

STRATEGIES FOR THE IMPROVEMENT OF FRUIT SET IN *VITIS VINIFERA* L. CV. 'CARMÈNÈRE' THROUGH DIFFERENT FOLIAR BIOSTIMULANTS IN TWO DIFFERENT LOCATIONS

ESTRATÉGIAS PARA A MELHORIA DA FRUTIFICAÇÃO EM *VITIS VINIFERA* L. CV. 'CARMÈNÈRE' ATRAVÉS DE DIFERENTES BIOESTIMULANTES FOLIARES EM DOIS LOCAIS DIFERENTES

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SUMMARY

'Carménère' is an important grapevine variety cultivated in Chile, which exhibits a high tendency to fruitlet abscission and "millerandage", reproductive disorders that seriously affect plant productivity. However, despite the existence of a lot of information about the effects of micronutrients on millerandage, few studies exist about the impacts of seaweed biostimulation on reproductive disorders in grapevines, which currently has been classified as elicitor. For these reasons, the aim of this work was to study the effect of different biostimulant applications to 'Carménère' grapevines on fruit set and cluster characteristics during 2011-2012 season. Viticultural parameters such as number and weight of berries, cluster weight, berry size and its distribution along the cluster were measured. In addition, physicochemical parameters in musts from the obtained grapevines were analyzed. The results showed that the treatments based on boron ethanolamine plus zinc oxide and brown algae (*Ascophyllum nodosum*) improved fruit set in 'Carménère' grapevines in both climate locations, mainly in the colder climate site. Different parameters such as weight and number of berries per bunch, among others, were improved after the application of this treatment. Therefore, the results suggest that it is possible to improve fruit set in 'Carménère' grapevines using a treatment based on certain microelements, such as boron and zinc, and an extract produced from seaweed algae.

RESUMO

'Carménère' é uma importante casta cultivada no Chile, que apresenta uma alta tendência para distúrbios reprodutivos durante a frutificação, como a abscisão dos bagos e a bagoinha, que afetam seriamente a produtividade da planta. Há muita informação sobre os efeitos dos micronutrientes nos distúrbios reprodutivos. Porém, existem poucos relatos sobre o impacto da bioestimulação a partir do uso de extratos de algas, que têm sido classificados como molécula elicitora, sobre a frutificação da videira. Por estas razões, o objetivo deste trabalho foi estudar o efeito da aplicação de diferentes bioestimulantes em videiras da casta 'Carménère' sobre os bagos e características do cacho durante o ciclo 2011-2012. Parâmetros vitícolas como o número e peso dos bagos, o peso do cacho, o tamanho do bago e sua distribuição no cacho foram medidos. Além disso, foram analisados alguns parâmetros físico-químicos em mostos. Os resultados mostraram que os tratamentos baseados na aplicação de etanolamina de boro, acrescido de óxido de zinco e algas castanhas (*Ascophyllum nodosum*) melhoraram a produção de frutos em videiras da casta 'Carménère' no local de clima frio. O valor de parâmetros como o peso e o número de bagos por cachos, entre outros, foi melhorado após a aplicação deste tratamento. Os resultados sugerem que, por vezes, é possível melhorar a produção de videiras da casta 'Carménère' usando um tratamento baseado em microelementos, como o boro e o zinco, e um extrato produzido a partir de algas marinhas.

Key words: *Ascophyllum nodosum*, biostimulation, 'Carménère' grapevines, fruit set, micronutrients, millerandage.

Palavras-chave: *Ascophyllum nodosum*, bioestimulação, videiras 'Carménère', frutificação, micronutrientes, bagoinha.

INTRODUCTION

‘Carménère’ (*Vitis vinifera*. L) is one of the most widely cultivated grapevine varieties in Chile since its arrival from Bordeaux, France around the year 1851, before the phylloxera (*Daktulosphaira vitifoliae*) devastation of European grapevines. It is nowadays an important part of the Chilean wine industry (Hernández, 2000). According to the report of the Servicio Agrícola y Ganadero (SAG, 2015), ‘Carménère’ accounts for 10,503 hectares, reaching a 7.65% of the national average surface. Currently, this variety is recognized as emblematic in Chile due to its economic importance and international recognition (Pszczółkowski, 2008). ‘Carménère’, with a long maturation period, was rediscovered in Chile around 1994, through ampelographic analysis, where it had been misidentified as ‘Merlot’ or ‘Cabernet Franc’ (Pszczółkowski, 2004). The presence of herbaceous and “green” aromas in ‘Carménère’ wines is mainly due to the synthesis of high concentrations of methoxy-pyrazines in grapes compared to ‘Cabernet Sauvignon’ grapevines, strongly related with the climatic conditions of the vineyard (Belancic and Agosin, 2007).

One of the most important problems in ‘Carménère’ vineyard management is the exhibition of a high tendency to fruitlet abscission and “millerandage”, reproductive disorders that seriously affect these traits (Alva *et al.*, 2015). This variety presents flowers with curved filaments, which limit the fruit set and production. In addition ‘Carménère’ is characterized by late entering into production and by very low yields due to the low fertility of the basal buds (Pszczółkowski, 2008). Millerandage is characterized by the presence of normal size seeded berries together with with small-size (<3mm) and mid-size (3–6mm) seedless berries in the same bunch (Alva *et al.*, 2015). Small seedless berries are presumably generated from defective, non-fertile ovules, mid-size seedless berries arise from a parthenocarpic event caused by a defective ovule fertilization due to a failure in pollen tube growth and a sperm cells release into ovaries (Keller, 2010; Alva *et al.*, 2015).

These physiological problems are commonly associated with deficiencies in essential micronutrients such as boron and zinc (Vasconcelos *et al.*, 2009). Boron deficiency is related to the aforementioned physiological problems negatively affecting pollen tube development, pollen germination, pollen tube growth, fruit set and seed formation (Tanaka and Fujiwara, 2008), whereas zinc is required as cofactor in several types of enzymes involved mainly in cell division, nucleic acid

metabolism and protein synthesis. Zinc assimilation by grapevines depends on ripening stage of berry, independent of the use of rootstocks (Volschenk *et al.*, 1999). Additionally, biostimulation through seaweed mainly by the *Ascophyllum nodosum* brown algae has allowed improving grapevine production and phenolics compounds in grapes, being classified by several authors as an elicitor (Khan *et al.*, 2012; Frioni *et al.*, 2018; Gutiérrez-Gamboa *et al.*, 2018). Based on the aforementioned, the aim of this work was to study different strategies to improve fruit set in *Vitis vinifera*. L cv. ‘Carménère’ through foliar biostimulation of a different combination of microelements and the use of *Ascophyllum nodosum* extracts in Maule and BioBio Valleys.

MATERIAL AND METHODS

Experimental site and plant material

The field study was conducted in two commercial vineyards cv. ‘Carménère’ during 2011-2012. One of the vineyards was located at San Javier, Maule Valley. The vineyard was planted in 2000 with a distance of 2.5 m between rows and 1.5 m between plants, accounting for a total of 2,666 vines/ha. The grapevines were trained to a vertically shoot positioned system, leaving around of 28-30 buds per vine. The vineyard has drip irrigation, with an output per plant of 4 L/hr. The soil is clay loam, and the climate is Mediterranean type with a prolonged dry season during summer. Maximum temperature during the warmest month (January) was 30.1 °C. Accumulated rainfall from September to April was around 84.8 mm. Annual precipitation reached around of 870 mm. The other vineyard was located at Yumbel, BioBio Valley. The vineyard was planted in 1999 with a distance of 3.0 m between rows and 1.5 m between plants accounting for a total of 2,222 vines/ha. The grapevines were trained to a vertically Scott Henry system, leaving around of 58-60 buds per vine. The vineyard has drip irrigation, with an output per plant of 4 L/hr. The soil is silty loam, and the climate is sub-humid Mediterranean type. Maximum temperature during the warmest month was 28.0 °C. Accumulated rainfall from September to April was around 161.7 mm. Annual precipitation reached around of 1,150 mm. The vineyards were managed under conventional soil tillage management system and were irrigated when the midday leaf water potential (ψ_l) reached 1.0 to 1.2 MPa. Respect to the nutritional management of the vineyards, 2 kg of nitrogen and 3 kg of potassium per ton of harvested fruit were partially applied.

Grapevine treatments

Five treatments were applied to the grapevines as well as control. Three commercial biostimulants were applied to the grapevines. The first, a product based on the *Ascophyllum nodosum* seaweed combined with boron and zinc (BM86, Goëmar, Saint -Malo, France). The second, a product based on boron ethanolamine and zinc oxide (Zintrac 700 + Bortrac 150, Yara, Oslo, Norway). The third, a product based on molybdenum (Molitrac, Yara, Oslo, Norway). A mixture of BM86 and Zintrac 700 + Bortrac 150 and sugar at 2% v/v were also applied to the vineyards. Treatments were applied with a back sprayer in different phenological growth stages depending on the recommendations of the product, with a wetting

of 667 L/ha. Treatments were performed in triplicate and were distributed as a complete randomized block design. Each replication had 16 grapevines, and therefore a total of 48 plants were used in each treatment, leaving 10 untreated plants in the same row and two rows between replicates to avoid treatment contamination. Treatments consisted of applying the recommended amount of the product, divided in two or three moments, the first at seven days after flowering, the second ten days after the first application (flowering) and the third, at ten days after the second application. Additionally, 48 grapevines, distributed in the same way, were kept untreated (Control). Composition and content of the products are shown in Table I.

TABLE I

Composition, concentration, level and time of application of the treatments

Composição, concentração, dose e época de aplicação dos tratamentos

Treatment	Commercial product	Active ingredient	Dose	Time of application
T1	Untreated	Water	-	1) Seven days after beginning of flowering. 2) Flowering. 3) Ten days after the second application.
T2	BM86 (Goëmar, Saint -Malo, France)	1) 260 g/L ascophyllum nodosum 2) 26 g/L B 3) 60 g/L Mg 4) 2.5 g/L Mo	3 L/ha	1) Seven days after beginning of flowering. 2) Flowering. 3) Ten days after the second application.
T3	Zintrac 700 + Bortrac 150 (Yara, Oslo, Norway)	1) Boron ethanolamine 2) Zinc oxide	1) 1L/ha 2) 1.25 L/ha	1) Seven days after beginning of flowering. 2) Flowering.
T4	T2 + T3	1) Active ingredient T2 2) Active ingredient T3	1) Dose T2 2) Dose T3	1) Seven days after beginning of flowering. 2) Flowering.
T5	Molitrac (Yara, Oslo, Norway)	25 g/L Mo	0.25 L/ha	1) Seven days after beginning of flowering.
T6	Sugar	Sucrose	at 2%	1) Seven days after beginning of flowering. 2) Flowering.

Sample evaluations

Ten clusters by grapevine were harvested when the grape berries reached pea grain size, and different parameters such as weight of clusters, number of fruit set berries and distribution of berries by weight and caliber along the clusters were evaluated. The rest of the clusters were collected at harvest stage and these same measurements were performed together with the analysis of technological maturity (°Brix, pH and total acidity), according to the methodology described by the OIV (2003). Weight of clusters was performed using an analytical balance (Cubis® Precision Balance, Sartorius, Göttingen, Germany). Distribution and weight of grape berries by size was measured using a sieve with three different levels, after separation of all berries from the bunch. Separation by size had three ranges: the first from 16 to 12 mm (as large diameter), the second from 12 to 8 mm (as medium diameter) and the third from 8 to 4 mm (as

small diameter). Subsequently, weight of the obtained berries of each range was measured using an analytical scale (Cubis® Precision Balance, Sartorius, Göttingen, Germany).

Statistical analysis

The statistical analysis in relation to the analyzed parameters was performed using variance analysis (ANOVA), by Statgraphics Centurion XVI.I (Warrento, Virginia, United States). Differences between samples were compared using the Duncan test at 95% confidence level.

RESULTS AND DISCUSSION

Effects of micronutrients and bioestimulant applications to 'Carménère' grapevines at pea sized stage

First evaluations were made at pea size stage with the aim to determine if the treatments allowed improving bloom and fruit set in ‘Carménère’ grapevines. No significant differences were found between the evaluated parameters, after micronutrients and bioestimulants applications to the ‘Carménère’ grapevines in the warm climate (Maule Valley) and the control plants (Table 2). However, the number of small berries (8 to 4 mm) was higher in untreated (control) and treated with sugar (T6) grapevines (Table II). These differences may be attributed to a yield compensation effect observed early in ripening since the number of berries of medium diameter (12 to 8 mm) was higher in untreated and treated with T6, although without significant differences. Micronutrients and bioestimulants applications to the ‘Carménère’ grapevines in the cold climate (BioBío Valley) affected weight of cluster, number of berries per cluster, number and weight of berries of medium diameter (Table III). These parameters were higher in grapes treated with BM 86 (T2) and Zintrac 700 + Bortrac 150 (T3) than in the grapes from untreated grapevines and treated with Molitrac (T5) and Sugar (T6), with the exception of weight of berries of medium diameter (Table III). Samples treated with a combination of T2+T3 presented higher number of berries per cluster and weight of berries of medium diameter than the grapes from untreated grapevines (Table 3). Based on these results, micronutrients such as boron and zinc and the extract of *Ascophyllum nodosum* allowed to improve fruit set in the grapevines planted in the cold site (BioBío Valley). Boron ethanolamine is sold a fertilizer which is

quickly absorbed by plant tissues, stimulating growth of cambium tissues and apical meristems, promoting calcium mobility and the production of pollen and bloom fertility (Brown and Shelp, 1997; Arrington and DeVetter, 2017). Reproductive tissues of vines are most susceptible to boron deficiency, which results in reduced fruit-set, small “shot berries” that are around to pumpkin-shaped, and flower and fruit cluster necrosis (Christensen *et al.*, 2006). Deficiencies in boron are also associated with higher rainfall areas and irrigated soils (Christensen *et al.*, 2006). On the other hand, zinc is involved in berry development, the synthesis of growth regulators and chlorophyll (Volschenk *et al.*, 1999). Based on our results, it was observed that biostimulation was more effective in the vineyard planted in BioBío Valley, where the climatic conditions were colder and a greater amount of rainfall falls. On the other hand, different results have been reported by certain authors in respect to the effects of boron applications to some plant crops. Biostimulation through boron / zinc applications improved seed set, seed yield and seed quality in Alfalfa (*Medicago sativa* L.), number, length and weight of berries, length and weight of clusters, and number and size of seeds in grapevines (*Vitis vinifera* L.), whereas it did not increase fruit set or yield in Northern Highbush Blueberry (*Vaccinium corymbosum*) plants (Dordas, 2005; Nikkhah *et al.*, 2013; Arrington and DeVetter, 2017). Besides, foliar applications of seaweed extracts from *Ascophyllum nodosum* improved growth and physicochemical parameters in grape berries (Khan *et al.*, 2012).

TABLE II

Viticultural parameters after biostimulation at pea-sized stage in ‘Carménère’ grapevines growing in a warm climate (Maule Valley)
Parâmetros vitícolas após bioestimulação, ao bago de ervilha, em vinhas ‘Carménère’ em clima quente (Vale do Maule)

	Weight of bunch ^a	Number of berries / bunch	Number of berries of 16-12 mm	Number of berries of 12-8 mm	Number of berries of 8-4 mm	Weight ^a of berries of 16-12 mm	Weight ^a of berries of 12-8 mm	Weight ^a of berries of 8-4 mm
Control (T1)	52.4	98.7	15	55.9	31.3b	15.2	27.9	6.3
BM 86 (T2)	59.0	87.7	23.2	48.7	16.9a	25.8	27.6	2.4
Zintrac 700 + Bortrac 150 (T3)	63.6	92.5	24.3	53.5	17.3a	25	31.6	2.7
T2 + T3 (T4)	73.7	92.5	17.5	47.5	20.0ab	23.7	30.9	4.2
Molitrac (T5)	49.7	92.9	17.8	45.6	27.9ab	19.3	23.5	3.6
Sugar (T6)	53.4	108.9	17	57.5	35.3b	17	28.7	4.3
Coefficient of variation (%)	13.9	9.8	19.5	9.4	28.7	20.5	10.7	40.7
Significance	ns	ns	ns	ns	*	ns	ns	ns

For each parameter, different letters in the same row indicate significant differences between treatments (*p≤0.05); (**p≤ 0.01); ns: Not significant; ^aAs g.

TABLE III

Viticultural parameters after biostimulation at pea-sized stage in ‘Carménère’ grapevines growing in a cold climate (BioBio Valley)

Parâmetros vitícolas após bioestimulação, ao bago de ervilha, em vinhas ‘Carménère’ em clima frio (Vale do BioBio)

	Weight of bunch ^a	Number of berries / bunch	Number of berries of 16-12 mm	Number of berries of 12-8 mm	Number of berries of 8-4 mm	Weight ^a of berries of 16-12 mm	Weight ^a of berries of 12-8 mm	Weight ^a of berries of 8-4 mm
Control (T1)	51.8a	108.4a	1.4	81.8a	26.8	1.6	40.2a	3.9
BM 86 (T2)	77.0b	159.0c	1.8	122.9c	34.4	2.3	64.2c	4.9
Zintrac 700 + Bortrac 150 (T3)	77.3b	156.0c	1.3	119.8bc	34.5	2.1	64.0c	4.8
T2 + T3 (T4)	65.8ab	141.9bc	1.4	100.9ab	39.6	1.7	55.1bc	5.1
Molitrac (T5)	63.9a	128.4ab	2	95.4a	29.9	2.2	52.5bc	5.2
Sugar (T6)	55.8a	117.0ab	0.6	89.8a	28.5	0.8	45.6ab	4.1
Coefficient of variation (%)	16	15.3	34.1	16.2	14.7	36.7	17.8	12.5
Significance	*	*	NS	*	ns	ns	*	ns

For each parameter, different letters in the same row indicate significant differences between treatments (*p≤0.05); (**p≤ 0.01); ns: Not significant; ^aAs g.

Effects of micronutrients and bioestimulants applications to ‘Carménère’ grapevines at harvest

Evaluations were also performed at harvest, since it is possible that bloom and fruit set is normally developed. However, at the beginning of fruit growth, the “millerandage” phenomenon may be developed and grape bunches may contain berries that differ greatly in size and maturity. Besides, it has been reported that cool conditions during bloom caused a greater occurrence of “millerandage” (Neethling *et al.*, 2017).

In the warm site (Maule Valley) Foliar applications of the treatments to ‘Carménère’ grapevines affected the number of berries per bunch and the number of berries of minimum diameter (Table 4). Grapes harvested from grapevines treated with Zintrac 700 + Bortrac 150 (T3) presented higher number of berries per bunch than grapes from grapevines untreated and treated with BM 86 + Zintrac 700 + Bortrac 150 (T4), Molitrac (T5) and sugar (T6) (Table 4). Moreover, all the treatments increased the number of small berries compared to untreated plants (Table IV).

TABLE IV

Viticultural parameters after biostimulation at harvest in ‘Carménère’ grapevines growing in a warm climate (Maule Valley)

Parâmetros vitícolas após bioestimulação, à colheita, em videiras ‘Carménère’ produzidas em clima quente (Vale do Maule)

	Weight of bunch ^a	Number of berries / bunch	Number of berries of 16-12 mm	Number of berries of 12-8 mm	Number of berries of 8-4 mm	Weight ^a of berries of 16-12 mm	Weight ^a of berries of 12-8 mm	Weight ^a of berries of 8-4 mm
Control (T1)	91.8	78.7a	37.7	23.6	16.8a	65.5	17.1	2.7
BM 86 (T2)	102.9	92.9ab	46.9	32.3	49.9bc	73	23	2.1
Zintrac 700 + Bortrac 150 (T3)	102.3	106.8b	47.8	24.7	50.9c	75.2	20.3	3.3
T2 + T3 (T4)	96.5	88.1a	43	27	41.8bc	72.3	17.1	2.6
Molitrac (T5)	93.5	87.2a	40.6	28.2	37.9b	67.9	18.1	2.8
Sugar (T6)	85.5	84.0a	42.2	13.9	38.6b	71.2	18.6	3.2
Coefficient of variation (%)	6.1	10.8	8.9	13.9	31.1	6.9	15	18.1
Significance	ns	*	ns	ns	*	ns	ns	ns

For each parameter, different letters in the same row indicate significant differences between treatments (*p≤0.05); (**p≤ 0.01); ns: Not significant; ^aAs g.

In the cold climate (BioBio Valley), treatments applied to the grapevines affected all viticultural parameters, except the number and weight of the

berries of medium and small diameter (Table V). Grapes from grapevines treated with T3 presented the highest weight of cluster and number of berries per

bunch. In addition, these grapes presented the highest number and weight of berries of large diameter. The same happened with grapes from grapevines treated with T2. Due to the aforementioned, it is possible that the effectiveness of biostimulation, mainly through boron plus zinc treatment was higher at harvest than at pea size phase, when the phloem and gas exchange

is less active. Brown *et al.* (1999) reported that boron mobility is due to the production of sugar alcohols, enabling the cotransport of boron-polyol complexes in the phloem. Keller *et al.* (2014) indicated that the decrease in xylem inflow at the onset of ripening may be a consequence of the sink-driven increase in phloem inflow.

TABLE V

Viticultural parameters after biostimulation at harvest in ‘Carménère’ grapevines growing in a cold climate (BioBio Valley)
Parâmetros vitícolas após bioestimulação, à colheita, em videiras ‘Carménère’ produzidas em clima frio (Vale do BioBio)

	Weight of bunch ^a	Number of berries / bunch	Number of berries of 16-12 mm	Number of berries of 12-8 mm	Number of berries of 8-4 mm	Weight ^a of berries of 16-12 mm	Weight ^a of berries of 12-8 mm	Weight ^a of berries of 8-4 mm
Control (T1)	110.0a	106.9a	53.6a	42.8	14.3	75.0a	27.4	2.8
BM 86 (T2)	130.8b	127.0b	65.2ab	44.9	18.5	94.3bc	29.5	2.2
Zintrac 700 + Bortrac 150 (T3)	150.0c	149.3c	76.7b	44.4	14.5	112.8c	31	2.4
T2 + T3 (T4)	120.8ab	117.3ab	59.1a	43.3	16.8	85.1ab	28.5	3
Molitrac (T5)	115.0ab	111.3ab	56.9a	38.4	17.4	82.3ab	26	3
Sugar (T6)	106.4a	103.6a	56.8a	32.1	7.4	78.5ab	22.3	1.4
Coefficient of variation (%)	13.4	14.2	13.7	12	26.9	16.2	12.1	24.7
Significance	*	*	*	ns	ns	*	ns	ns

For each parameter, different letters in the same row indicate significant differences between treatments (*p≤0.05); (**p≤ 0.01); ns: Not significant;
^aAs g.

Oenological parameters

According to Table VI, none of the must oenological parameters measured was affected by the biostimulation through the different foliar applications. These results are similar to those reported by several authors (Gutiérrez-Gamboa *et al.*, 2017a, Gutiérrez-Gamboa *et al.*, 2017b, Frioni *et al.*, 2018), who showed little effects of biostimulation

through nitrogen fertilizers, algae extracts, among others, on must oenological parameters. Contrary to these results, Khan *et al.* (2012) showed that the application of *Ascophyllum nodosum* extracts application to the grapevines improved soluble solids, titratable acidity, pH, total sugars, reducing sugars and ascorbic acid as compared to unsprayed grapevines.

TABLE VI

Must oenological parameters from untreated and treated grapevines
Parâmetros enológicos obtidos em videiras não tratadas e tratadas

	Maule Valley			BioBio Valley		
	Soluble solids (°Brix)	Total acidity (g/L)	pH	Soluble solids (°Brix)	Total acidity (g/L)	pH
Control (T1)	25.2	4.08	3.3	25.6	4.38	3.8
BM 86 (T2)	25.6	3.8	3.4	25.6	4.81	3.6
Zintrac 700 + Bortrac 150 (T3)	23.4	4.6	3.2	25.2	5.2	3.5
T2 + T3 (T4)	23	4.1	3.3	24.8	4.62	3.8
Molitrac (T5)	23.2	4.1	3.4	26.2	4.7	3.7
Sugar (T6)	23.6	4.03	3.4	24.5	4.73	3.7
Significance	ns	ns	ns	ns	ns	ns

ns: Not significant

CONCLUSIONS

These preliminary results suggest that a combination of boron plus zinc can improved the fruit set levels on ‘Carménère’ bunches, compared with others treatments in the cold climate site. Biostimulation through seaweed brown algae *Ascophyllum nodosum* extract can allow an improvement of fruit set only when compared to untreated grapevines. On the contrary, molybdenum applications to the grapevines did not affect fruit set in ‘Carménère’ grapevines. The vineyard planted in the cold climate site presented a

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