nodosum extract delayed grape ripening and elevated the balance of berry traits

In grapevine, temperature-driven effects on anthocyanin and sugar accumulations have been widely investigated (Bergqvist et al., 2001; Spayd et al., 2002; Mori et al., 2005; Yamane et al., 2006; Tarara et al., 2008; Sadras and Petrie, 2011), because of the pivotal importance of phenolic content for wine quality, especially in regions suitable for premium grape production (e.g. the Chianti Classico production area). However, over the last few decades, grape composition has been altered by global warming, sometimes resulting in poorly balanced wines characterized by an increment of berry sugar content uncoupled with anthocyanin accumulation (Jones et al., 2005; De Orduña, 2010; Sadras and Moran, 2012).

This was also observed in our study in 2017, when the 1 °C higher temperature respect to 2016, drove the acceleration of sugar accumulation, particularly in CTRL berries (Table 3). This effect was less evident in SWE vines, in which we observed a significant lower sugar content accompanied with higher total anthocyanin content (Fig. 3), compared to CTRL plants. This is partially in accordance with previous reports (Sabir et al., 2014; Frioni et al., 2018) and may supports the hypothesis that *A. nodosum* treatments could be involved in delaying ripening, leading to both lower sugar and higher anthocyanin contents in treated berries. However, these positive effects on berry quality were not paralleled by a concomitant increment in yield, probably because *A. nodosum* extract was sprayed in medium-late phenological stages, on berries/clusters fully developed in size (Sabir et al., 2014; Frioni et al., 2018). On the contrary, as previously reported in table grape, early biostimulant applications during berry cell division and enlargement could even induce an improvement in vine productivity and berry weight (Norrie et al., 2002; Norrie and Keathley, 2006; Khan et al., 2012).

4.3. A. nodosum extract affected the content and partitioning of anthocyanins in berry skin

The total amount of anthocyanins and the relative abundance of single anthocyanins are under genetic control (Ortega-Regules et al., 2006). However, the interaction between genotype and environment plays an important role in determining the berry qualitative traits such as the accumulation of distinctive anthocyanins of a given variety (Tarara et al., 2008). This is particularly evident in some grape cultivars, such as Sangiovese, which shows a broad phenotypic plasticity in response to environmental variables (Dal Santo et al., 2018).

In our study, a large seasonal variation of anthocyanin content in berry skins was observed in two consecutive years. The higher amount of total anthocyanin in 2017 compared to 2016 (Supplementary Table 2) may have been driven by higher global radiation and temperatures combined with lower precipitations (Castellarin et al., 2007a; Braidot et al., 2008; Koyama et al., 2012; Romboli et al., 2017) and/or be consistent with a passive accumulation due to water loss during berry dehydration (Moreno et al., 2008). The harsh climatic conditions of 2017 may have also caused a higher ratio of methoxylated to non-methoxylated anthocyanins in berry skins, than in 2016. Furthermore, low water availability, high solar radiations and temperatures promote the conversion of hydroxylated anthocyanins (cyanidin and delphinidin) into their methoxylated derivatives (peonidin, petunidin and malvidin) (Castellarin and Di Gaspero, 2007; Zarrouk et al., 2016). Methoxylation represents a metabolic process that increases anthocyanin stability since the methoxylated derivatives are less sensitive to enzymatic and non-enzymatic oxidation under stressful conditions (Jackman and Smith, 1996; He et al., 2010). A compelling evidence that *A. nodosum* mitigated stressful conditions, particularly in 2017, is the sharp decrease in the ratio of methoxylated to non-methoxylated anthocyanins in SWE than in CTRL (Fig. 4). In particular, in CTRL berry skins we observed a major flux of the anthocyanin pathway towards more stable malvidin-based derivatives (Azuma et al., 2012), partly compensating the reduction in anthocyanin biosynthesis when P_n resulted depressed by stomatal closure (Zarrouk et al., 2016). By contrast, SWE plants showed higher P_n and accumulated preferentially cyanidin-based anthocyanins in berry skins. We suggest that *A. nodosum* might promote the activity of flavonoid 3′-hydroxylases (F3′H) enzyme, catalyzing the biosynthesis of cyanidin-based anthocyanins, instead of activating the delphinidin parallel branch. This could be due to a direct effect of *A. nodosum* treatments on the activation of specific gene expression (Jayaraman et al., 2011; Santaniello et al., 2017) or to an indirect effect on the improvement of plant physiological responses following environmental constraints. We cannot exclude that this finding may be also related to the treatments protocol, since the first *A. nodosum* application was carried out one week after full *véraison*, when cyanidin and peonidin are the prevalent anthocyanins synthesized in grape berry skins (Downey et al., 2006).

4.4. A. nodosum extract promoted the biosynthesis and accumulation of phenolic compounds in leaf and berry skin

There is substantial literature on the importance of seaweed extracts in triggering the biosynthesis of phenolic compounds in many species, leading to high production of these secondary metabolites with multiple functions (Lakhdar et al., 2010; Kumari et al., 2011; Fan et al., 2011; Krajnc et al., 2012; Lola-Luz et al., 2013, 2014). In grapevine, hydroxycinnamic acids and flavonols constitute two major groups of UV-absorbing phenolic compounds (Kolb et al., 2003; Doshi et al., 2006) and are involved in the stabilization of the flavilium ion of anthocyanins in red wines (Boulton, 2001). In addition, similarly to anthocyanins, their accumulation in berry skins and leaves is strongly affected by environmental conditions of the vineyard (Azuma et al., 2012). In our experiment, non-anthocyanin phenolic compounds in berry skins and leaves were more abundant in 2017 than in 2016 (Figs. 5 and 6), probably because of the most stressful conditions registered in 2017 (Castellarin et al., 2007a) and of the dehydration effect mentioned in the previous paragraph.

The phenolic contents in berry skins and leaves were also affected by treatments with *A. nodosum* extract. In fact, at full maturation, SWE berry skins and leaves showed higher contents of hydroxycinnamic acids (Figs. 5A and 6A) and quercetin derivatives (Figs. 5B and 6B), compared to CTRL, whereas fewer variations were found in kaempferol derivatives (Figs. 5C and 6C). The higher content of antioxidant flavonoids, in particular of di-hydroxy B-ring-substituted flavonoids (i.e. quercetin derivatives), may have provided photo-protection to PSII and improved vines performances in SWE leaves, as demonstrated by higher F_v/F_m and P_n values, at full maturation in 2017 (Table 2). Moreover, in the berry, the biosynthesis of flavonols is closely related to that of anthocyanins, depending on the same enzymes for both classes of flavonoids (Mattivi et al., 2006): F3′H and F3′5′H (flavonoid 3′-5′-hydroxylases), are involved in the biosynthetic pathway of cyanidin- and delphinidin-based anthocyanins, respectively, as well as of quercetin and myricetin derivatives (Jeong et al., 2006). In our berry samples, quercetin derivatives (Fig. 5B) were the major flavonols, while traces of myricetin derivatives were only detected in 2017. These results may again suggest that *A. nodosum* treatments is selectively involved in the activation of F3′H enzyme, rather than F3′5′H, thus favoring the accumulation of di-hydroxy B-ring-substituted flavonoids rather that their tri-hydroxy counterparts.

5. Conclusions

Our data indicate that *A. nodosum* treatments during the last stages of berry development may enhance the performances of *V. vinifera* by improving eco-physiological parameters and affecting secondary metabolism, resulting in improved grape quality.

Applications of *A. nodosum* extract had significant effects on phenylpropanoid biosynthesis, both in berry skins and in leaves, and ameliorated leaf gas exchanges, maximum photosystem II efficiency and grape maturity balance. Furthermore, the biochemical analyses revealed that the *A. nodosum* extract likely acted selectively in the phenylpropanoid pathway, influencing anthocyanin partitioning and lowering the biosynthesis of methoxylated compounds. Overall, these results support previous findings on the beneficial effects of *A. nodosum* treatments on plant acclimation to stressful environmental conditions. Furthermore, for the first time in our knowledge, our study shows the potential effect of seaweed extracts in promoting higher tolerance to stress in vines. In order to understand the mechanisms of action of seaweed extracts, it is crucial to focus future research on the identification of seaweed chemical compounds with potential effect on treated plants phenylpropanoid pathway.

Author contributions

The work was conceived and planned by GBM, LS, and CB. LS and EC performed treatments with the *A. nodosum* extract, gas exchange measurements and collected leaf and berry samples. Data processing and analysis were carried out by LS and EC. LS and CB performed the HPLC analysis of phenylpropanoids. The *A. nodosum* extract was chemically characterized by AN. LS, CB and AN drafted the initial manuscript, which was critically revised by all authors.

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Appendix A. Supplementary data

Supplementary data to this chapter can be found online at https://doi.org/10.1016/j.plaphy.2019.03.002.

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