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Effect of Shading Nets on Yield, Leaf Biomass and Petiole Nutrients of a Muscat of Alexandria Vineyard Growing under Hyper-Arid Conditions

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Abstract: *Background*: Currently, viticulture is exposed to extreme weather fluctuations and global warming, thus the implementation of short-term adaptation strategies to mitigate climate change impacts will be of a wide importance for the sustainability and competitiveness of wine industry. This research aimed to study the effect of shading nets on the viticultural performance of a Muscat of Alexandria vineyard growing under hyper-arid conditions. *Methods*: Three treatments were randomly arranged in the vineyard: (i) a control (without shading), (ii) a white shading net (25% of shading), and (iii) a black shading net (40% of shading). Subsequently, yield, vine vigor, berry composition, leaf biomass and petiole nutrient content were assessed. *Results*: Both shading nets decreased the incidence of solar radiation in vines. The application of white shading nets induced a high bunch weight and a higher number of berries per bunch than the black shading nets. Black shading nets increased pruning weight, decreased Ravaz index and induced a considerably accumulation of soluble solids in grapes. This treatment also decreased bunch weight and the number of berries per bunch, and increased rachis length compared to control. Black shading nets decreased Mg petiole content, leaf dry weight and leaf biomass at flowering compared to uncovered vines. *Conclusions*: Shading considerably affected the viticultural performance of Muscat of Alexandria vines growing under hyper-arid conditions, modifying yield, leaf biomass and petiole nutrients.

Keywords: colored shade nets; global warming; solar radiation exclusion; *Vitis vinifera*; sustainable viticulture

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1. Introduction

Grapevine surface accounts for 7.4 Mha around the world, and it represents an important concern for many agricultural research areas [1]. Viticulture has developed in different climatic zones, however, as far as quality is concerned, this only corresponds to a narrow climatic margin, represented mainly by regions that present a Mediterranean climate [2]. Due to this distribution, viticulture is an economic sector that is highly vulnerable to the current effects of climate change [3–6]. During the last few decades, the projected impacts of climate change in viticulture have shown increases on thermal stress, water demand by evapotranspiration, growth inhibition, and changes in yield and berry quality [7,8]. Under this scenario, the new conditions in which viticulture it is going to develop, will be more stressful for the plants [9–11]. Due to this, the wine industry has been adapting to these changes around the world [12–14]. Since the last decade, a rapid development of sustainable adaptation strategies has been proposed in viticulture to cope climate change in the short to long term [10,11,15,16], in which shading nets has become an interesting alternative for grape production under the current climate change scenario [17–20].

Extreme heat events, water scarcity and high irradiance are increasingly observed in vineyards [21–23]. Prolonged exposition to high temperatures and UV-radiation may induce negative effects on vine physiology and berry composition [20]. By consequence, short-term adaptation strategies, that allows to keep or improve plant growth regulation, should be studied under these environmental conditions [10,20]. Sunscreen materials that form an inert particle film upon the leaves, such as calcium carbonate, kaolin, and potassium silicate have been studied to increase reflective capacity, allowing leaf cooling, and reducing leaf and cluster sunburns under severe summer stress [24–28]. Shading nets produce a similar effect, and they are proposed as adaptation technique to mitigate the impacts of global warming in viticulture since limit the effects of high temperatures and to limit evapotranspiration [9]. Shading nets installed over the vine allowed to reduce the photosynthetic photon flux at the leaf surface available for photosynthetic process, delaying berry ripening [10,29,30]. In a preliminary study, Lobos et al. [20] showed that shading nets may decrease the temperature of the whole-canopy and the fruit close to 7 ºC. Generally, overhead shade appears to be the most efficient way to decrease temperatures and water stress, as compared to full canopy shade, bunch shade, soil shade, and side-canopy shade [9,31]. However, more studies about timing and duration of shading, color, type, and specific canopy portion shading should be performed with a technical and economic decision [9,10,32].

Shade cloths utilization in apples can affect leaf nutrient concentrations because radiation can alter transpiration rates, changing the concentration of some nutrients and organic compounds [33]. To our knowledge, there are few studies that have evaluated the effects of different shading nets on vines much less in South America hyper-arid environments. Therefore, the aim of this research was to evaluate the effect of shading nets on yield, leaf biomass and petiole nutrients of a Muscat of Alexandria vineyard growing under hyper-arid conditions over three consecutive seasons.

2. Materials and Methods

2.1. Experimental Site and Plant Material

A field trial was conducted in a commercial Muscat of Alexandria (*Vitis vinifera* L.) vineyard, belonging to the Agricultural Research Institute (INIA) located at the Vicuña Experimental Center (Elqui Valley, Coquimbo Region, Chile; 30°02′ S, 70°41′ W, 630 m.a.s.l.) over three consecutive seasons (2006–07, 2007–08 and 2008–2009 seasons). The vines were own-rooted, planted during 1996 winter for Pisco elaboration at a planting distance of 2.0 m × 3.5 m, and trained on an overhead trellis system at a height of 2 m above the ground. The climate of the site is classified as hyper-arid since it rains lower than 100 mm per year [34–36]. Due to the low rainfall that was recorded during the season and the high vapor pressure deficit, it is necessary to apply water through irrigation. The soil family is classified as fine-silty over sandy-skeletal, mixed, thermic of the typic Camborthids and presents a loam-clay-sandy texture. The volumetric water content at field capacity and wilting point were 12.1 and 5.7%, respectively. The vines were managed according to the conventional viticultural practices used in the Elqui Valley, in terms of leaf management, fertilization, growth regulator applications, irrigation, pruning and disease control. An automatic weather station (AWS), located at 300 m from the vineyards was used to characterize the climatic conditions in terms of temperature, relative humidity, and precipitation during the studied seasons. Based on the data provided by the AWS, different bioclimatic indices, such as Growing Season Temperature (GST), Cool Night index (CI), Heliothermal index (HI), Growing Degree Days (GDD) and Biologically Effective Degree Days (BEDD) were calculated as is shown in Table 1.

Table 1. Bioclimatic indices calculated for each season under study.

Season	GST (°C)	CI (°C)	HI (Heat Units)	GDD (Heat Units)	BEDD (Heat Units)
2006–2007	18.28	9.0	2389	1756	1543
2007–2008	17.89	8.8	2311	1682	1473
2008–2009	18.45	9.1	2425	1798	1538
30-years (mean) [a]	18.5	10.0	2409.7	1808.2	1528.7

GST: Growing Season Temperature [37]. CI: Cool Night Index [38]. HI: Huglin's Heliothermal Index [39]. GDD: Growing Degree Days [40]. BEDD: Biologically Effective Degree Days [41]. [a] Mean of 1985–2015 years.

2.2. Experimental Design and Treatments

Three treatments were randomly arranged in the vineyard in a completely randomized design, with three replicates as follows: (i) a control, in which vines were managed without a shading net, (ii) a white shading net (Raschel mesh) that covers the vines with a 25% of shade, and (iii) a black Raschel net that covers the vines with 40% of shade. Each replicate covered an area of 98 m^2 (7 × 14 m) that corresponded to three rows and seven vines. The evaluations were carried out in the central row (seven vines), leaving the other vines as border among treatments. The shading nets were installed at a height of 4.0 m above the ground level, parallel to canopy and they were established since June 2006 until the end of the trial in June 2009. Therefore, the treatments were permanently installed during the three seasons under study. Fertilization and irrigation program were similar for all treatments and control and the irrigation criteria was based upon the water demand of the control plants. Harvest of the treatments and control was defined when control grapes reached a total soluble solids content between 21 and 22 °Brix. All treatments were harvested at the same time in each season.

2.3. Microclimate Variables

To characterize the microclimatic modifications induced by the shading nets, measurements of temperature, relative humidity, and solar radiation were made in each treatment. These measurements were carried out during non-consecutive twelve days, from bloom to harvest stages in the 2006–07 season. Temperature and relative humidity were measured using a HOBO sensor (model H08-0.32-08, HOBO Instruments, Bourne, MA, USA) located above the canopy level, recording data every 15 min, obtaining the daily average of each variable. Solar radiation was measured between 13:00 and 14:00 h on 100% clear days using a solar pyranometer (Apogee Instrument, Inc., Logan, UT, USA). Solar radiation measurements were made below the canopy and the shade nets, at the height of the bunches. All the measurements were made in each of the treatments, control, and replicates.

2.4. Yield Determination, Yield Components and Bunch Traits

At harvest, all the bunches of each replicate were manually harvested and weighed in a digital weight scale, recording the yield (kg $vine^{-1}$) and the number of bunches per vine. The bunch weight (g) was obtained, dividing the yield by the number of bunches per plant. In addition, a sample of 10 bunches per replicate was taken to the laboratory, where the number of berries per cluster, berry diameter, length and weight of rachis were measured.

2.5. Determination of Berry Physicochemical Parameters

Measurements of total soluble solids (TSS) were performed weekly to define harvest date. For each cluster, two berries were sampled at the top, the middle and the bottom of the cluster. Berry physicochemical parameters were determined at harvest by selecting 100 berries from each replicate. TSS measurement was done using a thermo-compensating refractometer (BRIX30 model, Leica, IFT 40, Fisher Scientific, Waltham, MA, USA), whereas pH and titratable acidity was determined using the OIV protocols [42]. Maturity index was calculated as the relationship between TSS and titratable acidity [43].

2.6. Determination of Vine Vigor Parameters

Determination of vine vigor parameters was performed according to the methodology exposed by Verdugo-Vásquez et al. [35]. Briefly, vines of each replicate were manually pruned in winter and the pruning weight (kg vine^{-1}) was determined. Based on yield and pruning weight, the Ravaz index was calculated as the ratio between yield and pruning weight, which represents the balance between vine reproductivity and vegetative activity.

2.7. Determination of Leaf Dry Weight and Leaf Biomass

Fifteen leaves were collected per treatment and replication between fruit set and veraison in the first two growing seasons. The determinations were performed at flowering, fruit set and veraison. The selected leaves were healthy, fully developed and illuminated and were brought to the laboratory. Three disks were cut from each leaf using a cork borer of 1.25 cm in diameter. The obtained samples were dried for 48 h in an oven at 60 °C, and then were weighed on a four-decimal scale. The biomass was estimated from the quotient between the averages of the weight and volume of the dry disks.

2.8. Determination of Petiole Nutrient Content

Determination of petiole nutrient content was also performed according to the exposed by Verdugo-Vásquez et al. [35]. Petioles of leaves opposite to the bunches were collected at flowering of each study season. Forty petioles per treatment and per replicates were sampled and then, dried in an oven at 65 °C until a constant mass was achieved. Subsequently, the samples were milled and sieved through a 1 mm mesh. N was analyzed according to The Kjeldahl method as was described by Nikolaou et al. [44]. P content of the leaf petioles was performed by the Olsen colorimetric method as reported by Verdugo-Vásquez et al. [35]. K was determined by atomic absorption spectrophotometry according to the method described by Garcia et al. [45]. Ca and Mg were determined by atomic absorption, while Zn, Mn, and Cu were analyzed by sample calcination and atomic absorption spectrophotometry [35]. Macronutrient concentration was expressed in terms of percentage (w w^{-1}), whilst micronutrients were expressed in ppm.

2.9. Statistical Analysis

Microclimate variables were analyzed using one-way analysis of variance, whereas the rest of the measured parameters were analyzed using two-way analysis of variance (ANOVA) to assess the influence of each treatment (control, white and black shading nets) and seasons (2006–07, 2007–08 and 2008–09). Statistical significance of the mean differences was determined by Tukey's test (p-value $\leq$ 0.05). ANOVAs were performed by Statgraphics Centurion XV Software (Statgraphics Technologies, Inc., The Plains, VA, USA).

3. Results

3.1. Climatic Conditions of the Study Site

A summary of rainfall and minimum and maximum temperature and relative humidity for the 2006–07, 2007–08 and 2008–09 seasons is presented in Supplementary Figure S1. Rainfall events were concentrated between the 151 and 211 days of year (DOY; end of May and June). The 2008–09 season accumulated the highest precipitation, recording 141 mm. Contrary to this, the accumulated rainfall during the first and second seasons was 52 and 39 mm, respectively (Supplementary Figure S1). The daily minimum temperature ranged between 6.7 and 7.5 °C, while the minimum relative humidity varied between 23.8 and 24.8% for the three growing seasons (from June to May). Moreover, the average temperature and relative humidity ranged between 15.1 to 15.9 °C and 59.6 to 61.6%, respectively. The maximum temperature and relative humidity varied between 24.5 to 25.8 °C and 87.1 and 92.3%, respectively. Bioclimatic indices calculated for each season showed that the 2008–09 season presented the highest values of GST, CI, HI and GDD, whereas the 2006–08 season exhibited the highest BEDD. The 2007–08 season presented the lowest values of GST, CI, HI, GDD and BEDD (Table 1).

3.2. Vine Microclimate Conditions

Temperature, relative humidity, and solar radiation determined in each treatment and control are presented in Figure 1. Shading nets did not affect temperature and relative humidity at any date of measurement. As expected, shading net treatments considerably (significantly) affected the incidence of solar radiation. In this fashion, control vines received an average value of 332 W m^{2-1}, while the white and black shading net treatments decreased the incident radiation to 245 W m^{2-1}, and 178 W m^{2-1}, respectively (26 and 46% less solar radiation than control, respectively).

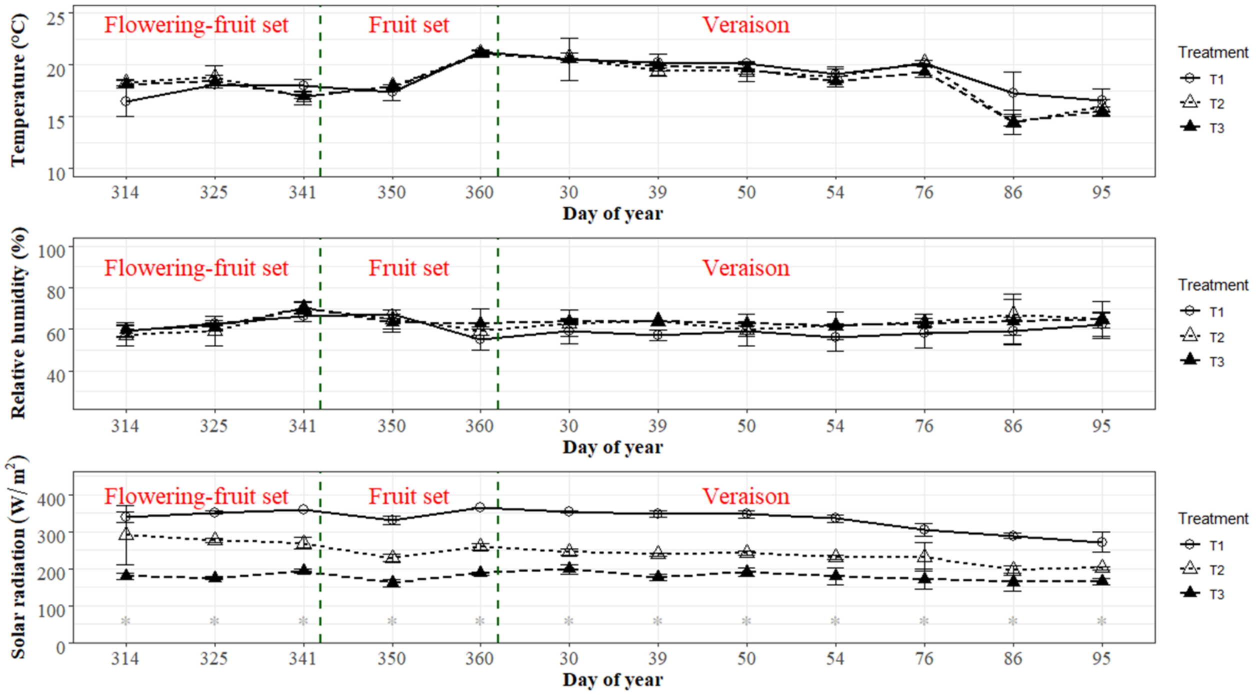

Figure 1. Seasonal pattern of temperature (°C), relative humidity (%), and solar radiation (W m^{2-1}) along the flowering-fruit set, fruit set; and veraison stages for control (T1), white shading net (T2) and black shading net (T3), during 2006–07 season. * Significant differences (Tukey's test, p-value ≤ 0.05).

3.3. Yield, Yield Components and Bunch Characteristics

Means of yield, yield components and bunch characteristics measurements after the installation of the white and black shading net treatments are shown in Table 2. Treatment factor did not affect yield, number of bunches per vine and rachis weight, but it had significant impacts on bunch weight, number of berries per bunch, berry diameter and rachis length. The vines covered by white shading nets presented the highest bunch weight and higher number of berries per bunch than the vines grown under the black shading net. Control vines presented the lowest berry diameter and lower rachis length than the vines grown under black shading net. Season factor affected most of the studied variables except for berry diameter and rachis weight. Second season induced the highest number of bunches per vine and lower yield than the first one, while the third season produced the highest bunch weight, and number of berries per bunch and the lowest rachis length in vines. Treatment and season interaction did not affect the measured parameters.

Table 2. Analysis of variance for yield, yield components and bunch characteristics by shading net treatments and control in 2006–07, 2007–08 and 2008–09 seasons.

Factor	Yield (kg Vine^{-1})	N° of Bunches Per Vine	Bunch Weight (g)	N° of Berries Per Bunch	Berry Diameter (mm)	Rachis Length (cm)	Rachis Weight (g)
Treatments (T)							
Control	6.3	22.1	218.6b	75.8ab	17.4b	17.5b	10.5
White shading net	6.8	21.6	277.6a	84.6a	18.3a	19.0ab	10.8
Black shading net	6.8	20.4	211.9b	66.6b	18.1a	20.4a	9.2
Season (S)							
2006–2007	5.1b	16.3b	204.7b	70.9b	17.9	20.7a	9.3
2007–2008	8.2a	30.9a	203.4b	69.8b	17.8	20.7a	10.6
2008–2009	6.5ab	16.8b	300.0a	86.4a	18.1	15.6b	10.5
Significance (*p*-value)							
T	0.751	0.835	0.002 [a]	0.003	0.003	0.001	0.343
S	0.004	0.000	0.000	0.002	0.497	0.000	0.463
T × S	0.233	0.256	0.578	0.439	0.183	0.479	0.631

For a given factor and significance $p \leq 0.05$, different letters within a column represent significant differences (Tukey's test, p-value ≤ 0.05). [a] In red, p-value lower than 0.05. If there were no statistical differences (p-value > 0.05, ns), the columns are presented without letters.

3.4. Yield, Yield Components and Bunch Characteristics

The analysis of berry physicochemical characteristics at harvest after the installation of the white and black shading net treatments are presented in Table 3. Treatment factor affected total soluble solids (TSS) content, while season affected all the studied parameters. The interaction between factors did not affect the measured variables. Thus, berries from vines covered with the black shading nets presented the highest TSS. The third season induced the highest TSS, pH and maturity index (TSS/TA), and the lowest titratable acidity (TA).

Table 3. Analysis of variance for berry physicochemical variables by shading net treatments and control in 2006–07, 2007–08 and 2008–09 seasons.

Factor	TSS (°brix)	TA (%)	pH	Maturity Index
Treatments (T)				
Control	21.8b	3.2	3.4	7.5
White shading net	21.9b	2.9	3.4	7.9
Black shading net	24.2a	3.0	3.5	8.7
Season (S)				
2006–2007	21.9b	3.3a	3.3b	7.0b
2007–2008	22.2b	3.6a	3.3b	6.1b
2008–2009	23.8a	2.2b	3.8a	11.0a
Significance (*p*-value)				
T	0.000 [a]	0.247	0.771	0.110
S	0.005	0.000	0.001	0.000
T × S	0.514	0.287	0.776	0.305

TSS: Total soluble solids. TA: Total acidity. For a given factor and significance $p \leq 0.05$, different letters within a column represent significant differences (Tukey's test, p-value ≤ 0.05). [a] In red, p-value lower than 0.05. If there were no statistical differences (p-value > 0.05, ns), the columns are presented without letters.

3.5. Vine Vigor Parameters

The analysis of vine vigor parameters after the installation of the white and black shading net treatments compared to control is shown in Table 4. The treatment factor affected pruning weight and Ravaz index, while season and the interaction of the factors did not affect vine vigor parameters. The vines located under the black shading net presented the highest pruning weight, whereas Ravaz index was higher in control vines than in the vines covered with the black shading net.

Table 4. Analysis of pruning weight and Ravaz index by shading net treatments and control in 2006–07, 2007–08 and 2008–09 seasons.

Factor	Pruning Weight (kg vine^{-1})	Ravaz Index
Treatments (T)		
Control	0.16b	46.6a
White shading net	0.17b	43.5ab
Black shading net	0.27a	25.6b
Season (S)		
2006–2007	0.19	30.2
2007–2008	0.21	48.3
2008–2009	0.20	37.2
Significance (*p*-value)		
T	0.003 [a]	0.040
S	0.750	0.110
T × S	0.478	0.634

For a given factor and significance $p \leq 0.05$, different letters within a column represent significant differences (Tukey's test, *p*-value ≤ 0.05). [a] In red, *p*-value lower than 0.05. If there were no statistical differences (*p*-value > 0.05, ns), the columns are presented without letters.

3.6. Leaf Dry Weight and Leaf Biomass

Results about leaf dry weight and leaf biomass analysis measured at flowering, fruit set and veraison after the installation of the white and black shading net treatments are shown in Table 5.

Table 5. Analysis of leaf dry weight and leaf biomass during flowering, fruit set and veraison affected by shading net treatments and control in 2006–07 and 2007–08 seasons.

Factor	Leaf Dry Weight (g)			Leaf Biomass (g cm$^{2-1}$)		
	Flowering	Fruit Set	Veraison	Flowering	Fruit Set	Veraison
Treatments (T)						
Control	0.125a	0.126	0.197	0.076a	0.077	0.126a
White shading net	0.092b	0.122	0.190	0.056b	0.075	0.116ab
Black shading net	0.086b	0.112	0.175	0.052b	0.068	0.107b
Season (S)						
2006–2007	0.095b	0.083b	0.203a	0.058b	0.051b	0.124a
2007–2008	0.107a	0.157a	0.172b	0.065a	0.096a	0.109b
Significance (*p*-value)						
T	0.000 [a]	0.193	0.074	0.000	0.193	0.002
S	0.030	0.000	0.001	0.030	0.000	0.001
T × S	0.003	0.529	0.027	0.003	0.529	0.040

For a given factor and significance $p \leq 0.05$, different letters within a column represent significant differences (Tukey's test, *p*-value ≤ 0.05). [a] In red, *p*-value lower than 0.05. If there were no statistical differences (*p*-value > 0.05, ns), the columns are presented without letters.

Treatment factor affected leaf dry weight at flowering and leaf biomass in flowering and veraison. The control vines presented the highest leaf dry weight at flowering and the highest leaf biomass at this same stage. Season factor affected all the studied parameters at flowering, fruit set and veraison. The vines in the 2007–08 season showed the highest leaf dry weight and leaf biomass at flowering and fruit set, and the lowest leaf dry weight and leaf biomass at veraison. The interaction of factors did not affect the measured parameters at fruit set. Control in the 2007–08 season presented the highest leaf dry weight at flowering and fruit set and the highest leaf biomass content at flowering (Supplementary Figure S2). Control in the 2006–07 season presented higher leaf dry weight and leaf biomass at veraison than most of the interactions except for white shading net in 2006–07 season.

3.7. Petiole Nutrient Content

The petiole nutrient content determined after the installation of the white and black shading net treatments is presented in Table 6. The treatment factor affected Ca, Mg and

Zn petiole content, whereas season factor influenced most of the measured nutrients except N and Mn. The interaction of the factors did not affect the petiole nutrient content. Petioles from control vines presented the highest content of Ca and higher content of Mg and Zn than the ones from the black shading net treatment. The vines presented the lowest content of P and K and the highest content of Zn in petioles in the 2008–09 season. Conversely, the vines presented the highest petiole content of K, Ca, Mg and Cu in the 2006–07 season.

Table 6. Analysis of petiole nutrient content at flowering affected by shading net treatments and control in 2006–07, 2007–08 and 2008–09 seasons.

Factor	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Zn (ppm)	Mn (ppm)	Cu (ppm)
Treatments (T)								
Control	0.68	0.12	3.13	1.38a	0.34a	86.7a	53.4	6.6
White shading net	0.61	0.11	2.81	1.26b	0.30ab	75.8ab	53.4	6.0
Black shading net	0.61	0.09	2.98	1.17b	0.26b	69.4b	46	6.2
Season (S)								
2006–2007	0.67	0.13a	3.47a	1.49a	0.38a	79.3b	50.2	8.3a
2007–2008	0.60	0.12a	2.98b	1.05c	0.23b	58.2c	50.3	5.4b
2008–2009	0.63	0.07b	2.47c	1.27b	0.28b	94.3a	52.3	5.0b
Significance (*p*-value)								
T	0.057	0.110	0.191	0.001 [a]	0.002	0.021	0.077	0.590
S	0.139	0.001	0.000	0.000	0.000	0.000	0.799	0.000
T × S	0.625	0.791	0.807	0.364	0.278	0.485	0.704	0.883

For a given factor and significance $p \leq 0.05$, different letters within a column represent significant differences (Tukey's test, p-value ≤ 0.05). [a] In red, p-value lower than 0.05. If there were no statistical differences (p-value > 0.05, ns), the columns are presented without letters.

4. Discussion

As far as we know, there are no previous studies on Muscat of Alexandria grape that evaluate the utilization of a permanent netting structure with a productive-nutritional approach. Nowadays, sunlight intensity has become one of the most important climatic parameters that influences dry matter production and fruit yield by its direct relationship with evapotranspiration and consequently with the vine water consumption [46]. However, this is a long-standing problem in vineyards and crops grown in hyper-arid areas in South America. As expected, our results showed that the average solar radiation in vines covered by shading nets was lower in all phenological stages assessed due to the solar radiation attenuation promoted by reflection and absorption of the nets (Figure 1). Subtle differences were observed in temperature and relative humidity that would be explained by the fact that the shading nets were installed on the row of vines, in the form of a continuous roof and without closing the edges, thus generating a structure that was not completely closed. Studies have established that a range between 1000–1500 µmol m^{-2} s^{-1} (217–326 W m^{2-1}) of photosynthetically active radiation (PAR) are needed to achieve the maximum CO_2 assimilation rate in vines [47].

Our results showed lower yields in the covered vines than those reported in the same cultivar by Buesa et al. [48]. Local experiences with own-rooted Muscat of Alexandria vines have pointed out that in the areas that present light-textured soils, plants decrease their vigor severely since the fifth productive season which directly affects yields. Black shading net treatments improved vine vigor parameters and induced a considerably higher total soluble solids in grapes (Tables 3 and 4). Gutiérrez-Gamboa et al. [49] reported that leaf to fruit ratios affected the accumulation of soluble solids in different grapevine cultivars, delaying it as the ratio decreased. In addition, these authors reported that the percentage of shoot lignification increased gradually as the leaf to fruit ratios raised. The leaf-to-fruit ratio or the light-exposed leaf area per fruit quantity (m kg^{-1}) is known as an essential parameter in grape development [50]. Leaf area affects the leaf gas exchange and the content of carbohydrates available through photosynthesis for vegetative growth and grape maturation, while the grapes, as a sink of nutrients, affect the accumulation of C and N required for their maturation [50–52]. Based upon this notion, Verdenal et al. [50]

reported that an excessive leaf area can induce N deficiency in the must. Therefore, a balanced leaf-to-fruit ratio (1 to 1.2 m kg^{-1}) should be defined to guarantee grape maturity and N recovery in the reserve organs. In this fashion, it is probably that black shading nets allowed to modify leaf to fruit ratio due to light exclusion, increasing the accumulation of soluble solids in berries. In this sense, in our study there were no significant differences in yield due to the use of shading nets. However, the pruning weight (estimator of the vegetative growth of the season) presented highly significant differences. Therefore, the higher accumulation of total soluble solids in the bunches under the black shading net may be due to presenting more vegetative growth for the same level of yield. Similar results were found by Verdugo-Vásquez et al. [53] in Cabernet Sauvignon and Chardonnay vineyards, where the zones within the vineyard with the highest accumulation of total soluble solids were related to the balance between vegetative growth and yield.

Some yield components seem to be enhanced with the use of a white shading net; however, the number of bunches per vine did not show statistical differences among the treatments and control. Vine yield considerably displayed a season influence and certain parameters such as the cluster number of the second season was higher than those observed in the first and third seasons (Table 2). Despite that pruning was similar among the treatments, the evaluation of bud fertility could be an auxiliary data to support the conclusions, but it seems to be that shading nets improved certain yield parameters as the vigor declines (Tables 2 and 4).

Black shading net considerably decreased solar radiation incidence, which resulted in low bunch weight and number of berries per bunch, and longer rachis. Muscat of Alexandria presents female flowers with recurved stamens and tends to develop millerandage [54,55]. Black shading net induced high pruning weight and Ravaz index, which represents an excess of vigor over crop production. Acimovic et al. [56] reported that decreasing leaf foliar area in Pinot Noir considerably reduced the number of berries per cluster, which was associated with transport and carbohydrate translocation. Additionally, it has been reported that reduction of intercepted light (for example, shading nets) generates abscission of flowers, which induces alterations in the signaling and transport pathways of sugars because of a global upregulation pattern in the genes that encode sugar metabolizing enzymes [57].

In addition, Mg petiole content decreased in the vines covered with the black shading nets (Table 6). This microelement is a structural component of chlorophyll and is involved in the protein production and in enzymatic processes [58]. Mg deficiency influences the partitioning of dry matter and carbohydrates between shoots and roots, resulting in a massive accumulation of carbohydrates in source leaves, especially of sucrose and starch [59]. These authors suggested that the accumulation of carbohydrates in Mg-deficient leaves is caused by Mg deficiency stress and not because of reduced sink activity.

Generally, black shading net decreased leaf dry weight and leaf biomass measured at flowering compared to uncovered vines (Table 5). The spectra of absorption, reflection and transmission of radiation varies with leaf thickness, age, water content, surface morphology and orientation [60]. In this way, leaf transpiration rate was decreased by shading at flowering, which was associated with a decrease in specific leaf dry weight [61]. The rate of photosynthesis in leaves depends on the concentration of CO_2 in the chloroplast, but the affinity for CO_2 of Rubisco is low [62]. To perform efficient CO_2 fixation, it is essential to increase the stomatal conductance for CO_2 diffusion from the ambient air to the chloroplasts [61]. Thickness of leaves is one of the important determinants in conductance for CO_2 diffusion and the reason why the leaves more exposed to the sunlight are thicker than the leaves in the shade [63].

A concerning issue that was not covered in this study is the effects of shading net on gas exchange and vine water status. Shading nets can decrease vine water requirements, resulting in vines with high vigor. High solar radiation and increased temperatures have modified the common conditions of viticulture in Mediterranean climates. However, it is necessary to extend the knowledge about the effect of these techniques on abiotic stress, such as sunburn in grapes, and by consequence, to evaluate their effects on the metabolic profile of the grapes and wine obtained from them.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae7110445/s1, Figure S1: Weather parameters recorded during study: rainfall (mm), maximum and minimum daily temperature (°C) and relative humidity (%) registered in the 2006–07, 2007–08, and 2008–09 seasons in Vicuña, Elqui Valley, Coquimbo Region (Chile). Figure S2: Significant interactions plot of analysis of variance for leaf dry weight and leaf biomass (Table 5) according to three shading net treatments in 2006–2007 (S1) and 2007–2008 (S2) seasons. A: Leaf dry weight at flowering, B: Leaf dry weight at veraison, C: Leaf biomass at flowering and D: Leaf biomass at veraison.

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