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Foliar *vs.* soil application of *Ascophyllum nodosum* extracts to improve grapevine water stress tolerance

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ABSTRACT

Biostimulants have recently been used in sustainable agriculture systems to improve plant growth and resilience to biotic and abiotic stress. In this study, foliar (ANEfl) and soil (ANEsl) *A. nodosum* extract applications were studied to elucidate the impact of different delivery methods on grapevines physiology either under well-watered conditions (WW) or under a water deficit period and a subsequent water recovery (WS). ANEfl increased leaf soluble sugars and photosynthesis of WW vines. Under progressive WS conditions, ANEfl positively impacted leaf gas exchange and water use efficiency (+35 % as compared to untreated vines) at Ψ_{stem} about -0.65 MPa. Photosynthesis was also improved during the re-watering period (+2.7 µmol $CO_2\,m^{-2}\,s^{-1}$) via preserved photochemical efficiency (Fv/Fm +0.19 as compared to untreated vines) and enhanced leaf anatomical and biochemical traits (+8% leaf dry matter and +27.3 mg/g DW of leaf soluble sugars). Contrarily, when soil applied, *A. nodosum* extracts did not significantly improve vine physiology during the reduced water supply period and only mild effects were detected at re-watering. Results demonstrates that foliar applications of *A. nodosum* extracts could be an alternative sustainable tool to improve grapevine physiological performances under mild-to-moderate water deficit and to preserve photosystems integrity and vineyard resilience when water limiting conditions get more severe.

1. Introduction

Viticulture is currently impacted by climate change. Many wine districts are recording drastic increases in air temperatures coupled with significant changes in rainfall distribution, especially during the growing season (Jones et al., 2005; Schultz, 2000). Under those scenarios, growers frequently face long periods of water shortage in their vineyards, negatively affecting yield and fruit composition (Palliotti et al., 2014; Poni et al., 2018). A main impact of reduced water availability on vine physiology is the decline of canopy assimilation and transpiration rates (Poni et al., 1994; Schultz, 1996; Flexas et al., 1998). In turn, vegetative and reproductive growth are hindered, as well as canopy thermoregulation by transpiration (Poni et al., 1994; Stevens et al., 1995; Girona et al., 2009; Flexas et al., 2010). When water limiting conditions become severe, leaves undergo non-reversible photoinhibition and yellowing, leading to leaf abscission (Palliotti et al., 2015). Due to warming trends, these phenomena and symptoms are common in vineyards where irrigation is not available or permitted. Therefore, grape growers are seeking new solutions to prevent water shortage, reduce water stress severity, and improve vine water use efficiency (Palliotti et al., 2014). For this reason, several long-term adaptation strategies, such as modified training systems, alternative cultivars and rootstocks have been recently proposed and currently tested (Palliotti et al., 2014). However, the short-term adaptations (i.e. flexible techniques timely applied during the season) are limited to soil and canopy management strategies or the application of foliar sprays (Palliotti et al., 2014).

Biostimulants are gaining interest for their ability to ameliorate biotic/abiotic stress tolerance, as well as for their use in sustainable crop management systems (Du Jardìn, 2015; Van Oosten et al., 2017; Rouphael and Colla, 2020). In particular, the extracts of the brown seaweed *Ascophyllum nodosum* L. LeJol (ANE) have recently been proposed as a promising tool to increase grapevine growth and productivity (Frioni et al., 2018, 2019; Salvi et al., 2019; Taskos et al., 2019;

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Gutiérrez-Gamboa et al., 2019, 2020). According to an extensive review by Shukla et al. (2019), ANE positively impact plant performance under water shortage via the modulation of plant hormonal signaling and the promotion of the biosynthesis of antioxidant and/or osmotically active compounds, such as flavonoids, carotenoids, betaines or carbohydrates. Several works have investigated the physiological effects exerted by ANE on different crops subjected to water deficit, none of which involved grapevines (Van Oosten et al., 2017; Santaniello et al., 2017; Shukla et al., 2019). To date, ANE have only been studied in grapes from the perspective of improving fruit ripening and technological maturity (Salvi et al., 2019; Frioni et al., 2018, 2019, Taskos et al., 2019; Gutiérrez-Gamboa et al., 2019, 2020). Thus, research evaluating the impact of ANE on water stressed grapevines is lacking in the literature.

Additionally, an aspect of debate regarding the use of ANE - and biostimulants in general- surrounds the most effective method of application (Paul et al., 2019; Basile et al., 2020). Several authors investigated the effects of foliar ANE application (Zhang and Ervin, 2004; Goñi et al., 2016, 2018, Frioni et al., 2018; Salvi et al., 2019; Gutiérrez-Gamboa et al., 2019), while others focused on the effects of ANE when incorporated in the soil or in the growing substrate (Rayorath et al., 2008; Wally et al., 2013; Martynenko et al., 2016; Santaniello et al., 2017). Nevertheless, most of cultivated crops, including wine grapes, allow for ANE application using either delivery method. To the best of our knowledge, only two published papers encompassed a comparison between foliar and soil application of ANE. Xu and Leskovar (2015) found that both foliar and soil ANE application reduced inhibition of stomatal conductance in spinach plants and promoted higher vegetative growth when compared to untreated control. Spann and Little (2011) showed that under water stress, leaf water potential and physiological performance of orange nursery trees were improved by ANE soil drench application. Contrarily, foliar sprays did not report a positive effect on leaf gas exchange but improved plant growth.

The objectives of the present work were to: 1) asses if ANE could improve grapevine physiological performance under a progressive water deficit and subsequent re-watering, and 2) elucidate whether foliar or soil ANE application is more effective at achieving 'objective 1′. Based on the available literature, our hypothesis was that ANE application could improve grapevine resilience to reduced water supply and that the efficiency could vary according to the delivery system.

2. Materials and methods

2.1. Plant material and treatments

The experiment was carried out in 2014 in the greenhouse facility of the Department of Horticulture at Michigan State University (42.7018 °N, 84.4822 °W) on 24 two-year old Pinot noir (*Vitis vinifera* L.) vines (clone 777 grafted onto C3309 rootstock) grown indoors in 30 l pots. Pots were filled with a mix of peat and sandy soil (20:80 by volume). All vines were fertilized at budburst with half-strength Hoagland nutrient solution (Hoagland and Arnon, 1950). During dormancy, vines were pruned to retain two spurs, each with four count-nodes. In May, vines were made uniform by retaining three of the most vigorous shoots per vine and removing all developing inflorescences. During vine growth, shoots were directed upright using stakes. Then, the 24 vines were randomly assigned to six treatments (four vines per treatment) in accordance to the water regime and the ANE treatment: well-watered control vines (WW-C), well-watered vines subjected to ANE foliar sprays (WW-ANEfl), well-watered vines subjected to ANE soil drench (WW-ANEsl), water-stressed control vines (WS-C), water-stressed vines subjected to ANE foliar sprays (WS-ANEfl) and water stressed vines subjected to ANE soil drench (WW-ANEsl). Before the beginning of the experiment, the greenhouse roof was painted in white in order to reduce radiation to a maximum of about 1400 PAR. Ambient relative humidity and air temperature were not conditioned during the experiment (air temperatures and PAR during water deficit imposition and recovery are provided in supplemental Table 1).

All vines were kept well-watered (WW) until the day of the year (DOY) 187 (5 July) through the manual supply of a daily quantity of 3 l in a unique irrigation at 8:00. Irrigation was withheld in all water stressed (WS) vines from DOY 188 until DOY 209 at 8:00, when all WS vines were re-watered and water supply was similar for all vines throughout the remainder of this season. ANE foliar (ANEfl) and soil drench (ANEsl) applications were applied at the same time. ANE treatments were repeated six times during the season, on DOY 155, 168, 174, 182, 187 before the WS imposition and DOY 209 the time of the re-watering application. The formulate used in the experiment was Acadian Marine Plant Extract Powder (Acadian Seaplants Limited, Dartmouth, NS, Canada), an alkaline extract of the brown seaweed *A. nodosum*, and application doses in both ANEfl and ANEsl followed the label doses of 3 g plant^{-1} after previous works on grapevine and other species (Santaniello et al., 2017; Frioni et al., 2019; Salvi et al., 2019). ANEfl was applied at 8:00 with a hand pump by diluting the seaweed extract in deionized water with the addition of a surfactant (Twin 20). ANEsl was applied at the same time of the day of the ANEfl by diluting the seaweed extract in the 3 l of water daily provided with irrigation. Pots were kept clean from weeds and standard pest management was carried out based on monitoring and expertise. No shoot trimming or hedging was executed.

2.2. Vegetative growth and tissues soluble solids and starch concentration

During the experiment, total length of each retained shoot was periodically measured. At the end of the experiment, leaf area was measured using a leaf area meter (LI−COR Portable Area Meter model LI-3000; LI−COR Environmental, Lincoln, NE). A sample of three median leaves per vine was washed and weighed before being frozen and stored at – 20 °C for the determination of leaf total carbohydrates concentration. Similarly, in the subsequent winter, at dormancy, a sample of 5 g of fine roots (diam. about 1.5 mm) per vine and three portions of one-year-old canes (internode between nodes 2 and 3) per vine were sampled and stored at -20 °C. The frozen samples were then used to determine dry matter (%) and soluble solids and starch concentration. Samples were lyophilized and weighed; specific leaf area (cm^2 g^{-1}) and dry matter (%) were then calculated. All samples were then ground to powder for analysis of soluble sugars and starch. 0.01 g of powder was placed in 15 mL tubes and mixed into a solution of 80 % ethanol and placed in a warm bath at 80 °C for 1 h. After 10 min of centrifugation at 10,000 rpm, 10 μl of supernatant was sampled and used for the determination of alcohol soluble sugars by the anthrone method (Loewus, 1952). For starch determination, pellet material was then washed with sodium acetate, buffer and then added with 0.5 mL of sodium acetate buffer. Tubes were placed in warm bath with temperature set at 80 °C for 1 h. One milliliter of solution of amyloglucosidase and α-amylase in 0.05 M sodium acetate buffer was added as described by Chow and Landhäusser (2004) and bath temperature was set at 50 °C. Sugar content was measured on the supernatant by the anthrone method as previously described Absorbance was read with a UV–vis spectrophotometer (Model UV-1800, Shimadzu Corporation, Kyoto, Japan) at values of 620 nm.

2.3. Stem water potential, leaf gas exchanges and photosystems efficiency

Stem water potential (Ψ_{stem}) was measured after Deloire et al. (2020) at midday on DOY 187 (the day before WS imposition), 194, 201, 207 (WS progression) and 209 (re-watering) on three vines per treatment on one mature leaf per vine that had been wrapped in plastic film and aluminium foil 2 h prior to the measurements using basis using a pressure chamber (Model 3005, Soil moisture, Corp. Sta. Barbara, CA, USA).

Leaf assimilation rates (leaf A), stomatal conductance (leaf g_s) and transpiration (leaf E) were measured from DOY 187 to 213 every two/four days on a mature well-exposed leaf per vine. Measures were taken at 13:00, at saturating light conditions (PAR > 1000 mmol photon m^{-2} s^{-1}) using a CIRAS-2 portable photosynthesis system (PP Systems Version 2.02; Amesbury, MA). Readings were taken on a leaf area of 2.5 cm^2 when steady state conditions in gas exchange were achieved (about 2 min). The CO_2 concentration (375 ppm) inside the leaf cuvette was controlled by the CIRAS-2. Leaf instantaneous water use efficiency (WUE) was calculated as the ratio of leaf A on Leaf E.

Chlorophyll fluorescence was quantified on one leaf per vine using a Handy PEA chlorophyll fluorimeter (Hansatec Instrument, United Kingdom). The initial fluorescence yield (Fo), the variable fluorescence (Fv), and the maximum fluorescence yield (Fm) were assessed in leaves that were dark-adapted for 45 min. The photosystem II quantum yield (Fv/Fm) was automatically calculated by the instrument.

2.4. Statistical analysis

Two-way analysis of variance (water supply, ANE application) was carried out and when the F-test was significant, mean separation was performed by the Student Newman Keuls (SNK) test at $P < 0.05$. Data taken over time for Ψ_{stem}, leaf A, leaf E, leaf gs, Fv/Fm, and WUE were analysed with the repeated measure analysis of variance routine embedded in the XLSTAT software package (Addinsoft, Paris, France). Least squared mean method at $p < 0.05$ was used for multiple comparisons within dates. Equality of variances of the differences between all possible pairs of within-subject conditions was assessed via Mauchly's sphericity test.

The correlations existing between variables were analysed by regression analysis, using SigmaPlot 11 (Systat Software Inc., San Jose, Ca, USA).

3. Results

3.1. Vegetative growth and tissue morphological and biochemical traits

Independently of the delivery method, multiple ANE applications did not affect the early stages of shoot growth (DOY 154–181, Fig. 1). Once irrigation was suspended (DOY 195–203), shoot growth of WS vines suddenly slowed down resulting in 320 cm of cumulated shoot length *vs* 354 cm recorded in WW vines, on DOY 203). However, vine growth in ANEsl and ANEfl treatments did not differ from respective C vines. Re-watering did not restore a rapid resumption of shoots growth rates, and at the end of the experiment (DOY 214) WS vines reported a lower cumulated shoot length when compared to WW. The reduction of shoot length in WS was not linked to the number of nodes per shoot at the end of the experiment, which was similar between WW and WS vines (Supplemental table 2).

Similarly, vine leaf area at the end of the experiment was unaffected by ANE treatments, while WS did impose a reduction in leaf area (Table 1). ANEfl significantly affected WS vines specific leaf area (81.21 cm^2 g^{-1}) when compared to WS-C and WS-ANEsl (91.13 cm^2 g^{-1} and 96.23 cm^2 g^{-1}, respectively). No difference was found between WS-ANEfl and WW vines (76.01 cm^2 g^{-1}, if pooled among WW treatments). ANEfl also promoted higher leaf dry matter percentage and leaf soluble sugars when compared to the respective C treatments. In particular, WW-ANEfl showed +5% leaf dry matter and +31 mg g^{-1} DW soluble sugars when compared to WW-C. WS-ANEfl reported an increase of +8% leaf dry matter and +27.3 mg g^{-1} DW of soluble sugars in relation to WS-C. Conversely, ANE application had no effects on cane and roots soluble sugars at the end of the experiment, nor on root starch concentration (Table 1).

3.2. ANE effects on stem water potential and leaf gas exchange

WW vines Ψ_{stem} ranged between -0.4 and -0.5 MPa during the experiment, with no effects ascribed to ANE application (Fig. 2a). WS caused a progressive reduction in Ψ_{stem} up to -1.57 MPa recorded on DOY 207 (mean of the three WS treatments), yet ANE did not cause any significant effect. At re-watering, all WS vines resumed Ψ_{stem} values comparable to WW vines. Considering only WW vines, a clear trend for higher leaf assimilation rates in ANEfl than any other treatment was discernible through the experiment, with significant differences between ANEfl and C on DOY 194, 201 and 204 (Fig. 2b). Similarly, in WS vines, ANEfl showed significantly higher leaf A than C on DOY 194 (+1.4 μmol CO_2 m^{-2} s^{-1}), with ANEsl reporting intermediate values. At increasing water deficit severity, ANE had no effects on the drastic decline of leaf A.

Upon re-watering, WS-ANEfl showed a prompter Leaf A resumption than any other WS treatment, with WS-ANEsl and WS-C behind by 1.2 μmol CO_2 m^{-2} s^{-1} and 2.7 μmol CO_2 m^{-2} s^{-1}, respectively. On DOY 212, four days past re-watering, all WS vines restored Leaf A rates comparable to WW vines. ANE applications did not affect WW vines leaf g_s (Fig. 2c). Under progressive WS no clear effects on g_s were related to ANE. However, at re-watering (DOY 209) WS-ANEfl and WS-ANEsl showed significantly higher g_s than WS-C (+110 mmol m^{-2} s^{-1} and

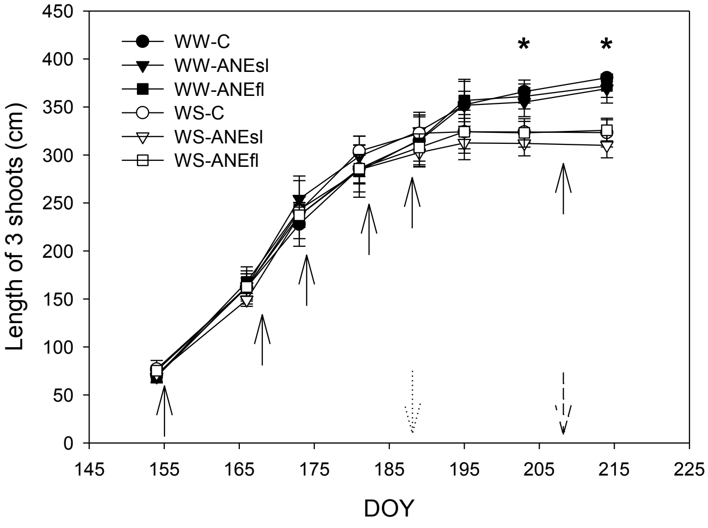

Fig. 1. Seasonal trend of vine total shoot length in response to *Ascophyllum nodosum* extract (ANE) application and different water regime. Vertical bars represent standard errors (n = 4). The solid arrows indicate ANE application dates. The dotted arrow indicates the imposition of water stress and the dashed arrow indicates re-watering. WW = well-watered, WS = water stressed, C = untreated controls, ANEfl = multiple ANE foliar sprays, ANEsl = multiple ANE soil drench.

Table 1

Effects of *Ascophyllum nodosum* foliar (ANEfl) and soil (ANEsl) application on leaf area and vegetative parameters of well-watered (WW) and water stressed (WS) vines (C = untreated control, DW = dry weight).

Treatment	Vine leaf area (m^2)	Specific leaf area (cm^2/g)	Leaf dry matter (%)	Leaf soluble sugars (mg/g DW)	Cane[a] soluble sugars (mg/g DW)	Roots[b] soluble sugars (mg/g DW)	Roots[b] starch (mg/g DW)
WW-C[d]	0.461 a[c]	74.34 bc	38 c	80.89 b	96.12	56.32	146.11
WW-ANEsl	0.466 a	72.45 bc	37 c	89.18 b	98.57	61.12	132.12
WW-ANEfl	0.409 a	81.24 b	42 b	111.83 a	95.11	58.32	129.99
WS-C	0.318 b	96.23 a	48 b	76.58 b	77.12	40.23	118.89
WS-ANEsl	0.302 b	91.13 a	52 ab	88.91 b	79.18	51.31	122.11
WS-ANEfl	0.269 b	81.21 b	56 a	103.88 a	81.33	50.12	103.32
W	***	**	**	ns	ns	ns	ns
ANE	ns	*	*	***	ns	ns	ns
W x ANE	ns	***	ns	ns	ns	ns	ns

[a] Internode between nodes.2–3.

[b] Fine roots about 2 mm diam.

[c] Different letters indicate significant difference per $P < 0.05$ (SNK test). ns = not significant; * $P < 0.05$, **$P < 0.01$, ***$P < 0.005$.

[d] WW = well-watered, WS = water stressed, C = untreated controls, ANEfl = multiple ANE foliar sprays, ANEsl = multiple ANE soil drench. W = water regime; ANE= *A. nodosum* extract application.

+63 mmol m^{-2} s^{-1}, respectively). Leaf E (Supplemental Fig. 1) followed a similar pattern to g_s.

The correlation between leaf A and Ψ_{stem} in the three WS treatments during the water deficit period (Fig. 3) shows higher leaf A values in WS-ANEfl at Ψ_{stem} comprised between -0.6 and -0.8 MPa. Moreover, there was a linear correlation between leaf A and leaf soluble sugars concentration for data pooled over the WS treatments on DOY 194 (leaf A_{194}) (Fig. 4).

Leaf WUE was unaffected by ANE application in absence of water deficit (Fig. 5). In WS vines, no difference in WUE was found until DOY 191. On DOY 194, when WS-C and WS-ANEsl still had a leaf WUE similar to WW vines (about 1.08 μmol CO_2 m^{-2} s^{-1} / mmol H_2O m^{-2} s^{-1}), WS-ANEfl reported a significant increase up to 1.46 μmol CO_2 m^{-2} s^{-1} / mmol H_2O m^{-2} s^{-1}). At increasing WS severity, no difference was found between WS-C and WS-ANEfl, even though on DOY 198 WS-ANEsl exhibited higher WUE than WS-C. Immediately after re-watering (DOY 209), WS-ANEfl showed the prompter leaf WUE resumption (0.81 μmol CO_2 m^{-2} s^{-1} / mmol H_2O m^{-2} s^{-1}) of all the treatments with WS-C reporting a slow response, behind WS-ANEfl and WS-ANEsl by 0.19 and 0.08 μmol CO_2 m^{-2} s^{-1} / mmol H_2O m^{-2} s^{-1}, respectively.

3.3. *Photo-chemical efficiency of PSII*

In WW vines, Fv/Fm did not vary during the experiment (Fig. 6). In all WS treatments, Fv/Fm remained steady until DOY 204, with values similar to WW. At the peak of WS (DOY 207) all WS treatments showed a sudden decrease of Fv/Fm, yet WS-ANEfl maintained significantly higher values than WS-ANEsl (+0.13) and WS-C (+0.19). At re-watering (DOY 209), the differences in Fv/Fm between WS-ANEfl and other WS treatments increased up to 0.23. Additionally, no correlation was found between Ψ_{stem} and leaf A at re-watering in WS treatments (Fig. 7a), yet a significant linear model was fit between leaf A of WS vines and the respective Fv/Fm values (Fig. 7b).

4. Discussion

Our data show that ANE foliar or drench application were substantially ineffective in boosting grapevine vegetative growth, either in absence or presence of WS (Fig. 1, Table 1). Research reports by Khan et al. (2009); Battacharyya et al. (2015) and Shukla et al. (2019) depicted a scenario where ANE application clearly contributed to herbaceous plant growth and leaf expansion. Conversely, in perennial species, including *V. vinifera*, reports are more controversial and most of the field experiments outlined minimal or no effects on shoot growth, leaf area or pruning weight (Spann and Little, 2011; Sabir et al., 2014; Frioni et al., 2018).

In WW vines, ANEsl had no effect on leaf gas exchange, whereas ANEfl slightly improved leaf A (Fig. 2b). Several authors associated positive effects of ANE to higher chlorophyll concentration, nutrient uptake and nutrient use efficiency (Khan et al., 2009; Battacharyya et al., 2015; Basile et al., 2020). However, in field grown grapevines and other tree crops, in absence of limiting conditions, ANE application did not cause clear effects on main physiological components (Spann and Little, 2011; Frioni et al., 2018; Salvi et al., 2019). In our work, the ANEfl higher leaf assimilation rates under WW conditions were associated to the higher leaf soluble sugars concentration (Table 1).

On DOY 194, in presence of mild-to-moderate WS (Deloire et al., 2020) (i.e. midday Ψ_{stem} comprised between -0.6 and -0.8 MPa), ANEfl maintained higher leaf A and improved WUE when compared to C vines (Figs. 2a,b,). These results are in partial agreement with those by Spann and Little (2011) on Swingle orange nursery trees, by Santaniello et al. (2017) on *Arabidopsis* and by Goñi et al. (2018) on tomato. However, positive effects of ANE on moderately water stressed grapevines were never observed before. In literature, just one paper investigated the effects of a seaweed extract on vines subjected to progressive water deficit, but the formulation was added of a foliar fertilizer and the raw seaweed specie processed to produce the biostimulant was not specified (Mancuso et al., 2006). The higher leaf A of WS-ANEfl as compared to other treatments was likely due to changes in leaf anatomical traits and was correlated to leaf soluble sugars concentration (Table 1 and Fig. 4).

Soluble sugars are the main contributors to grapevine leaf osmotic potential, together with inorganic ions (Patakas, 2000). Their concentration varies during the season and according to water status (Kliewer and Nassar, 1966; Rodrigues et al., 1993). Adjustments in leaf carbohydrates concentration are considered a plant strategy in order to regulate turgor pressure, to maintain positive assimilation rates under stress conditions and to prevent damages to photosystem II (Ackerson, 1981; Sánchez et al., 1998). In detail, the higher the leaf carbohydrates concentration, the higher the leaf relative water content and the assimilation rates at decreasing water potentials (Ackerson, 1981). Theoretically, the higher leaf carbohydrates concentration found in ANEfl was partially involved in the higher photosynthetic rates at Ψ_{stem} comprised between -0.6 and -0.8 MPa (Fig. 4).

At more severe water deficit, differences among WS treatments tended to shrink and all vines reached full stomatal closure on the same day (DOY 201) (Fig. 2b and c). This is in agreement with Spann and Little (2011), who found no difference in physiological performance of orange nursery trees at Ψ_{stem} of about -1.6 MPa, after ANE foliar application. On the other hand, on DOY 207, at peaking water deficit severity, ANEfl exhibited a significantly higher Fv/Fm value than WS-C (Fig. 6). An improvement of Fv/Fm by ANE was observed in *Arabidopsis* (Santaniello et al., 2017), creeping bentgrass (Zhang et al., 2003) and also in field-grown grapevines (Salvi et al., 2019) but never with the magnitude reported in this work.

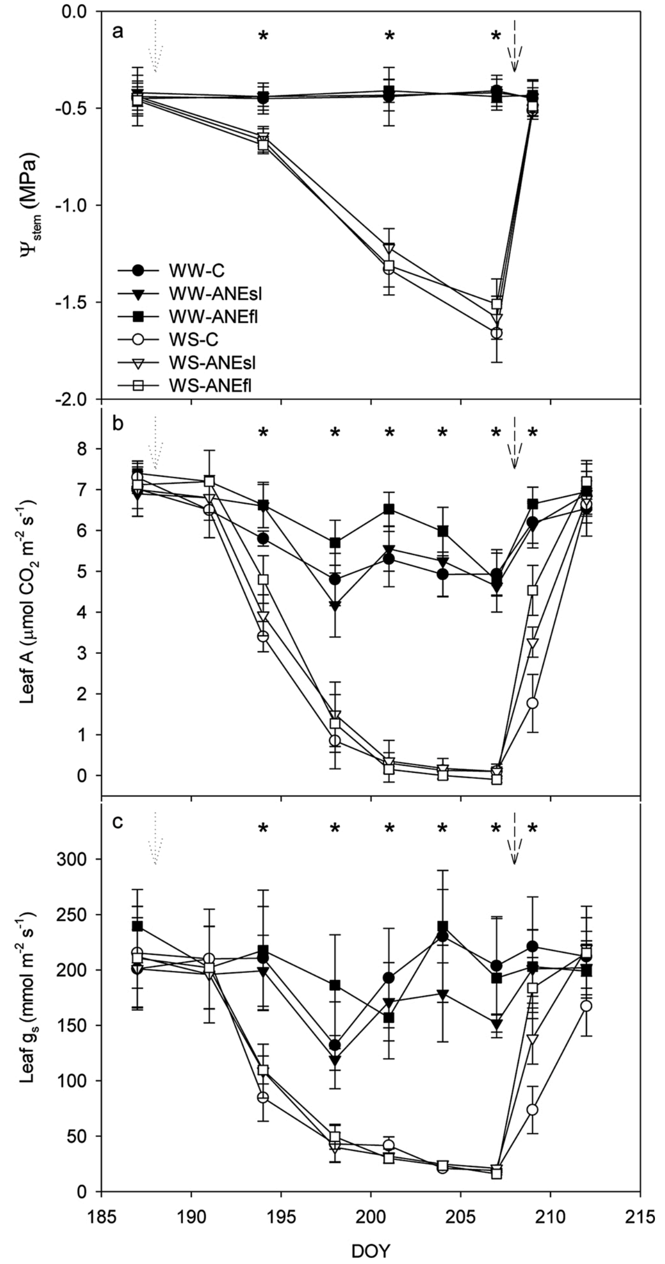

Fig. 2. Stem water potential (Ψ_{stem}, panel a), leaf assimilation rates (Leaf A, panel b), and leaf stomatal conductance (g_s, panel c) in response to *Ascophyllum nodosum* extract (ANE) application and different water regime. Vertical bars represent standard errors ($n = 3$ for Ψ_{stem}, $n = 4$ for leaf A and g_s). Asterisks indicate significant difference between treatments within date ($P < 0.05$). The dotted arrow indicates the imposition of water stress and the dashed arrow indicates re-watering. WW = well-watered, WS = water stressed, C = untreated controls, ANEfl = multiple ANE foliar sprays, ANEsl = multiple ANE soil drench.

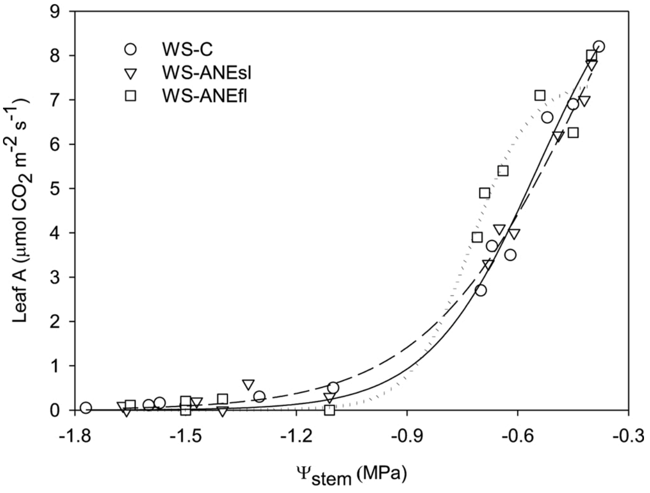

Fig. 3. Correlation between stem water potential (Ψ_{stem}) and leaf assimilation rates (Leaf A) of water stressed vines during the experiment in response to *Ascophyllum nodosum* extract (ANE) application. WS-C = untreated controls, $y = 10.846/(1+\exp(-(x-(-0.549))/0.148))$, $R^2 = 0.988$, $P < 0.05$; WS-ANEfl = multiple ANE foliar sprays, $y = 14.159/(1+\exp(-(x-(-0.431))/0.213))$, $R^2 = 0.994$, $P < 0.05$; WS-ANEsl = multiple ANE soil applications $y = 7.440/(1+\exp(-(x-(-0.727))/0.080))$, $R^2 = 0.984$, $P < 0.05$.

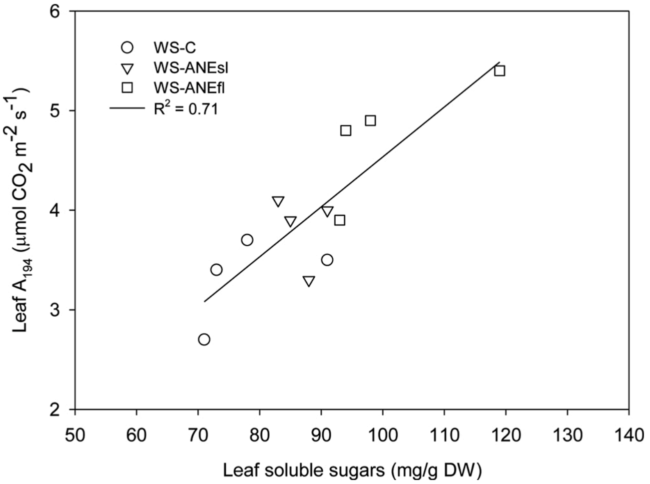

Fig. 4. Correlation between leaf soluble sugars and leaf assimilation rates on DOY 194 (Leaf A_{194}) of water-stressed vines in response to *Ascophyllum nosodum* extract (ANE) application, $y = 0.05x - 0.48$, $R^2 = 0.71$, $P < 0.05$. WS-C = untreated controls; WS-ANEfl = multiple ANE foliar sprays; WS-ANEsl = multiple ANE soil applications.

The most relevant effect of ANE in this study was the prompt restore of leaf gas exchanges at re-watering (Figs. 2a,b, 5). Both delivery systems of ANE promoted a faster physiological resumption as compared to WS-C, yet data indicate that WS-ANEfl was more effective than WS-ANEsl. The effect was related to the ANEfl ability to delay the inception of non-reversible photoinhibition damages and to preserve the leaf photochemical functioning when severe WS occurs, as demonstrated by the lack of correlation at re-watering between leaf A and Ψ_{stem} and the concurring significant correlation between leaf A and Fv/Fm (Fig. 7). In turn, the protection of photosystem II integrity can be ascribed again to the above-mentioned role of WS-ANEfl higher leaf soluble solids concentration (Table 1) (Ackerson, 1981). Conversely, in WS-ANEsl, it is more difficult to understand what promoted the prompter recovery than WS-C. Considering also the lack of positive effects of ANEsl during WS progression, the higher leaf A and g_s in WS-ANEsl vs. WS-C detected on DOY 209 could be a short-term direct effect of the seaweed extract application executed right at rewatering. This is also in agreement with previous work by Martynenko et al. (2016) and Kałużewicz et al. (2017).

Finally, the comparison between foliar vs. soil application of ANE highlighted that foliar application of ANE was overall more effective than soil ANE application in improving grapevines physiological performances either under moderate WS or at re-watering. In orange nursery trees, Spann and Little (2011) found that, under prolonged WS conditions (50 % ET), soil drench ANE applications were more effective than foliar treatments in terms of water potential maintenance and shoot growth. Xu and Leskovar found that both foliar and soil drench ANE

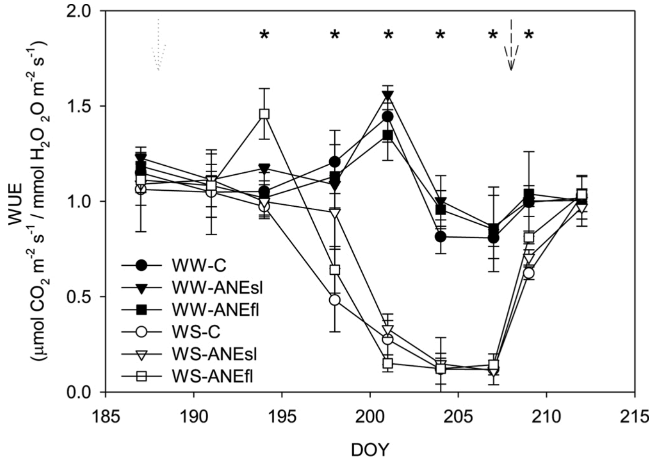

Fig. 5. Leaf water use efficiency (WUE) in response to *Ascophyllum nodosum* extract (ANE) application and different water regime. Vertical bars represent standard errors ($n = 4$). Asterisks indicate significant difference between treatments within date ($P < 0.05$). The dotted arrow indicates the imposition of water stress and the dashed arrow indicates re-watering. WW = well-watered, WS = water stressed, C = untreated controls, ANEfl = multiple ANE foliar sprays, ANEsl = multiple ANE soil drench.

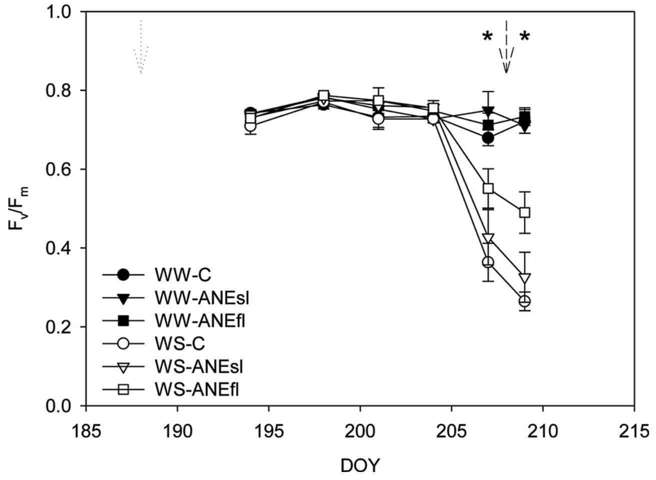

Fig. 6. Leaf photosystems efficiency (Fv/Fm) in response to *Ascophyllum nodosum* extract (ANE) application and different water regime. Vertical bars represent standard errors ($n = 4$). Asterisks indicate significant difference between treatments within date ($P < 0.05$). The dotted arrow indicates the imposition of water stress and the dashed arrow indicates re-watering. WW = well-watered, WS = water stressed, C = untreated controls, ANEfl = multiple ANE foliar sprays, ANEsl = multiple ANE soil drench.

application improved spinach leaf gas exchanges when compared to untreated plants. Given these two works, as well as all the other ones investigating the sole soil ANE application (Wally et al., 2013; Martynenko et al., 2016; Santaniello et al., 2017; Rayorath et al., 2008), it remains quite unclear why in our experiment ANEsl did not give positive results, especially as compared to ANEfl. However, two relevant aspects need to be taken into account: i. none of these authors used grafted plants, as we did; ii. none of the above-mentioned works was conducted on trees, yet on annual species or one-year old nursery trees. These differences suggest the hypothesis that in trees, ANE soil applications could be negatively affected by rootstocks or permanent organs buffering effects.

Overall, our data demonstrate that, under controlled conditions, foliar ANE applications improve vine physiological performances under mild-to-moderate water deficit and could preserve the integrity of photo-chemical leaf functioning under severe limiting conditions. Though field trials will be essential to validate the efficacy of ANE in non-controlled conditions, our data suggest that integrating foliar applications of ANE into the vineyard management system could be a smart approach in those regions where Ψ_{stem} frequently ranges between -0.6 and -0.8 MPa in summer, if maintaining high CO_2 assimilation rates is something desirable.

5. Conclusions

The application of foliar versus soil *A. nodosum* extracts on water-stressed grapevines produced contrasting results. Repeated foliar sprays improved vine physiological performances and water use efficiency when water deficit was moderate (i.e. Ψ_{stem} of about -0.65 MPa) and fostered the resumption of full leaf gas exchanges at re-watering thanks to the preservation of higher photochemical efficiency and changes in leaf anatomical and biochemical features. Conversely, soil application resulted in a weak physiological recovery upon re-watering. Both types of application seemed ineffective in preventing stomatal closure at severe water stress, though foliar sprays reduced the severity of non-reversible photoinhibition. Overall, our results suggest that foliar application of *A. nodosum* extracts can be a valuable technique to improve grapevine resilience in temperate regions, where water shortages lead to moderate, but rarely severe, water deficit conditions in vineyards.

CRediT authorship contribution statement

Tommaso Frioni: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft. **Joshua VanderWeide:** Writing - review & editing. **Alberto Palliotti:** Conceptualization, Visualization, Investigation, Writing - review & editing. **Sergio Tombesi:** Methodology, Writing - review & editing. **Stefano Poni:** Writing - review & editing. **Paolo Sabbatini:** Supervision, Visualization, Methodology, Resources, Writing - review & editing.

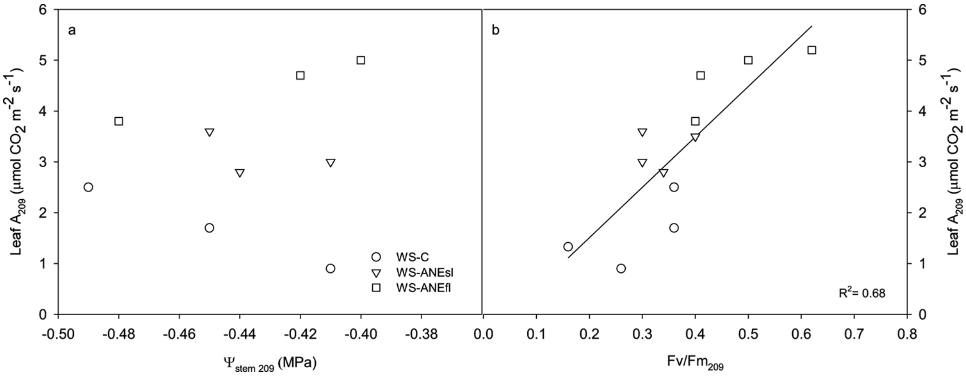

Fig. 7. (a) Dispersion of stem water potential (Ψ_{stem}) and leaf assimilation rates (Leaf A_{209}) of re-watered vines on DOY 209 in response to *Ascophyllum nodosum* extract (ANE) application. (b) Correlation between Fv/Fm and leaf assimilation rates (Leaf A_{209}) of re-watered vines on DOY 209, according to *Ascophyllum nosodum* extract (ANE) application, $y = 9.917x - 0.475$, $R^2 = 0.675$, $P < 0.05$. WS-C = untreated controls; WS-ANEfl = multiple ANE foliar sprays; WS-ANEsl = multiple ANE soil applications.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.scienta.2020.109807.

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