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Responses of four winegrape varieties to managed water stress and partial defoliation in an arid environment

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Summary

In viticulture, the imposition of managed water deficits is a strategy which has been used to increase both water use efficiency and winemaking quality in arid climates. Partial defoliation early in the season is another innovative practice that may also be used as an aid in regulating yield components and improving fruit quality. The aim of this work was to evaluate the effects of managed water stress and early season partial defoliation on crop yield and quality in two autochthonous ('Frappato' and 'Nero d'Avola') and two international varieties ('Syrah' and 'Cabernet Sauvignon') growing in an arid environment. The four treatments were: (i) no leaf removal, un-irrigated, (ii) no leaf removal, irrigated at 30 % of estimated crop evapotranspiration (ETc), (iii) partial leaf removal, un-irrigated, and (iv) partial leaf removal, irrigated at 30 % of estimated ETc. The results confirm the effectiveness of partial defoliation in yield management which leads to smaller clusters. Managed water stress was also an effective strategy for reducing berry size, improving must quality and generally enhancing anthocyanin accumulation.

Key words: *Vitis vinifera* L., irrigation management, partial defoliation, winemaking quality.

Introduction

Sicily's climate is typically Mediterranean. It is characterised by rainfall (500-1,500 mm) during a cool winter and by insignificant rainfall during a hot summer. In modern viticulture, it is widely recognised that under these conditions the careful management of irrigation and of the canopy can play a key role in determining must and wine quality. Maintaining the right balance between vegetative and reproductive growth under irrigated conditions is one of the more difficult problems in viticulture (De Souza *et al.* 2005).

Irrigation during the growing season increases vine vigour and can also increase the quality of the fruit (Dos Santos *et al.* 2007). However, a number of authors (Dokoozlian and Kliewer 1996, Keller and Hrazdina 1998) have shown that too much irrigation stimulates excessive vegetative growth, creating a dense canopy and thus poor exposure of the fruit to light. This has the result that fruit is of reduced quality. Moreover, lateral shoot growth is stimulated with the result that competition for photosynthates is increased. An overly-dense canopy also creates conditions that predispose berries and leaves to disease and delays ripening (Schreiner *et al.* 2007, Dos Santos *et al.* 2007). Under conditions of inadequate irrigation, severe water deficits develop and the vines respond by closing stomata which reduces both water loss and photosynthetic gas exchange (Esteban *et al.* 1999). In grapes, the phenological stages most sensitive to water deficit are during early vegetative growth and during the first phases of berry growth. Leaf photosynthetic function and post-veraison berry growth are less sensitive to water deficits (Lu and Neumann 1998).

In recent years, a number of novel irrigation strategies have been proposed, including regulated deficit irrigation (RDI), whereby irrigation is reduced during a well-defined period of berry development, and partial root-zone drying (PRD) in which water is applied to alternating areas of the root system to help manage the balance between vegetative and reproductive growth (Loveys *et al.* 2000).

To increase water use efficiency (yield per unit of water applied), Mc Carthy *et al.* (2000) suggested that RDI was an effective strategy for improving fruit composition. RDI can be done either during the whole season or during only certain phenological stages. Beneficial effects can include the control of canopy vigor, improvements in fruit exposure to light and reductions in berry growth which helps to reduce any undesirable dilution effects of irrigation on berry composition (Intrigliolo and Castel 2009).

The effects of a managed water deficit during the growth period are controversial. Some studies have reported beneficial effects on berry quality with increases in anthocyanin and polyphenol concentrations as well as in total soluble solids (Roby *et al.* 2004). However, others have reported higher berry quality in irrigated vines than in rain-fed ones (Esteban *et al*. 1999; Reynolds *et al.* 2007).

Early leaf removal (Poni *et al.* 2006; Intrieri *et al.* 2008), is another innovative viticultural practice which may be used to help regulate yield components and to improve fruit quality (Diago *et al.* 2010; Nicolosi *et al.* 2012). Direct interception of sunlight by the fruit is usually associated with improved quality and is widely recognised as being desirable by viticulturalists under certain climatic conditions (Austin and Wilkox 2011). In addition

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to increasing direct exposure to sunlight, removing leaves around the fruit also enhances the evaporative potential within the fruit zone, lowering humidity and making the cluster microclimate less conducive to the development of the familiar fungal diseases (English *et al.* 1990). Furthermore, increased exposure to sunlight raises fruit temperature (Smart and Sinclair 1976), which has the potential to alter aspects of berry physiology (Downey *et al.* 2006).

The aim of our study was to evaluate the effects of water stress and early defoliation on fruit yield and quality, by monitoring the water status of both autochthonous and international winegrape cultivars growing in an arid climate.

Material and Methods

Plant materials, site and experimental design: The experiment was conducted over two growing seasons in 2011 and 2012 in a commercial vineyard in the Ragusa district of Sicily (lat. 37°01' N; long. 14°32' E; elevation 220 m) bordered on all sides by other vineyards. The vineyards had been established in 2001 on a deep, sandy soil with the following four *Vitis vinifera* L. varieties: 'Frappato', 'Nero d'Avola' (autochthonous), and 'Syrah' and 'Cabernet Sauvignon' (international). All varieties were grafted onto '140 Ru.' rootstocks. The vines were spaced at intervals of 2.50 m between rows and 0.9 m within rows. The rows were oriented east-west and trained on a unilateral cordon system at a height of 0.5 m with the top of the canopy at approximately 1.60 m. The experimental area was served by an irrigation system. Each irrigation treatment plot was equipped with its own timed valve to control water delivery. All vines were pruned to between three and five nodes per vine, with shoots pruned to two buds. All shoots derived from *bourillon* and adventitious buds were hand-pruned to retain six to ten shoots per vine. The shoots were positioned vertically and were not hedged during the growing season. Annual rainfalls of 618 mm (2011) and 438 mm (2012) were recorded.

Treatments consisted of: (i) no leaf removal and rainfed with no irrigation (NLR-NI); (ii) no leaf removal and irrigated at 30 % of estimated crop evapotranspiration (ETc) (NLR-I); (iii) leaf removal and rain-fed with no irrigation (ELR-NI); (iv) leaf removal and irrigated at 30 % of estimated ETc (ELR-I). The treatments were applied to four replicate plots each containing five vines (20 vines per treatment) and arranged in a completely randomised design.

Early leaf removal, irrigation treatments and vine water status: Three weeks after full bloom, all main and lateral leaves from the cordon up to the leaf of the last cluster in each shoot were removed by hand (fruit-set: BBCH 71). At this stage the berries were approximately 6-7 mm in diameter (Koblet *et al.* 1994). This stage occurred on 3rd June 2011 and on 28th May 2012 for 'Frappato' and 'Syrah', and on 17th June 2011 and 14 June 2012 for 'Cabernet Sauvignon' and 'Nero d'Avola'. Leaf area per vine was measured just after leaf removal on both main and lateral shoots using a leaf area meter (model LI-3100; Licor, Inc., Lincoln, Nebraska).

Weather parameters were measured with a meteorological station and reference evapotranspiration (ETo) was calculated by the Penman-Monteith equation (Allen *et al.* 1998). Rainfall over the (warm) June-September period was of 58 mm (2011) and 18 mm (2012), with ETo values of 611 mm (2011) and 578 mm (2012). Crop evapotraspiration was estimated as the product of ETo and a crop coefficient (Kc). The values of Kc used were 0.30 from flowering (early June) to veraison (end of July) and 0.15 from veraison to harvest. Irrigation was applied every 15 d and started on 23 June (2011) and on 18th June (2012). Irrigation was discontinued at the end of August in both years.

Midday stem water potential (Ψ_{stem}) at solar noon was measured by a Scholander pressure chamber (Soil moisture Equipment Corp., Sta. Barbara, CA, USA) on the day before and on the day after irrigation from 1st August to 15th September in both years according to Matthews *et al.* (1987). Briefly, a leaf was enclosed in a small black-plastic bag covered with aluminum foil, at least 1 h before detachment for water potential measurement. Five replicates leaves were measured per treatment and these were taken from different shoots in each plot.

Crop yield and analysis of berry quality: The yield of each cultivar was harvested at maturity stage on the last decade of September in both years. For yield assessment, the number of clusters on each vine was counted (n) and these were then weighed to determine total yield per vine (kg·vine^{-1}). In the laboratory, a sample of 18 clusters per treatment was dissected and used to determine the average weights of clusters, berries and skins.

In each experimental unit, three replicate samples of 100 berries were taken. These berries were randomly separated into two, equal subsamples. One subsample was used to determine total sugars, glucose, fructose, pH, titrable acidity (TA), and tartaric, malic and citric acids.

Sugars (mg·g^{-1}) were determined according to the procedure described by Commission Regulation (EEC) determining Community methods for the analysis of wines. Juice was extracted according to Miron and Schaffer (1991) and injection volumes of 20 µL were used for HPLC analysis (Agilent, 1100 series). Sugar and acid separations were performed on a (250 × 4.6 mm, *i.d.* 5 µm) reverse-phase NH_2 analytical column (Econosphere C18, Alltech) operated at 40 °C, with a flow rate of 1 mL·min^{-1}. For sugars, elution was isocratic with acetonitrile:water (3:1) and for acids with 0.5 % aqueous meta-phosphoric acid. Components were identified by comparison of their retention times under the same conditions with those of authentic standards. Detection was obtained with a sensitivity of 0.1 absorbance units full scale, between 210 nm wave lengths. For the stock solution of organic acid standards, tartaric, malic, and citric acid were dissolved in methanol at a concentration of 1 mg·mL^{-1}. Sugar standards were dissolved in water at a concentration of 1 mg·mL^{-1}. Samples and standards were injected three times and average values were calculated. Measurements of pH and TA were made using an automatic titrater (Titrino model 798, Metrohm, Riverview, FL). The TA was measured using a 5.0 mL aliquot of juice and titrating against 0.1 N NaOH to pH 8.2 and was expressed as g·L^{-1} of tartaric acid equivalents. The second berry subsample was used to measure total anthocyanins and flavonoids and expressed as mg·kg^{-1} of fresh weight (fw) as reported by Corona *et al.* (2010). Aliquots of skin extracts (0.5 mL) were diluted to 25 mL with ethanol:H_2O:HCl [70:30:1]. The spectra from 230 to 700 nm and the absorbances at 540 or 536 nm were recorded and the E'_{280} was calculated. Total anthocyanins (Tot. Ant.) and total flavonoids (Tot. Flav.) were calculated according to the equations: Tot. Ant. (mg L^{-1}) = $16.17\times E_{540}\times 50$; Tot. Flav. (mg L^{-1}) = $82.4\times E'_{280}\times 50$; where the value 16.17 was calculated from ε of malvidin-3-glucoside in ethanol-HCl deduced from ε = 33700 in methanol-HCl conc. (Wulf and Nagel 1979) and the value 82.4 was concentration/E'_{280} determined for a 10 mg·L^{-1} solution of (+)-catechin. The dilution coefficient of the extracts was 50; the length as absorbance units of the segment joining the peak at 280 nm of the spectrum of the skin extract diluted in ethanol-HCl, with the intersection point between the perpendicular drawn from the peak at 280 nm to the λaxis and the tangent to the spectrum in the UV region, was E'_{280}. For skin extracts values measured in units of mg·L^{-1}were converted to mg·kg^{-1}.

Statistical analyses: Analyses of variance (ANOVA) were carried out using STATISTICA 6.0 and used to test the significance of each variable ($p \leq 0.05$), and mean separations were made using Fisher's test. Significant treatment and genotype effects were shown by a factorial analysis of variance ($p \leq 0.05$ and $p \leq 0.001$).

Results

Early leaf removal and vine water status: Values reported are the means for both years because no significant year-effects were observed. Un-defoliated vines had average leaf areas (LA) of 25,861 cm^2 per vine. 'Frappato' showed the highest total leaf area and 'Nero d'Avola' the lowest. The defoliation at three weeks after full bloom left a maximum of 14,564 cm^2 for 'Nero d'Avola' and a minimum of 6,625 cm^2 for 'Syrah', the average area of leaves removed was 14,697 cm^2 (Fig. 1). The total leaf area (TLA) per main shoot was significantly higher in 'Cabernet Sauvignon' than in the other cultivars amongst which no significant differences emerged. Of the defoliated vines, leaf area was significantly lowest in 'Nero d'Avola'. Values of TLA for the lateral shoots were not significantly different between the un-defoliated and the defoliated vines (Fig. 2).

Vine water status was monitored during both growing seasons. In 2011, differences between treatments were not significant because unusually abundant rain occurred in the middle and latter part of August (data not shown). In 2012, summer rainfall was more usual and the irrigation treatments had a positive effect on vine water status even though the amounts of water applied in the irrigated treatments from June to August were relatively small (less than 50 mm). The two autochthonous cultivars both showed a significant recovery of Ψ_{stem} after irrigation, while in 'Syrah' the recovery was less significant and in 'Cabernet Sauvignon' it was not significant (Fig. 3). After veraison, no significant differences were observed between the ELR and NLR treatments, the irrigation effect being the predominant one. The recovery recorded in September is attributed to late summer rainfall events, somewhat usual in this climate. Consistent with the irrigation response, the strongest responses to rainfall in terms of Ψ_{stem} were observed in 'Frappato' and 'Nero d'Avola'.

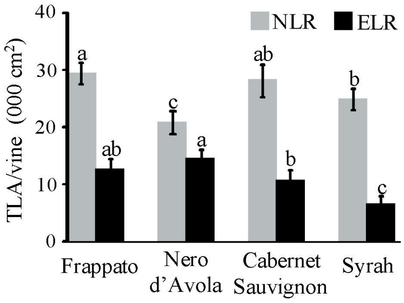

Fig. 1: Total leaf area (TLA) of non-defoliated (NLR) and defoliated (ELR) vines recorded for each cultivar. Measurements were made before the irrigation treatments. Values are the means of two years of experimentation. Means indicated by different letters are significantly different (p ≤ 0.05) based on Fisher's least significant different (LSD) test between treatments.

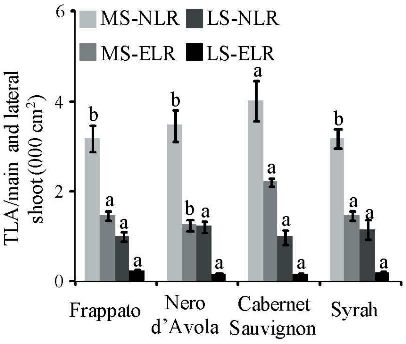

Fig. 2: Total leaf area (TLA) of main shoots (MS) and lateral shoots (LS) in non-defoliated (NLR) and defoliated (ELR) vines in each cultivar. Measurements were made before the irrigation treatments were imposed. Values are the means of two years of experimentation. Means indicated by different letters are significantly different (p ≤ 0.05) based on Fisher's least significant different (LSD) test between treatments.

Vine yield, cluster and berry characteristics: 'Frappato' and 'Cabernet Sauvignon' exhibited similar production (yield/vine) in the defoliated and un-irrigated (ELR-NI) and the un-defoliated and irrigated (NLR-I) treatments. The defoliated and un-irrigated combination was associated with a significant decrease in production in 'Nero d'Avola' and 'Syrah'. Due to its natural resilience, 'Frappato' was not particularly affected by drought and did not show signs of stress when defoliated. Indeed, the clusters in the ELR-NI treatment were heavier. However, in 'Frappato', berry weight was significantly reduced in the NLR-NI treatment. A similar decrease in berry weight was recorded in the 'Cabernet Sauvignon' ELR-NI samples. 'Syrah' behaved similarly under all treatment conditions and did not show significant changes in skin weight when subjected to the major stresses of no irrigation and defoliation (Fig. 4).

As shown in Tab. 1, the main effects of the various treatments and their interactions is confirmed in the sig-

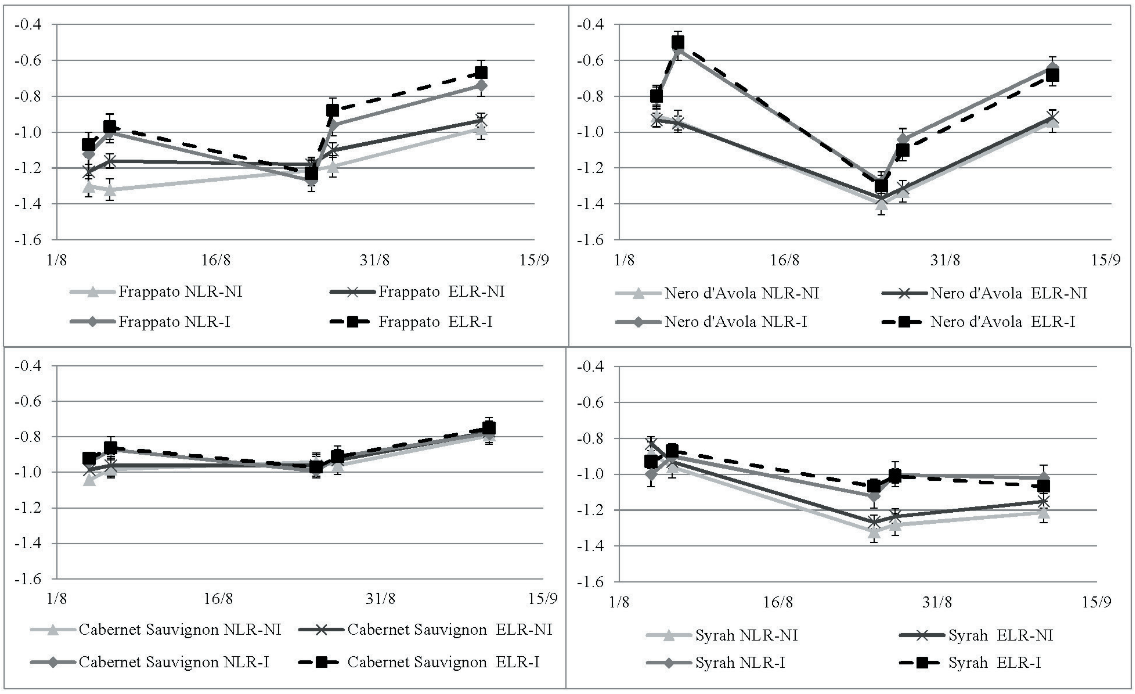

Fig. 3: Midday stem water potential (Ψstem) measured in 2012 in each of four cultivars under the treatments: no leaf removal and no irrigation (NLR-NI), no leaf removal and irrigation (NLR-I), leaf removal and no irrigation (ELR-NI), leaf removal and irrigation (ELR-I). Each symbol represents the mean of five measurements with standard errors.

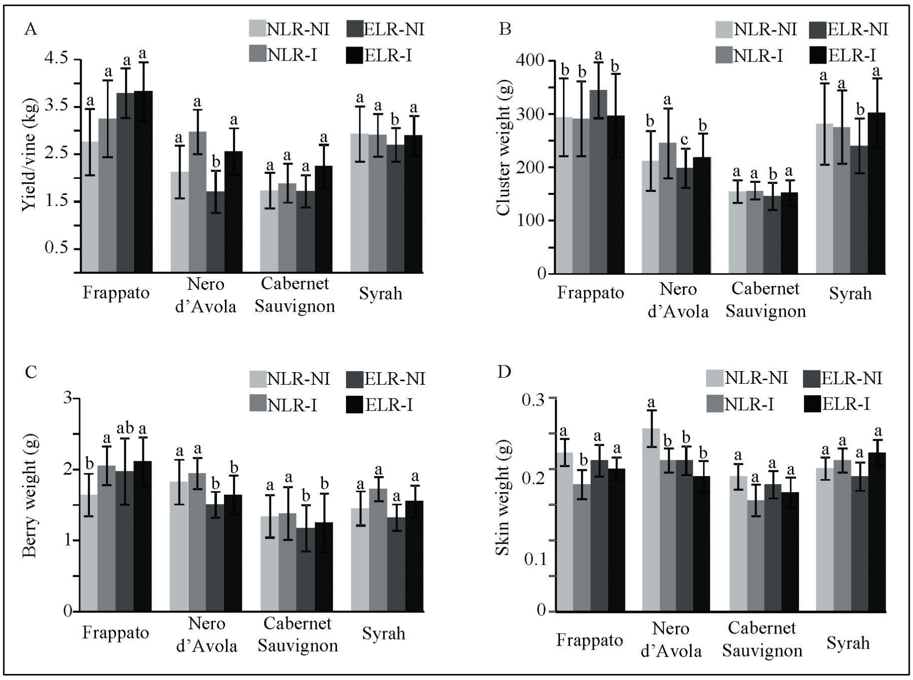

Fig. 4: Yield, cluster and berry characteristics observed in each of four cultivars under the treatments: no leaf removal and no irrigation (NLR-NI), no leaf removal and irrigation (NLR-I), leaf removal and no irrigation (ELR-NI), leaf removal and irrigation (ELR-I). Values shown represent the mean of the two years of experimentation. Means indicated by different letters are significantly different ($p \leq 0.05$) based on Fisher's least significant different (LSD) test for each cultivar and treatment.

nificant influences of variety on yield/vine ($p \leq 0.05$), and cluster and berry weights ($p \leq 0.001$). Canopy management (M) and irrigation (I) affected only berry weight, but year (Y) had no significant effects. The cultivar x canopy management interaction (C x M) was highly significant for yield/vine and berry-weight parameters, whereas the cultivar x irrigation interaction (C x I) was significant only for cluster weight. Cluster weight was the only yield component significantly affected by the interaction of all the experimental factors (C x M x I x Y).

Accumulation of sugars and organic acids: Qualitative data are reported in Tab. 2. 'Frappato' and 'Syrah' showed higher accumulations of reducing sugars under un-irrigated conditions. While 'Syrah' under

Table 1

Main effects and significant interactions of treatments, cultivar and year on three yield components

	Yield/ vine	Cluster weight	Berry weight
Cultivar (C)	*	**	**
Canopy management (M)	ns	ns	*
Irrigation (I)	*	ns	**
Year (Y)	ns	ns	ns
C x M	**	ns	**
C x I	*	*	ns
C x Y	ns	*	ns
M x I	ns	ns	ns
M x Y	ns	ns	ns
I x Y	ns	ns	ns
C x M x I x Y	*	*	ns

ns = not significantly different;
* = significantly different ($p < 0.05$);
** = significantly different ($p < 0.01$).

NLR-NI had the highest total sugars content. 'Frappato' reached a maximum value when un-irrigated conditions were combined with defoliation. Defoliation resulted in a significant decrease in berry sugar content in 'Cabernet Sauvignon' (NLR-NI) and the combination of defoliation and non-irrigation (ELR-NI) reduced sugar accumulation. None of the treatments affected 'Nero d'Avola' significantly. In all varieties, fructose was more abundant than glucose, except for 'Syrah' which showed similar values for these two sugars under all treatments. Irrigation reduced fructose and glucose contents of 'Frappato' and 'Syrah' berries, whereas the effect was minor in 'Nero d'Avola'. Only in 'Cabernet Sauvignon' did defoliation combined with irrigation (ELR-I) increase glucose and fructose accumulation.

Values of berry pH did not show significant differences among varieties, or among treatments but titrable acidity (TA) was highly variable. 'Frappato' berries had the highest TA values under all treatment conditions and 'Syrah' had the lowest. The latter attained its highest TA values in the irrigated treatments (NLR-I and ELR-I), whereas TA levels were almost halved under water-deficit conditions, either with or without defoliation (NLR-NI and ELR-NI). Lowered TA was due almost entirely to a lower malic acid content. 'Cabernet Sauvignon' berries had low TA values except in the ELR-NI treatment. Similarly low TA values were observed in 'Nero d'Avola' in all treatments. Qualitative analyses revealed that this was due to consistently low malic acid levels. In the un-irrigated 'Syrah' vines malic acid content was halved, the same was true also in 'Frappato'. Tartaric acid contents rose significantly in the un-irrigated treatments of 'Frappato' (NLR-NI and ELR-NI) and in 'Syrah' ELR-I while citric acid contents were very stable, showing similar values that were not significantly different between any treatments or between varieties.

Polyphenol content: The highest levels of anthocyanin accumulation (Fig. 5) were in the treatments that did not involve defoliation in both 'Frappato' and 'Cabernet Sauvignon'. Here, anthocyanin contents were slightly higher when defoliation was associated with irrigation, suggesting that leaf removal and irrigation positively influence anthocyanin levels. 'Syrah' showed the opposite behaviour; defoliation was associated with raised levels of anthocyanins whereas accumulation was reduced in treatments where vines were neither defoliated nor irrigated. In 'Nero d'Avola' anthocyanins were accumulated especially in the ELR-NI treatment.

In both 'Frappato' and 'Syrah' (Fig. 6), the combination of defoliation and irrigation drastically decreased flavonoid content. 'Cabernet Sauvignon' showed a similar pattern for anthocyanin and flavonoid content with the highest values in NLR-NI. 'Syrah' had the highest flavonoid content for the defoliation treatments under both irrigated and non-irrigated conditions. 'Cabernet Sauvignon' showed a different behaviour, with the defoliation treatment being associated with a lower flavonoid content. In 'Nero d'Avola', flavonoid accumulation was elevated in both the defoliation and non-irrigated treatments.

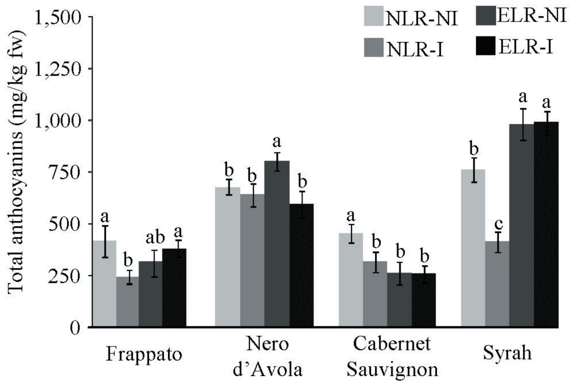

Fig. 5: Total anthocyanin (mg/kg fresh weight) content in each of four cultivars and under the treatments: no leaf removal and no irrigation (NLR-NI), no leaf removal and irrigation (NLR-I), leaf removal and no irrigation (ELR-NI), leaf removal and irrigation (ELR-I). Values represent the means of two years of experimentation. Means indicated by different letters are significantly different ($p \leq 0.05$)based on Fisher's least significant different (LSD) test for each cultivar and treatment.

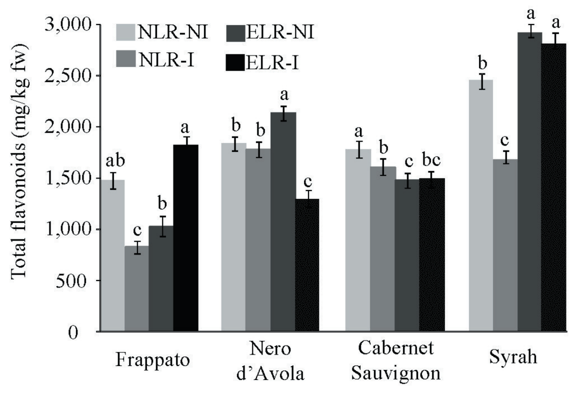

Fig. 6: Total flavonoid content in each cultivar under the treatments: no leaf removal and no irrigation (NLR-NI), no leaf removal and irrigation (NLR-I), leaf removal and no irrigation (ELR-NI), leaf removal and irrigation (ELR-I). Values shown represent the means of two years of experimentation. Means indicated by different letters are significantly different ($p \leq 0.05$) based on Fisher's least significant different (LSD) test for each cultivar and treatment.

Table 2

Main qualitative parameters observed in each cultivar under the treatments: no leaf removal and no irrigation (NLR-NI), no leaf removal and irrigation (NLR-I), leaf removal and no irrigation (ELR-NI), leaf removal and irrigation (ELR-I). Values represent mean of the two years of experimentation

	Frappato				Nero d'Avola			
	NLR-NI	NLR-I	ELR-NI	ELR-I	NLR-NI	NLR-I	ELR-NI	ELR-I
Total sugars (mg·g^{-1})	187.21b ± 4.11	167.78c ± 1.21	197.12a ± 3.30	168.44c ± 2.45	196.32b ± 2.12	197.24ab ± 2.21	200.25a ± 3.11	199.83a ± 2.03
Glucose (mg·g^{-1})	90.43a ± 5.67	82.65b ± 4.98	94.42a ± 3.11	79.21b ± 1.34	96.12a ± 1.01	95.23a ± 1.45	98.44a ± 2.76	97.33a ± 2.56
Fructose (mg·g^{-1})	93.44b ± 1.58	85.30c ± 2.21	97.81a ± 3.17	82.23c ± 3.94	101.61ab ± 2.22	99.80b ± 1.45	105.12a ± 3.21	102.26ab ± 1.43
pH	3.81a ± 0.21	3.52a ± 0.16	3.68a ± 0.13	3.91a ± 0.13	3.86a ± 0.16	3.74a ± 0.21	3.82a ± 0.13	3.98a ± 0.18
Titratable acidity (g·L^{-1} tartaric acid)	10.72a ± 0.34	9.84a ± 1.02	9.68a ± 0.78	10.68a ± 0.33	7.98a ± 0.44	7.82a ± 0.87	8.12a ± 0.61	7.16a ± 0.45
Tartaric acid (g·L^{-1})	7.86a ± 0.27	6.12b ± 0.42	7.82a ± 0.34	7.02ab ± 0.45	7.24a ± 0.24	7.68a ± 0.36	7.56a ± 0.42	6.98a ± 0.34
Malic acid (g·L^{-1})	3.64b ± 0.21	4.48a ± 0.43	3.23b ± 0.26	4.22a ± 0.33	1.28a ± 0.22	0.98a ± 0.14	0.82a ± 0.20	0.98a ± 0.09
Citric acid (g·L^{-1})	0.62a ± 0.14	0.68a ± 0.11	0.52a ± 0.10	0.54a ± 0.09	0.64a ± 0.07	0.56a ± 0.13	0.62a ± 0.05	0.64a ± 0.09

	Cabernet Sauvignon				Syrah			
	NLR-NI	NLR-I	ELR-NI	ELR-I	NLR-NI	NLR-I	ELR-NI	ELR-I
Total sugars (mg·g^{-1})	183.27a ± 3.34	183.82a ± 2.11	166.62c ± 4.54	174.71b ± 2.32	204.23a ± 4.23	171.24d ± 2.12	198.64a ± 4.56	188.87c ± 4.43
Glucose (mg·g^{-1})	85.92a ± 3.21	84.81a ±3.14	78.27b ±2.87	81.60ab ± 1.47	96.82a ± 4.76	82.12c ± 3.43	98.15a ± 4.21	90.42b ± 2.98
Fructose (mg·g^{-1})	90.13a ± 2.32	88.93a ± 1.76	80.62b ± 3.32	85.02ab ± 2.21	99.04a ± 2.45	83.82c ± 4.14	98.31a ± 3.52	92.81b ± 3.09
pH	4.22a ± 0.33	4.18a ± 0.11	4.22a ± 0.16	4.48a ± 0.12	4.38a ± 0.20	3.99a ± 0.19	3.90a ± 0.16	3.78a ± 0.15
Titratable acidity (g·L^{-1} tartaric acid)	7.84b ± 0.41	8.02ab ± 0.37	9.24a ± 0.68	8.42a ± 0.35	6.26b ± 0.99	9.45a ± 0.48	5.82b ± 0.63	10.42a ± 0.62
Tartaric acid (g·L^{-1})	6.98a ± 0.45	6.64a ± 0.31	7.22a ± 0.37	6.94a ± 0.51	5.48b ± 0.43	5.26b ± 0.46	5.46b ± 0.55	6.68a ± 0.53
Malic acid (g·L^{-1})	3.14b ± 0.30	2.62c ± 0.07	4.26a ± 0.10	2.96b ± 0.19	2.72c ± 0.29	5.26a ± 0.23	2.18c ± 0.36	4.12b ± 0.33
Citric acid (g·L^{-1})	0.68a ± 0.12	0.76a ± 0.04	0.68a ± 0.06	0.72a ± 0.13	0.66a ± 0.07	0.68a ± 0.04	0.64a ± 0.10	0.52a ± 0.07

For each cultivar and parameter means indicated by different letters are significantly different ($p \leq 0.05$) based on Fisher's least significant different (LSD) test.

Discussion

This work reports the influences of early defoliation and water stress on grape quality. It shows that, when considered in relation to the productive potentials of the four grape varieties examined, satisfactory productivity can be achieved in Sicily's arid, Mediterranean climatic even when deliberate stress conditions (defoliation, restricted irrigation) are imposed. This result is also to be related to the number of buds left after winter pruning in which those from the crown and *bourillon* are eliminated. It should be recognised that after green pruning, only shoots from the previous growing season's budwood remain. It is not easy to determine the importance of the interactions between environmental factors and cultural practices on the productivity of these genotypes. Some authors (ESCALONA *et al.* 1999; CHAVES *et al.* 2007) have reported that the combined effects of drought, high air temperatures and high evaporative demand during summer limit grapevine yield and also reduce berry and wine quality. Conversely, LOVISOLO *et al.* (2002) has shown that grapevines adapt well to the Mediterranean's semi-arid climate as a result of their efficient stomatal responses - especially where stocks and scions are appropriately matched. However, this adaptive response is highly variable both with genotype and with the phenological stage in which a water stress occurs (CHAVES *et al.* 2010). The reduced production found here for defoliated and un-irrigated 'Nero d'Avola' and 'Syrah' vines could be due to the combined effect of the two treatments. KOBLETT *et al.* (1994) reported that fruit yield decreases with increasing levels of defoliation as a consequence of reductions in both cluster and berry weight. Also, as a result of looser clusters which nevertheless remain long in 'Nero d'Avola'. Working in a climate similar to ours, Dos Santos *et al.* (2007) noted that cluster weights in 'Moscato di Alessandria' increased with increases in irrigation and that reduced production in un-irrigated conditions was due principally to reductions in cluster weight. In our study, lowered production was independent of the number of clusters per vine and of the number of berries per cluster as the defoliation and water stress treatments were imposed after fruit-set and there was no significant fruit drop. Therefore, berry weight was the only yield component able to respond to stress during the growing season. The most critical stage of berry growth (stage I) occurs during the hottest period. Here, the impact of water stress on berry growth is thought to occur directly through reductions in water import through the xylem (Chaves *et al.* 2010). This could well decrease mesocarp cell turgor (Thomas *et al.* 2006) and result in a reduction in cell and thus berry expansion (Petrie *et al.* 2000). Another possibility is a reduction in the cell division rate of the skin (Mc Carthy 1999). It is also known that berry shrinkage can occur during the final stages of ripening (Crippen and Morrison 1986). Changes in berry development caused by defoliation are also likely due to increased light exposure. Dokoozlian and Kliewer (1996) reported differences in berry size between exposed and shaded fruit. While berries grown in the shade during stages I and II were significantly smaller than the controls, whereas berries grown without light during stage III were similar in size ('Pinot noir'), or only slightly smaller ('Cabernet Sauvignon') than the controls. It is clear that genetic factors predominate over agronomic factors (canopy, irrigation) in determining berry size. As reported by Nicolosi *et al.*(2012), 'Frappato' is much more productive than the other varieties, with large clusters and large berries and that 'Cabernet Sauvignon' has lower productivity and smaller berries.

The enhanced sugar accumulation in the ELR-NI treatment in 'Frappato' is probably due to an extended vegetative-productive cycle that allows accumulation over a longer period and one that continues into a later time of year when environmental stresses become less severe. Similarly, the absence of differences among the treatments in sugar accumulation in 'Nero d'Avola' is probably due to the high vigour of this variety that, despite quite severe soil water deficits during periods of high evapotranspirative demand, managed to maintain relatively high water potentials. The rather variable behaviour reported here between treatments and varieties, derives from the fact that the severity of the effects of water deficit on soluble sugar content tend to be variety dependent (Gaudillère *et al.* 2002). For example, Castellarin *et al.* (2007) found different behaviours in sugars accumulation in 'Merlot' and 'Cabernet Sauvignon' when subjected to identical water stresses. This may be explained either by varietal differences in vigour, and therefore source/sink equilibrium, or by distinctive mechanisms underlying the response of berry development to water deficit depending on the timing and intensity of the stress (Chaves *et al.* 2010). Indeed, it has been shown that water stress has more pronounced effects on berry sugar accumulation when imposed before véraison (Keller 2005). Crippen and Morrison (1986) report that sugar accumulation is greater in light-exposed fruit than in shaded fruit. Our results on the qualitative analysis of sugars differ from those of De Souza et al. (2005) where the amounts of glucose and fructose per berry were increased by irrigation, indicating an enhancement in berry sink strength and/or increased sugar availability.

The stress conditions we induced here seem to have been well tolerated in 'Frappato', perhaps due to its high vigour and an ability to correct any earlier imbalances in the final stages of berry ripening. As reported above for sugars, 'Frappato' seems well able to degrade acid. Our observation of variability in acidity does not agree with the report by Dos Santos *et al.* (2007) that shows a more drastic lowering of acid levels under high levels of imposed water stress. Contrasting with the sugar and acid results, there was a decline in flavonoid accumulation in 'Frappato' under high-stress conditions, in spite of its high vigour. Flavonol biosynthesis is closely related to that of anthocyanins (Jeong *et al.* 2006). More recently, flavonol concentration has been reported to increase under water stress in the white variety, 'Chardonnay', but not in the red 'Cabernet Sauvignon' (Deluc *et al.* 2009). This suggests a greater need for photoprotection in the berries of these varieties, as previously shown in apples with low levels of anthocyanin (Merzlyak *et al.* 2008).

This study confirms the effectiveness of early defoliation in yield management, leading to smaller clusters. Leaf removal at earlier stages of cluster development appear to be an effective strategy that could be used to achieve smaller clusters and thus reduce the crop load when coupled with other agronomic practices. Water stress had positive effects on yield and fruit quality, therefore indicating that production of winegrapes having high winemaking quality appears to be possible under arid conditions and with minimal irrigation. The imposition of managed water deficits, through careful regulation of the irrigation system, would seem to be an effective strategy for reducing berry size, improving must quality and enhancing the accumulation of anthocyanins. This may not apply to some high vigour varieties where flavonoid accumulation may create difficulties. Incidentally, a particularly low incidence of the common fungal diseases was noted throughout our study.

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