Scientia Horticulturae 232 (2018) 97–106

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Effects of a biostimulant derived from the brown seaweed *Ascophyllum nodosum* on ripening dynamics and fruit quality of grapevines

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ARTICLE INFO

Keywords:
Ascophyllum nodosum
Vitis vinifera
Seaweed extract
Anthocyanins
Phenolic maturity
Viticulture

ABSTRACT

Most modern and traditional grape-growing regions are facing challenging times due to the unpredictability of weather conditions and warming trends. Innovative and sustainable tools such as seaweed-based biostimulants may play a key-role in the development of environment-friendly viticultural strategies to improve yields, biotic/abiotic stress tolerance and fruit and wine quality. A sprayable *Ascophyllum nodosum* extract was tested on grapevines cv. Sangiovese grown under Mediterranean conditions (central Italy) and on grapevines cv. Pinot Noir and Cabernet Franc within a cool-climate viticulture region (Michigan, USA). The product was sprayed on the canopies at label doses (1.5 kg/ha) five times during the season, starting two weeks before veraison. The seaweed extract did not affect leaf gas exchanges, yield or cluster and berry size, but hastened veraison, improved anthocyanins accumulation in all cultivars and increased phenolic content particularly in Sangiovese. Therefore, medium-late application of the seaweed extract can be a simple way to favour chromatic and chemical proprieties of grapes and wines. This is the first report of positive effects of *Ascophyllum nodosum* extracts on the quality of cultivated wine grapes. The adoption of the technique can be particularly suitable to cool-climate viticulture, especially as it pertains to short growing seasons and genotypes with a limited phenolic profile.

1. Introduction

Efforts to improve agricultural sustainability are being encouraged worldwide. Sustainable production includes ensuring yield with particular attention to food safety and conservation of rural ecosystems (Pretty, 2008). Biostimulants, natural fertilizers and plant defense activators/elicitors, are tools gaining consideration within modern crop management (Colla and Rouphael 2015). Seaweed extracts are natural compounds described by Du Jardin (2015) as one of the main groups of biostimulants. Concentrates obtained by different marine plants have been studied for their positive effects in different agricultural systems (Battacharyya et al., 2015; Khan et al., 2009). The brown seaweed *Ascophyllum nodosum* (L.) Le Jol. is one of the more interesting seaweed species given its widespread application and potential in agriculture (Khan et al., 2009). *Ascophyllum nodosum* (AN) extracts have been reported to promote growth and yield in many crops and to increase quality. They can trigger specific metabolic pathways in treated plants and provide organic compounds having diverse effects in plant metabolism (Battacharyya et al., 2015; Khan et al., 2009). Foliar applications of AN extracts have been reported to increase crop tolerance towards pathogens (Battacharyya et al., 2015; Khan et al., 2009) and to affect plant hormone biosynthesis (Wally et al., 2013). Moreover, experiments on model plants such as *Arabidopsis thaliana* (L.) suggested that AN extracts can modulate genetic signalling related to secondary metabolism and phenolic biosynthesis (Goñi et al., 2016). Molecular and genomic studies are supported by several researches that reported an increased content of anthocyanins, phenolics, flavonoids and anti-oxidant compounds in response to AN extract treatments (Fan et al., 2011, 2013; Lola-Luz et al., 2013, 2014a,b; Ochmian et al., 2008; Roussos et al., 2009).

In premium red wine grape production, phenolic content is of pivotal importance for wine quality and economic return. This remains a challenge for growers given wide seasonal climatic variability due to climate change (Jones et al., 2005; Schultze et al., 2016b).

Abbreviations: AN, *Ascophyllum nodosum* (L.) Le Jol; T min, Minimum temperature; T max, Maximum temperature; T avg, Average temperature; GDD, Growing Degree Days; TSS, Total soluble solids; TA, Titratable acidity; DOY, Day of the year; DAFB, Days after full bloom

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https://doi.org/10.1016/j.scienta.2017.12.054
Received 18 August 2017; Accepted 22 December 2017

Furthermore, the extensive range of climatic areas where thousands of grapevine cultivars are spread imposes different challenges to growers. In warmer climate regions, advanced phenological stages and the uncoupling of technological and phenolic maturity in red grape cultivars depletes grape composition at harvest (Jones et al., 2005; Palliotti et al., 2014). On the other hand, in cool climate regions winter and spring minimum temperatures, short growing season and high disease pressure challenge vineyard productivity and grape quality (Jones et al., 2005; Schultze et al., 2016a). Such variability causes different problems and can influence the effectiveness of cultural techniques (Frioni et al., 2017). Although several experiments report on positive effects of AN extracts applications in grapevines (Khan et al., 2012; Kok et al., 2010; Norrie et al., 2002; Norrie and Keathley 2006; Sabir et al., 2014), a comprehensive evaluation of the potentiality of AN-based products on *Vitis vinifera* under different climatic conditions is lacking.

The aim of this work was to evaluate canopy applications of a AN extract on grapevines grown in two different viticultural areas: central Italy, a typical Mediterranean environment, and Michigan, a cool-cold viticulture region (Schultze et al., 2016b). More specifically, this study examined the effect of AN extract on phenolic maturity and tested its replicability under different climatic conditions and on different genotypes. Taking into account the promotion of anthocyanins and polyphenols reported on other crops and considering the challenge that climatic conditions represent in different environments for achieving an optimal ripening, our general hypothesis was that AN extracts can be a useful tool to improve grape quality for red wine production.

2. Materials and methods

2.1. Experiment 1: site, plant material and experimental design

The first experiment (Exp. 1) was conducted in 2013 in central Italy (Deruta, Umbria, 42° 96′ 15″ N, 12° 40′ 78″ E, 405 m asl, loamy soil type, south exposition, north-south row orientation) on 48 fifteen-years-old vines of *Vitis vinifera* L. cv. Sangiovese (clone VCR30) grafted on 420A. Vines were planted at 1.00 × 2.50 m between vines and rows, respectively, and trained with vertical shoot positioned trellis system (VSP), spur-pruned during winter to ~10 buds per vine. Cordons were trained 0.9 m aboveground and three pairs of catch wires were forming canopy walls of 1.2 m above the cordons. The plot was organized using a Randomized Complete Block Design (RCBD), consisting of four blocks of 12 vines each and one factor (AN extract foliar application), with 16 vines per treatment. Two weeks after full bloom all vines were adjusted to a crop load of about 13 clusters per vine. Shoot trimming was performed when the shoot tips reached a length of ~30 cm higher than the top wire and standard pest management practices were applied, according to local standards. Three weeks after the pea-size stage (as described by Coombe, 1995), vines were assigned to the following treatments: 16 vines, four per block, were assigned to the first treatment, consisting of multiple applications of the AN extract Acadian Marine Plant Extract Powder (Acadian Seaplants Limited, Dartmouth, NS, Canada) at label rates, 1.5 kg/ha (SWE1); another set of 16 vines were assigned to SWE2, consisting of multiple applications of the same AN extract at 3.0 kg/ha; the remaining 16 vines were assigned to CONTROL, consisting in application of water. A surfactant was added to all treatments as suggested on the product's label. Treatments were repeated on the same vines four times before harvest, at application intervals of ten to twenty days. Treatment dates were 22 Jul 2013 (56 Days After Full Bloom – DAFB), 5 Aug 2013 (70 DAFB), 23 Aug 2013 (88 DAFB), 2 Sep 2013 (98 DAFB), 15 Sep 2013 (105 DAFB).

2.2. Experiment 2: site, plant material and experimental design

A second experiment (Exp. 2) was carried out in 2014 on 64 five-years-old vines of *Vitis vinifera* L., divided into two plots. The first plot was composed of grapevines cv. Pinot Noir (clone 114 grafted on 101-14 MGt) while the second one of grapevines cv. Cabernet Franc (clone 332 grafted on 101-14 MGt). Plots were situated in a commercial vineyard in Benton Harbor, MI, USA (42° 13′ 30″ N, 86° 37′ 36″ W). Soils were spinks sandy loam (USDA, 1957) and the vineyard had a barely perceptible slope, with south exposition and a north-south row orientation. Each plot consisted of 32 vines of the same cultivar, planted with a spacing of 1.50 × 3.00 m for Pinot Noir and 1.80 × 3.00 m for Cabernet Franc between vines and rows, respectively. Both cultivars were trained with a vertical shoot positioned trellis system (VSP), cane-pruned during winter to about 30 nodes per vine for Pinot Noir and 50 nodes per vine for Cabernet Franc. Multiple trunks were retained to ensure survival during low winter temperatures and re-trained after severe damage from extreme freezing temperatures recorded during winter 2012/2013. The two sections were organized with a Randomized Complete Block Design (RCBD), consisting of four blocks of eight vines each and one factor (AN extract application), with 16 vines per treatment. Two weeks after full bloom all vines were adjusted to a crop load of about 45 clusters per vine in Pinot Noir and 100 clusters per vine in Cabernet Franc. Shoot trimming was performed when the shoot tips reached a length of ~30 cm higher than the top wire. Standard commercial disease management was applied based on experience and weather conditions.

Phenological stages were identified as described by Coombe (1995). Grape veraison was considered when 50% of the berries presented full color change. For Pinot Noir, vines were assigned to the two treatments three weeks after vines reached the pea-size stage. For the late-ripening Cabernet Franc, vines were assigned to the two treatments four weeks after the pea-size stage. Half of the vines of each cultivar were assigned to the AN extract application (SWE) and the remaining 16 vines to the untreated control (CONTROL). On the same day the first application was performed. At application, SWE vines were treated with a full canopy spray at 1.5 kg/ha of the AN extract (Acadian Marine Plant Extract Powder, Acadian Seaplants Limited, Dartmouth, NS, Canada), diluted in water, including the addition of an adjuvant, as suggested in the product's label. CONTROL vines were sprayed only with water and the adjuvant. Treatments were repeated on the same vines four times before harvest, at ten to twenty day intervals. Treatment dates for Pinot Noir were 30 Jul 2014 (44 DAFB – Days After Full Bloom), 6 Aug 2014 (51 DAFB), 16 Aug 2014 (61 DAFB), 26 Aug (71 DAFB), 7 Sep 2014 (83 DAFB) and for Cabernet Franc 16 Aug 2014 (57 DAFB), 30 Aug (71 DAFB), 7 Sep 2014 (80 DAFB), 20 Sep 2014 (93 DAFB), 11 Oct 2014 (114 DAFB).

2.3. Weather data

Environmental conditions during both experiments were data-logged by two automated weather stations located nearby the vineyards. Daily maximum (T max), average (T avg) and minimum temperature (T min) and precipitation from 1 Apr to 31 Oct of 2013 (Exp. 1) and 2014 (Exp. 2) were collected. Cumulative growing-degree-days (GDD) (Baskerville and Emin 1969) were then calculated. Same data were obtained also for the same period of the ten previous years, to calculate the ten-year running average.

2.4. Gas exchanges parameters, leaf composition and canopy architecture

Throughout Exp. 2, two weeks after full bloom shoots were counted and three representative shoots were identified, tagged and numbered. Leaf net photosynthesis (P_n), stomatal conductance (g_s) and transpiration rate (E) were measured on 22 Aug 2014 for Pinot Noir (T max = 29.4, T min = 21.2, T avg = 25.3) and on 3 Sep 2014 for Cabernet Franc (T max = 28.7, T min = 15.2, T avg = 22.0), which corresponded to about one week after veraison. Gas exchange parameters were measured between 1200 h and 1300 h on the third leaf of first tagged shoot of each vine, using a CIRAS-2 portable photosynthesis machine (PP Systems Version 2.02; Amesbury, MA, USA). Readings