CANOPY MICROCLIMATE: THE EFFECT OF EXPOSURE ON THE NUTRACEUTIC PROPERTIES OF *VITIS VINIFERA* L CV. DOLCETTO GRAPE BERRIES.

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Introduction

Several studies report the importance of the effect of canopy microclimate on berry composition (Smart et al. 1985, Carbonneau et al.1987, Spayd et al. 2002). These studies show the effect of light exposure on specific phenolic compounds of the berries. In grapevine canopies, depending on canopy architecture, leaves and bunches can develop in conditions varying from heavily shaded to middle shaded to fully exposed canopies. The relathionship between sunlight exposure and temperature cluster is important to berry composition and metabolism (Spayd et al. 2002). Generally, berries that develop in fully exposed conditions have higher juice sugar concentration, lower juice pH, higher titratable acidity and an increased of berry phenolics.

Considering the relathionship between phenolic compounds and antioxidant activity, aim of this work was to evaluate the nutraceutical proprierties in red Dolcetto grape skin and seeds from three different canopy treatments.

Material and Methods

The study was carried out using V*itis vinifera* L. red grape Dolcetto cultivar grown in the experimental field of the Research Unit for Enology of Velletri in Lazio region (Italy) (41° 40.5' N latitude, 12° 50.7' E longitude) at 355 m above sea level, during 2011 and 2012 vintages. The climatic is described as temperate climate. The trials were made in triplicate. Grapes were harvested at technological maturation. In both years the same cultural practise were found in vineyard. Three replicates for each treatments were applied to 12 vines, giving a total of 36 replicates. Vines were trained to the Single Cordon Spur with plant density of 2,60 x 2.00 m and pruned approximately 10 nodes per meter of cordon. Three levels of bunch exposure were imposed: heavily shaded; middle shaded; fully exposed. All of the treatments were made after winter vine pruning. Bunches on the cordon were used for all treatments.

Bunches on shoots where leaves had not been removed represent the heavily shaded treatment Bunches on shoots where leaves had been removed represent the fully exposed treatment. Bunches on shoots where leaves had been partially removed represented the middle shaded treatment.

In the fully exposed treatment the leaves were removed from shoot in the vicinity of the bunches lower and upper cordon. In the middle shaded treatment the leaves were removed only lower shoot and infertile shoots were removed. All the treatments were applied when berries were at the veraison stage.

Climatic data. Climatic data were supplied by the weather station belonging to the Regional Institute of Meteorology. This weather station is located at "Research Unit for Enology in Velletri" (Rome) and it is representative of the weather in the area. GDD (Growing Degree Days), daily mean temperature, monthly mean temperature, day-night temperature difference, radiation, rainfall and number of rainy days were considered.

Polyphenol extraction. Three groups of 50 berries for each treatment were randomly sampled from the bunches (vintage 2011 and 2012) for phenols extraction and DPPH analysis. The extraction solution (buffer to pH 3,2) composition per liter was: tartaric acid 5g; NaOH 1N 22 mL, Na2SO2O5 2 g; 120 mL ethanol 96% and distilled water up to 1000 mL. Skins, and seeds were manually separated, and dried in incubator at *105°C* until constant weight. Then dried skin and seeds were incubated with 125 ml of 3.2 pH Tartaric buffer , for 48 hours for skins , and 144 hours for seeds at 30°C. Then the samples were homogenized and centrifuged at 4000 rpm, for 15 min, and the supernatant was collected for analysis. Some grape berries for each treatment were pressed and the juice analyzed to determine oenological parameters.

Total Polyphenols. The amount of total polyphenols (TP) in the grape skin extract and in grape seeds extract were determined according to the Folin-Ciocalteau method. Catechin was employed as a calibration standard and results were expressed as catechin equivalent (CE) of dry matter (mg CE/g of skin or seeds DM). The absorbance was measured using a spectrophotometer at the wavelength of 750 nm.

Total Flavan 3-ols. The total flavan 3-ols (FL) content were determined by the vanillin assay (Di Stefano & Cravero, 1991) using catechin as the standard and expressed as catechin equivalent of dry matter (mg CE/g of skin or seeds DM) through the calibration curve of catechin. The absorbance was measured at the wavelength of 500 nm.

Total anthocyanin (TA) contents of the skin extract was determined using the method of Di Stefano *et al. (1991)* Malvidin 3-0-glu was employed as a calibration standard and results were expressed as malvidin-30-glu equivalent (ME) of dry matter (mg *MEI* g of skin DM).

Antiradical activity: The antioxidant activity (AA) was measured employing free stable radical 2,2-diphenyl-1-picryl-hydrazyl ($DPPH^{\cdot}$) spectrophotometric assay (Brand-Williams *et al.,*1995; Sanchez Moreno, 2002), and slightly modified.
Briefly: 0.1 mL of sample, at various concentration, was added to 3.9 ml of DPPH solution 4.73 x 10^{-2} g/L) and to 3.9 mL of methanol (spectrophotometric blank). The kinetics of absorbance of DPPH for different sample concentrations were monitored at a constant temperature of 23°C, wavelenght of 515 nm, every 10 minutes until reaching the plateau. The initial concentration of $DPPH^{\cdot}$ was controlled by using a calibration curve made by measuring the absorbance at 515 nm of standard samples of $DPPH^{\cdot}$ at different concentrations. The antiradical activity was defined as mg of skin or seeds necessary to decrease the initial DPPH concentration by 50% (EC50). All the measurements were made in triple, the results were expressed as ARP (1/EC50).

Statistical analysis. The statistical analysis was carried out with analysis of variance (ANOVA) and the means were compared with Least Significant Difference (LSD) test.
For data analysis, the “Statistical package” (version 7.1, StatSoft., Italy) was used.

Results and Discussion

In both years the grape was harvest to technological maturation. There were no significant differences in the juice oenological parameters between three treatments (table 1). In agreement with other works (Crippen and Morrison 1986), the light had no effect on juice oenological parameters even if the fully exposed grape was harvest three and six days before in middle shaded and in heavily shaded respectively.

Table 1. Oenological parameters

	2011 year		
	soluble solids g/L	***pH***	***titratable acidity g/L***
Heavily Shaded	196 ± 0.07	3.25 ± 0.01	6.70 ± 0.20
Middle Shaded	198 ± 0.12	3.30 ± 0.02	7.02 ± 0.15
Fully Exposed	200± 0.10	3.28 ± 0.01	6.92 ± 0.21
		2012 year	
Heavily Shaded	195 ± 0.09	3.30 ± 0.01	6.55 ± 0.12
Middle Shaded	196 ± 1.30	3.29 ± 0.02	6.98 ± 0.09
Fully Exposed	198 ± 1.15	3.31 ± 0.02	6.75 ± 0.13

Titratable acidity expressed as tartaric acid. The values are the mean of three replicates for each treatments ± standards deviation.

Data of grape berry morphology (table 2) showed significant differences ($p \leq 0.05$) in the morphological characteristic of the berry among heavily shaded, middle shaded and fully exposed and the significant differences ($p \leq 0.05$) between the same treatments in two years. Grapes developed on bunches fully exposed, for both years, showed a higher berry weigh and a higher number of seeds per berry. The differences observed between same treatment in 2011 and 2012 depended on exclusively from different climatic conditions in two years.
Both skin and seeds differences significant ($p \leq 0.05$) in phenolic content among three treatments and between same treatments in two years were also found (table 3). The amount of TA, FL and TP was significantly higher in skin berries from heavily shaded bunches in both years, even if differences were significant between two years. While TA, FL and TP were significantly higher in berries skin from fully exposed bunches in 2012 with respect to the 2011. In seeds the higher concentrations of TP and FL were found in all three treatments in the 2011 with only exception of the FL higher in the berries seeds from fully exposed bunches in 2012.

Table 2. Grape berry morphology

	2011 Year			
	Berry Weight g	***Skin Weight g***	***Seed Weigth g***	***N° seeds/berry***
Heavily Shaded	1.81 ± 0.03 ^{d,e}	0.33 ± 0.01 ^{c}	0.043 ± 0.02 ^{c}	1.45 ± 0.03 ^{a}
Middle Shaded	1.56 ± 0.01 ^{b}	0.41± 0.01 ^{d}	0.039 ± 0.02 ^{b}	1.45 ± 0.03 ^{a}
Fully Exposed	1.83 ± 0.05 ^{e}	0.37 ± 0.02 ^{d}	0.033 ± 0.01 ^{a}	1.78 ± 0.04 ^{b}
		2012 Year		
Heavily Shaded	1.69 ± 0.02 ^{c}	0.21 ± 0.01 ^{a}	0.031 ± 0.01 ^{a}	1.53 ± 0.02 ^{a,b}
Middle Shaded	1.35 ± 0.02 ^{a}	0.26 ± 0.01 ^{b}	0.037 ± 0.02 ^{b}	1.85 ± 0.05 ^{b,c}
Fully Exposed	1.71 ± 0.05 ^{c,d}	0.25 ± 0.01 ^{a,b}	0.044 ± 0.02 ^{c}	1.90 ± 0.05 ^{c}

The values are the mean of three replications ± standard deviation, the different superscript letters indicate the significant differences ($p \leq 0.05$).

Table 3. Phenols content in 2011 and 2012 years

	2011 Year				
	Skin			***Seeds***	
	TA	***FL***	***TP***	***FL***	***TP***
Heavily Shaded	12.67 ± 0.98 ^{e}	8.42 ± 0.80 ^{c}	25.70 ± 1.62 ^{b}	21.13 ± 1.26 ^{e}	40.96 ± 2.56 ^{d}
Middle Shaded	7.57 ± 0.51 ^{b,c}	5.95 ± 0.71 ^{a}	19.97 ± 1.25 ^{a,b}	21.21 ± 1.18 ^{e}	26.23 ± 1.21 ^{a,b}
Fully Exposed	6.84 ± 0.52 ^{a,b}	5.01 ± 0.09 ^{a}	16.25 ± 1.10 ^{a}	4.30 ± 0.42 ^{a}	21.79 ± 1.52 ^{a}
	2012 Year				
Heavily Shaded	9.65 ± 1.20 ^{d}	13.22 ± 0.48 ^{d}	37.45 ± 1.02 ^{d}	11.03 ± 0.98 ^{d}	44.71 ± 1.86 ^{d,,e}
Middle Shaded	6.39 ± 0.85 ^{a}	5.87 ± 0.51 ^{a}	29.30 ± 2.20 ^{c}	10.19 ± 0.51 ^{c,d}	46.91 ± 1.51 ^{e}
Fully Exposed	7.85 ± 0.82 ^{c}	7.42 ± 1.02 ^{b}	25.87 ± 1.98 ^{b}	6.36 ± 0.52 ^{b,c}	36.31 ± 1.05 ^{c}

A=Total Anthocyanins (mgMElg of DM); FL= Flavan-3-ols (mgCE/g of DM); TP= Total Polyphenols (mgCE/g of DM). The values are the mean of three replications ± standard deviation, the different superscript letters indicate the significant differences ($p \leq 0.05$).

The antioxidant activity in both skin and seeds (figure1 and figure 2) expressed as ARP (1/EC50) was significant higher ($p \leq 0.05$) in 2011 with respect to 2012 for all the treatments and was significantly higher ($p \leq 0.01$) in seeds with respect to the skin in agreement with other authors (BaliK et al. 2009, Giannini et al. 2011). In 2011 the berries skin from heavily shaded showed higher value of ARP significantly different from middle shaded and fully exposed. In 2012 the ARP value was not significantly different between heavily shaded and middle shaded treatments that showed lower value.

In seeds extract 2011 the ARP value was significantly higher ($p \leq 0.05$) in fully exposed and significant lower ($p \leq 0.05$) in middle shaded. Also in 2012 the ARP was significantly higher in fully exposed and there was not significant differences between heavily shaded and middle shaded. From the results of three experiments, it appeared that the plant metabolism responded to change in light condition. In conditions where bunches are heavily shaded the light should be a limiting factor in the accumulation of phenols during ripening, as reported by other authors (Haselgrove et al. 2000), on the contrary in the our results the higher content of all phenolic compounds in both years, even if with different concentrations, were found in berries skin and seeds of heavily shaded. In our research the heavily shaded represent a treatment where the leaves are not been removed, so low lighting is due exclusively to the shade made from leaves. While, in research of other authors, the heavily shaded treatment was made covering completely the bunches with aluminum foil. Therefore, in our case, the high content of phenolic compounds in heavily shaded is due as to strong natural abiotic stress response in which the vine was exposed, while in the bunches completely covered there is a inhibition in the accumulation of phenol compounds.

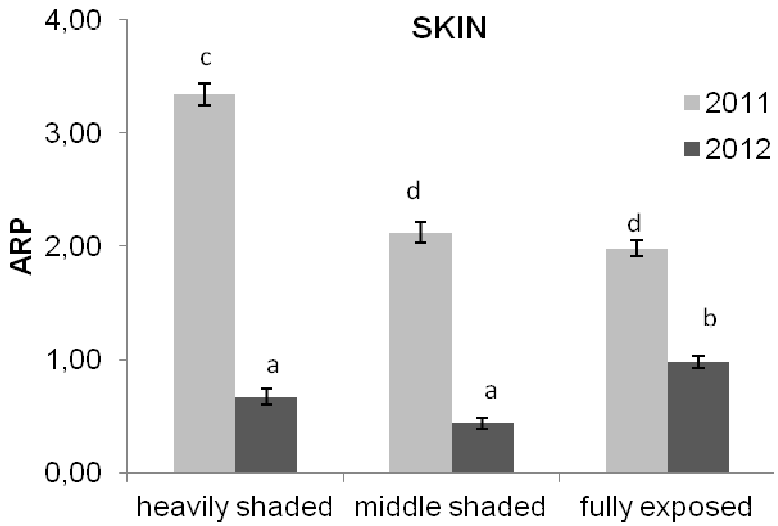

Figure 1. Antioxidant activity in grape skin extract expressed as ARP (1/EC50) in the three treatments. Bars indicate ± standard deviation. The different letters indicate significant differences (p ≤ 0.05).

The lower phenol content in fully exposed bunches among the three treatments was due to less stress by low lighting.

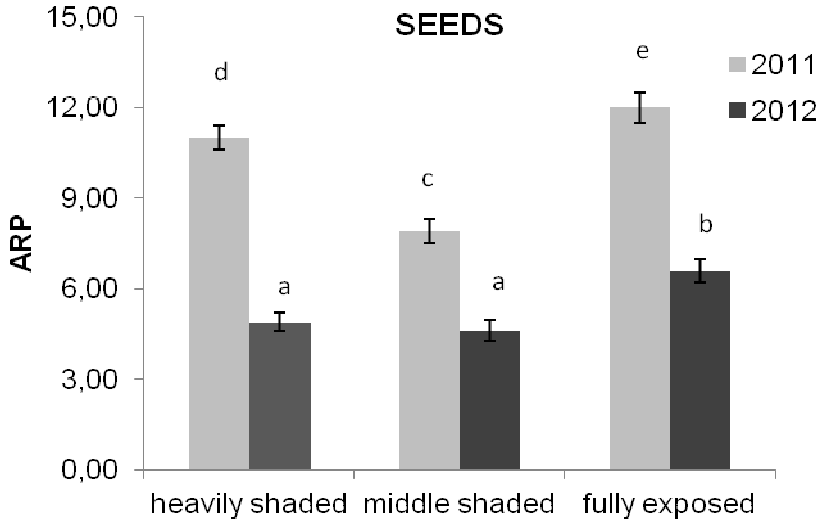

Figure 2. Antioxidand activity in grape seeds extract expressed as ARP (1/EC50) in the three treatments. Bars indicate ± standard deviation. The different letters indicate significant differences (p ≤ 0.05).

The AA in both skin and seeds in 2012 from grape bunches from fully exposed treatment was higher with respect to other two treatments. Although it is amply demonstrated the high correlation between polyphenols and antioxidant activity, however the seeds extract from grape fully exposed, in both years, showed the lower phenolic content and the higher antioxidant activity. While the skin extract from heavily shaded, for both years, showed the higher phenolic content, however, the antioxidant activity was higher only in 2011 year. This fact suggests that the light exposure of the bunches has effect both on the quantity and on the quality of polyphenols. In addition, the differences observed both in phenol content and in AA, between same treatment in two years were due exclusively to climatic differences.

The climatic analysis showed that climate conditions were different in the site analyzed in summertime. From veraison to ripening, in 2012 GDD (Growing Degree Days) values (Figure 3), daily and monthly mean temperatures, sum of radiation were generally higher from late of June to 20 August. In addition, the year 2012 showed a positive anomaly of about +4°C in the maximum temperature and of about +3°C in the minimum temperature with reference to the 1993-2010 means values. Year 2012 was more rainy (1198.4 mm) than 2011 (1082.2 mm). During the period from April to September rainy days were 39 in 2011 and 52 in 2012.
The interaction among climatic conditions and canopy treatments affect significantly the composition and concentration of phenolic compounds and as a consequence the antioxidant activity.

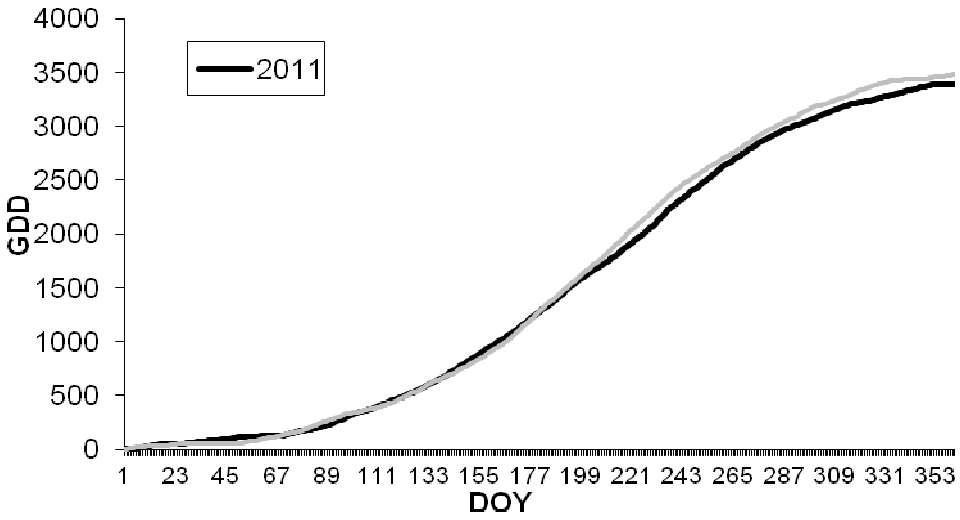

Figure 3. - GDD cumulated in the days of the year (DOY)

Conclusion

The interaction between climatic conditions, canopy treatments effect both on the morphological characteristic, and on the phenolics and on the antioxidant activity.
The response of total polyphenol, flavanol, anthocyanin contents and antioxidant activity to treatment conditions was variable and depending on the degree of bunch shading. Grapes that had developed on bunches receiving high levels of light (fully exposed) for both years, showed a higher berry weigh and a higher number of seed per berry.
The seed extract from grape fully exposed, in both years, showed the lower phenolic content and the higher antioxidant activity. The skin extract from heavily shaded, for both years, showed the higher phenolic content, however, the antioxidant activity was higher only in 2011 year. The effects of light on quali-quantitative berry phenolic depend on light exposure of the bunches.
Therefore in temperate climate conditions, if from the oenological point of view, the high degree of bunch light exposure may not be desirable for the production of phenol content particularly for the production of anthocyanins in berries, from the nutraceutical point of view the bunches from fully exposed showed higher antioxidant activity.

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Abstract

In grapevine canopies, depending on canopy architecture, leaves and bunches can develop in conditions varying from heavily shaded to fully exposed. Studies on the effect of light exposure on specific phenolic compound and on the nutraceutical properties, evaluated as antioxidant activity, in seed and skin extract of Dolcetto grape cultivar are reported. Three levels of bunch exposure were carried out: fully exposed, middle shaded and heavily shaded. The grape was harvest at technological maturation during 2011 and 2012 vintage.

The response of total polyphenol, flavanol, anthocyanin contents and antioxidant activity to treatment conditions was variable an depend on the degree of bunch shading. Grapes that had developed on bunches receiving high levels of light (fully exposed) for both years, showed a higher berry weigh and a higher number of seed per berry.

The seed extract from grape fully exposed, in both years, shows the lower phenolic content and the higher antioxidant activity. While the skin extract from heavily shaded, for both years, showed the higher phenolic content, but the antioxidant activity was higher only in 2011 year. The effects of light on quali-quantitative berry phenolic content are discussed.

***Keywords**: canopy microclimate, Dolcetto cultivar, phenol contens, antioxidant activity.*