EFFECTS OF MECHANICAL PRE-BLOOM DEFOLIATION ON CORDON DE ROYAT PRUNED SANGIOVESE (*VITIS VINIFERA* L.) VINES

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Abstract

Aims: Recent trials on Sangiovese vines have shown that hand defoliation of shoot basal leaves at pre-bloom is effective in reducing fruit set and yield, leading to better grape composition and quality. The present work was performed to assess whether similar outcomes could be obtained by a more economically viable mechanical approach, which appears to be extremely attractive in cultivars such as Sangiovese, marked by high or very high yield potential and heavy, fairly compact clusters quite sensitive to rot.

Methods and results: The trial was designed to compare pre-bloom mechanical defoliation (MD), hand defoliation (HD) and no defoliation (C) on Sangiovese vertical shoot positioned and spur pruned cordon de Royat trained vines. In the HD treatment, the first six basal leaves of each shoot were removed (70 % of leaf area), whereas in the MD treatment 33 % of the leaf area was removed from the basal part of the shoots. HD and MD compared to C reduced fruit set (HD = 29.8 % ; MD = 24.2 % ; C = 35.5 %), yield per shoot (HD = 546 g ; MD = 516 g ; C = 764 g), cluster weight (HD = 292 g ; MD = 272 g ; C = 382 g) and berry weight (HD = 2.17 g ; MD = 2.31 g ; C = 2.45 g), but improved total soluble solids (HD = 23.0 °Brix ; MD = 22.5 °Brix ; C = 20.8 °Brix) and total anthocyanins (HD = 837 mg/kg of grapes ; MD = 744 mg/kg of grapes ; C = 647 mg/kg of grapes). Leaf photosynthesis, measured in 2007, increased only temporarily after HD and MD as compared to control.

Conclusions: The pre-bloom HD of shoot basal leaves confirmed its positive effect on crop yield control and grape composition, leading to better grape quality. The pre-bloom MD of the basal part of the shoots maintained most of the advantages associated with HD, although only half of the leaf area removed by hand was removed by the machine.

Significance and impact of the study: Pre-bloom mechanical defoliation can replace hand defoliation and partially replace the time-consuming and costly manual cluster thinning technique, which is often used in high yield cultivars such as Sangiovese.

Key words: defoliation, fruit set, photosynthesis, mechanization, anthocyanins

Résumé

Contexte et objectifs : Des recherches récentes ont démontré que l'effeuillage manuel de la partie basale des rameaux de vigne avant floraison peut diminuer la nouaison, la production et améliorer la qualité des raisins. Or, l'effeuillage mécanique de la vigne est très intéressant par sa simplicité et par son coût réduit comparé à l'effeuillage manuel. Ainsi, une expérimentation sur le sujet a été mise en place à Bologne (44°30'N, 11°24 'E) en Italie, sur le cépage Sangiovese (*V. vinifera* L.) marqué par une production très élevée, avec des grappes serrées et sensibles à *Botrytis*.

Méthodes et résultats : L'expérimentation visait à comparer des souches témoins non effeuillées (C), ou effeuillées à la main (HD) ou mécaniquement (MD) avant floraison. Les essais ont été réalisés en 2006 et en 2007 sur une vigne conduite en espalier taillé en cordon de Royat. Dans la modalité HD, les six premières feuilles basales ont été éliminées manuellement sur chaque rameau (70 % de la surface totale par rameau). Pour le MD, l'effeuillage avec une machine aspirante a éliminé 33 % de la surface foliaire présente. Les HD et MD comparées au témoin C ont montré une diminution de la nouaison (HD = 29,8 % ; MD = 24,2 % ; C = 35,5 %), de la production par rameau (HD = 546 g ; MD = 516 g ; C = 764 g), du poids des grappes (HD = 292 g ; MD = 272 g ; C = 382 g) et du poids des baies (HD = 2,17 g ; MD = 2,31 g ; C = 2,45 g). Le rapport entre la surface foliaire finale et la production des HD et MD a été plus élevé que dans le témoin C (HD = 0,72 ; MD = 0,77 ; C = 0,56). Les raisins des HD et MD comparés au témoin C ont montré une quantité plus élevée de sucre (HD = 23,0 °Brix ; MD = 22,5 °Brix ; C = 20,8 °Brix) et d'anthocyanes totales (HD = 837 mg/kg de raisins ; MD = 744 mg/kg de raisins ; C = 647 mg/kg de raisins). En particulier, dans les modalités effeuillées HD et MD, la quantité d'anthocyane Cyanidine-3-G et de Péonidine-3-G est augmentée par rapport au témoin C. La photosynthèse des feuilles adultes de HD et MD, mesurée dans 2007, a été temporairement augmentée par rapport au témoin C.

Conclusions : L'effeuillage manuel de la partie basale des sarments avant floraison, a confirmé son efficacité pour diminuer la production et améliorer la qualité des raisins.

Impact de l'étude : L'effeuillage mécanique avant floraison, plus simple et d'un coût réduit par rapport à l'effeuillage manuel, permet d'obtenir les mêmes effets, malgré le fait que la machine n'a éliminé que la moitié de la surface foliaire éliminée par la modalité manuelle HD. Il est aussi évident que selon le cépage, les conditions de travail et le type de machine, l'effeuillage mécanique peut être réalisée d'une façon plus efficace. Cette méthode peut en tout cas se substituer à l'éclaircissage coûteux des grappes.

Mots clés : effeuillage, nouaison, photosynthèse, mécanisation, anthocyanes

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INTRODUCTION

Previous work on pre-bloom hand defoliation of main shoot basal leaves has shown that source limitation induced in potted and field vines is effective in reducing fruit set and thus to form looser clusters less susceptible to rot (Poni *et al.*, 2005 and 2006). Under high yield conditions, this approach has also turned out to be a powerful crop management technique while leading, at the same time, to improved must composition.

According to Poni *et al.* (2006) and Intrieri *et al.* (2008), the principal mechanism underlying grape quality improvement in defoliated vines involves increased leaf to fruit ratio, smaller berries with a higher skin to pulp ratio, and probably different temperature and light regimes, which could be positively involved in anthocyanin and flavonol synthesis (Kliewer, 1977 ; Haselgrove, 2000 ; Spayd *et al.*, 2002 ; Price *et al.*, 1995). These aspects need to be further investigated because it has been reported that excessive temperature may cause anthocyanin degradation (Yamane *et al.*, 2006 ; Mori *et al.*, 2007) and that even the anthocyanin profile can be influenced by different light regimes (Downey *et al.*, 2006 ; Guidoni *et al.*, 2008 ; Rustioni *et al.*, 2005) and different source-sink ratios (Guidoni *et al.*, 2002 ; Filippetti *et al.*, 2007).

Very promising results achieved with manual pre-bloom leaf removal promoted further studies aiming to assess if similar results could be obtained by a more economically viable mechanical approach, which appears to be extremely attractive in cultivars such as Sangiovese, marked by high or very high yield potential and heavy, fairly compact clusters quite sensitive to rot.

The first successful attempt to mechanize pre-bloom defoliation has been conducted on Sangiovese vines trained to a COMBI system (Intrieri *et al.*, 2008). Yet, on this trellis system, characterized by two horizontally divided canopy walls supported by a U-shaped frame, mechanical defoliation could only be performed on the external canopy side, with a probable lower efficiency in terms of amount of leaves removed compared to an application on both side of the canopy.

The present work aims to compare the performance as yield, cluster morphology and grape composition of pre-bloom mechanical defoliation, hand defoliation and non defoliated vines of cv Sangiovese, 15 years old and trained to a traditional, vertically shoot positioned (VSP) spur pruned cordon.

MATERIALS AND METHODS

The trial was conducted in 2006 and 2007 on Sangiovese vines (*V. vinifera* L. ; clone 12T grafted on SO4) planted in 1990 in Bologna, Italy (44°30'N, 11°24'E). Vines were spaced 1 m by 2.8 m, equivalent to a density of 3570 plants/ha, and they were trained to a vertical shoot positioned (VSP) spur pruned cordon (12 buds per vine).

Forty-eight vines in four blocks (12 vines per block) were set up within a single uniform row and randomly assigned within each block to one of the following treatments (total of 16 vines per treatment) : a) non-defoliated control (C) ; b) hand defoliation (HD) of six basal leaves at stage H (« separate flowers » according to Baggiolini *et al.*, 1952) ; and c) mechanical defoliation (MD) on the basal part of the shoots at the same phenological stage as for HD. In the HD treatment, any laterals growing in the 6 basal node shoot zone were also removed.

The MD was carried out by a tractor-mounted leaf plucker unit (Plucker, Tanesini Technology, Faenza, Italy), which operates using a suction force and rotating blades (Intrieri *et al.*, 1994). The tractor speed was about 1.5 km/hour and the rotating blades were set to about 2000 revolutions per minute. MD was performed on both sides of the canopy and on the same zone as for HD.

In both years, a few days before treatments, 20 shoots from extra vines in a parallel row were completely defoliated and the regression between each shoot length and their actual leaf area was calculated. The same procedure was used for the laterals of each shoot. The resulting regressions for main shoots and their laterals were, respectively, $y = 14.99 x$ ($R2 = 0.88$) and $y = 5.46 x$ ($R2 = 0.86$) in 2006 and $y = 12.39 x$ ($R2 = 0.98$) and $y = 5.02 x$ ($R2 = 0.86$) in 2007.

The day before defoliation, one shoot per vine with two inflorescences was chosen and tagged as sub-replicate in each block, and the length of each tagged shoot was recorded. The estimation of flower number for the basal cluster of each tagged shoot was obtained according to the photographic method reported by Poni *et al.* (2005). As the defoliation machine could trim some part of the inflorescences, digital pictures of the same inflorescences were also taken after the machine run for a post-defoliation estimation of retained flower number per cluster. Dates of defoliation were May 24, 2006 and May 14, 2007.

The total leaf area of all tagged shoots was calculated with the regressions reported above. The main and lateral leaf area removed in HD shoots was directly measured with a leaf area meter (LI-3000A, Li-Cor Biosciences, Lincoln, Nebraska, USA). The leaf area removed in MD shoots was estimated by visual assessment of the proportion of leaf blades left on the shoots and their laterals after machine runs.

A light shoot trimming was performed yearly over the first ten days of July, leaving a canopy height of about 1.3-1.4 m.

At harvest (September 25, 2006 and September 18, 2007), the tagged shoots were individually harvested and their main and lateral leaf area was measured. Their clusters were weighed and the number of normal berries, pea-size berries and live green shoot berries in each of the basal cluster was counted. Cluster compactness was visually estimated using code 204 (OIV, 1983). For each cluster, the incidence of rot was evaluated as the percentage of infected normal berries over total normal berries.

Twenty berries were sampled from each basal cluster. Anthocyanin analysis was carried out as follows: a 3 g berry sample, which was previously grinded, was weighted into a screw-capped tube and extracted in methanol (1 % HCl, v/v) and 0,1 % ascorbic acid (p/v). The extract was stored in a freezer at -20 °C until analysis. HPLC separation and quantification of anthocyanins was performed on a Waters 1525 instrument equipped with a diode array detector (DAD), using a reversed-phase column LiChrospher® RP-18 250 mm x 4 mm (5 µm) with precolumn. Separation of the five main free anthocyanins was done according to Mattivi *et al.* (2006). Each anthocyanin was quantified at 520 nm with a calibration curve of malvidin 3-glucoside chloride. The remaining berries of each tagged cluster were mixed and crushed with the distal cluster of each test shoot and the resulting must was analyzed for total soluble solid concentration (°Brix), pH and titratable acidity (TA). To evaluate the carry-over effects of defoliation on the following year's bud induction, the fruitfulness of shoots growing from the test vines was assessed in spring 2007 and 2008.

Weather data (mean daily air temperature and rainfall) were recorded from April to September in 2006 and 2007 at a meteorological station located close to the experimental site. Light and temperature at cluster level were not measured.

In 2007, leaf assimilation rate was measured at 22, 35, 49, 58, 74 and 108 days after defoliation, using a LI-6400 portable gas-exchange system (Li-Cor Biosciences). Readings were taken on every second one leaf above the sixth node along the tagged shoots and along laterals.

A combined analysis of variance over years (Gomez and Gomez, 1984) was performed using the mixed General Linear Model (GLM) procedure of the SAS statistical package (SAS Institute, Cary, North Carolina, USA).

RESULTS AND DISCUSSION

No significant climatic differences were recorded between the 2006 and 2007 growing seasons. In 2006, the April-September Growing Degree Days (GDD) were 1880 and rainfall was 325 mm, while in 2007 the GDD were 1950 and rainfall was 250 mm.

At defoliation, the shoot leaf area was very similar between treatments (around 1200 cm^2 per shoot). HD and MD respectively removed 70 % and 33 % of leaf area from tagged shoots (table 1). Despite that the machine did two runs per row, the percentage of removed leaf area did not differ from the value reported in a previous study using a COMBI trellis system (Intrieri *et al.*, 2008). The result is probably linked to the trellis structure which in COMBI is divided in two main permanent cordons showing, under the same environmental condition, a lower canopy density compared to the undivided cordon system used in the present trial. Total leaf area (main and lateral leaves) per shoot at harvest did not differ among treatments, indicating the capability of defoliated vines to partially regain the leaf area lost by defoliation during

Table 1 - Sangiovese grapevines: influence of manual and mechanical pre-bloom defoliation on shoot leaf area (LA) as compared to a non-defoliated control. (HD = manual defoliation; MD = mechanical defoliation). Data averaged over 2006-2007.

Source of variation	LA before defoliation /shoot (cm^2)	Removed LA /shoot (cm^2)	Removed LA /shoot (%)	LA after defoliation /shoot (cm^2)	Main final LA/shoot (cm^2)	Lateral final LA /shoot (cm^2)	Total final LA /shoot (cm^2)
Control	1237.6	0 c	0 c	1237.6 a	2398.9	1873.1	4272.0
HD	1256.3	881.9 a	70 a	374.4 c	2071.8	1887.1	3958.9
MD	1208.2	399.9 b	33 b	808.1 b	2238.6	1755.2	3993.8
Main	ns	**	**	**	ns	ns	ns

** P = 0.01 ; ns = not significant. Means within columns marked by different letters are significantly different by the Student-Newman-Keuls test. No significant year x treatment interaction was registered.

the growing season, and no significant interaction between year and treatment was recorded (Table 1).

Pre-defoliation flower number per cluster was not significantly different among treatments, varying from 497 to 570 (Table 2). In MD, the flower number after defoliation was reduced from 546 to 439. Both defoliation treatments resulted in a marked reduction of fruit set (ratio of normal berries to pre-defoliation flowers) and hence of berry number per cluster as compared to C vines (Table 2). It should be noted that the lower fruit set registered in MD (24.2 %) compared to HD (29.8 %) is due to the elimination of some inflorescence tips by the machine. The fruit set calculated in MD after machine runs accounts for 30.1 % (Table 2). The number of pea-size berries and live green shoot berries was negligible for all treatments and was not considered. There were no carry-over effects of defoliation on bud flower induction, as no difference in fruitfulness (clusters per shoot) was recorded in the test vines in spring 2007 and 2008 (Table 2).

HD and MD lowered yield per vine by 28.5 % and 32.5 %, respectively, and the same trend was observed for cluster weight (Table 3). Defoliation also reduced berry weight, although significance was reached in the HD treatment only. This result matches with the lower leaf area removed by the machine as compared to HD. We may speculate that in MD vines, the removal of source leaves has not been sufficient to induce, after flowering, a cell division limitation as strong as in HD vines. Overall,

Table 2 - Sangiovese grapevines : influence of manual and mechanical pre-bloom defoliation on flower number per cluster and fruit set characteristics compared to a non-defoliated control. (HD = manual defoliation ; MD = mechanical defoliation ; na = not applicable). Data averaged over 2006-2007. Shoot fruitfulness of Sangiovese grapevines averaged over 2007-2008.

Source of variation	Flowers/cluster before machine run (n)	Flowers/cluster after machine run (n)	Normal berries/cluster (n)	Fruit set (1) (%)	Shoot fruitfulness assessed in spring 2007 and 2008 (clusters/shoot)
Control	570.3	na	202.5 a	35.5 a	1.24
HD	496.7	na	148.0 b	29.8 b	1.30
MD	546.3	439.3	132.2 b	24.2 b	1.26
Main	ns	ns	*	*	ns
Year	**	**	*	ns	ns

* P = 0.05 ; ** P = 0.01 ; ns = not significant. Means within columns marked by different letters are significantly different by the Student-Newman-Keuls test. No significant year x treatment interaction was registered.
(1) The MD fruit set value is calculated from the number of flowers counted before machine runs. The MD fruit set value after machine runs is 30.1%.

Table 3 - Sangiovese grapevines : influence of manual and mechanical pre-bloom defoliation on yield components and cluster and berry characteristics as compared to a non-defoliated control. (HD = manual defoliation ; MD = mechanical defoliation). Data averaged over 2006-2007.

Source of variation	Yield/ shoot (g)	Cluster weight (g)	Berry weight (g)	Skin weight (g)	Cluster compactness (OIV rating)	Bunch rot (%)
Control	764.2 a	382.0 a	2.45 a	0.331	7.0	6.3
HD	546.7 b	292.5 b	2.17 b	0.324	6.1	0.8
MD	516.1 b	271.8 b	2.31 ab	0.339	5.9	3.5
Main	*	*	*	ns	ns	ns
Year	ns	ns	**	**	**	**

* P = 0.05 ; ** P = 0.01 ; ns = not significant. Means within columns marked by different letters are significantly different by the Student-Newman-Keuls test. No significant year x treatment interaction was registered.

it can be affirmed that the cluster constraint on HD and MD shoots was caused by the combined reduction in berry number and in berry size. Considering the limited climatic differences recorded in 2006 compared to 2007, the between-year differences found for some parameter seem to be more linked to the typical Sangiovese behavior, which normally tends to vary from year to year.

It is interesting to note that skin weight showed no differences among treatments despite the lower berry weight of MD compared to control (Table 3).

Cluster compactness and cluster rot values were not significantly affected by defoliation (Table 3). The lack of significant differences in cluster compactness among treatments could partially be attributed to the unusually low density of the control clusters during the two-year trial (7 OIV rating) compared to the higher value (8 OIV rating) normally registered in Sangiovese (Intrieri *et al.*, 2008). With regard to rot infection, it has to be noted that the summer weather conditions in both years of the trial were not favorable to rot, so even the control clusters showed very low *Botrytis* incidence (Table 3).

Berry composition data pooled from the two-year trial are presented in table 4. HD and MD treatments enhanced the concentration of soluble solids (more than 2 °Brix and 1.7 °Brix, respectively) and this result is coherent with the higher leaf to fruit ratio, which increased on a two-year basis by 28.6 % and 37.5 % for HD and MD, respectively, as compared to C. In accordance with previous works (Poni *et al.*, 2006; Intrieri *et al.*, 2008), these results may depend not only on the capability of defoliated vines to regain the lost leaf area during the growing season but also on the yield reduction per shoot, which offsets the effect of removing mature leaves.

Table 4 - Sangiovese grapevines: influence of manual and mechanical pre-bloom defoliation on sugar content, total acidity (TA) and pH of berries at harvest as compared to a non defoliated control. Data on source-sink balance are also presented. (HD = manual defoliation; MD = mechanical defoliation). Data averaged over 2006-2007.

Source of variation	Soluble solids (°Brix)	pH	TA (g/L)	LA shoot/yield (m^2/kg)
Control	20.8 b	3.27	7.72	0.56 b
HD	23.0 a	3.35	7.37	0.72 a
MD	22.5 a	3.35	7.42	0.77 a
Main	*	ns	ns	*
Year	**	**	ns	ns

* P = 0.05; ** P = 0.01; ns = not significant. Means within columns marked by different letters are significantly different by the Student-Newman-Keuls test. No significant year x treatment interaction was registered.

Table 5 - Sangiovese grapevines: influence of manual and mechanical pre-bloom defoliation on skin anthocyanin concentration as compared to a non defoliated control. (HD = manual defoliation; MD = mechanical defoliation). Data averaged over 2006-2007.

Source of variation	Anthocyanins (mg/kg of grapes)					
	Total	Delphinidin-3-Glucoside	Cyanidin-3-Glucoside	Petunidin-3-Glucoside	Peonidin-3-Glucoside	Malvidin-3-Glucoside
Control	647.5 b	85.6	156.1 b	88.5	110.9 b	206.3
HD	837.1 a	115.6	225.1 a	112.0	146.3 a	222.8
MD	744.0 ab	94.8	207.6 a	95.4	143.1 a	203.0
Main	*	ns	**	ns	**	ns
Year	ns	**	**	*	ns	*

* P = 0.05; ** P = 0.01; ns = not significant. Means within columns marked by different letters are significantly different by the Student-Newman-Keuls test. No significant year x treatment interaction was registered.

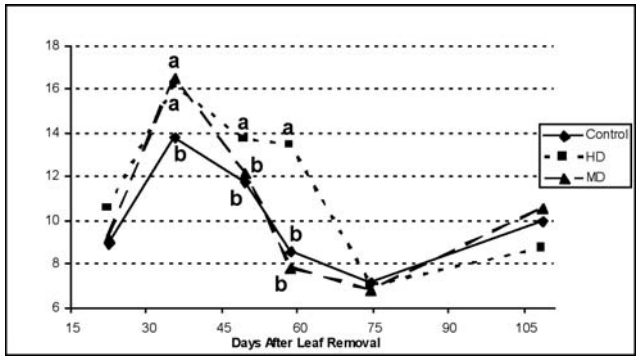

Figure 1 - Sangiovese grapevines: main leaf assimilation rate measured in 2007 at different time points after leaf removal. Readings were taken on well exposed leaves above the 6*th* node of the shoot.
Means marked by different letters within the same day are significantly different by the Student-Newman-Keuls test.

Must acidity and pH were unaffected by defoliation (Table 4). As previous pre-flowering leaf removal work on Trebbiano reported (Poni *et al.*, 2006), we may argue that a possible reduction of the malic acid fraction, due to an increasing cluster exposure to light and to higher temperature (Kliewer, 1977), may have been compensated by an increase of tartaric acid.

Total anthocyanins on a fresh-weight basis increased by about 30 % in HD, while intermediate values were obtained in MD (Table 5). Our trial did not evaluate the effect of defoliation on cluster light exposure and temperature, but it has been reported that these factors may have conflicting effects on anthocyanin concentration (Dokoozlian and Kliewer, 1996; Haselgrove *et al.*, 2000; Bergqvist *et al.*, 2001; Spayd *et al.*, 2002; Downey *et al.*, 2004; Yamane *et al.*, 2006; Tarara *et al.*, 2008). In the present study, pre-bloom leaf removal increased total anthocyanins probably because of the higher source to sink ratio during ripening (Pirie, 1976; Wicks *et al.*, 1983; Hunter *et al.*, 1991; Vanden Heuvel *et al.*, 2005), suggesting that in our conditions this physiological parameter may have had higher influence on berry composition than cluster microclimate. Besides, enhancement of grape color after pre-bloom defoliation has been reported in different cultivars (Poni *et al.*, 2006; Intrieri *et al.*, 2008).

Besides, source to sink ratio was probably involved even in anthocyanin composition, because the dihydroxylated anthocyanins (Cyanidin-3-Glucoside and Peonidin-3-Glucoside) increased in HD and MD treatments (Table 5), as found in Nebbiolo and in Sangiovese after cluster thinning (Guidoni *et al.*, 2008; Filippetti *et al.*, 2007).

In 2007, the main leaf gas exchange readings showed higher photosynthetic activity in HD versus control, from 35 to 58 days after leaf removal; in MD, similar results were detected only at 35 days after leaf removal (Fig. 1). Later in the season, no difference was detected among treatments. Lateral leaf assimilation rate was not affected by defoliation (data not reported). The assimilation compensation on main leaves showed a temporary nature, as observed in a previous study (Poni *et al.*, 2008), and does not seem responsible for improved berry composition in terms of sugar and anthocyanin content in the defoliated vines.

CONCLUSION

This study on Sangiovese vines, vertical shoot positioned and spur pruned cordon de Royat trained, confirmed the effectiveness of hand pre-bloom basal leaf removal in yield reduction, grape composition enhancement and cluster morphology improvement. Mechanical defoliation performed at the same phenological stage as hand defoliation maintained most of the advantages associated with the hand treatment, despite the partial leaf removal on the basal part of the shoots. Defoliation by machine can eventually replace the time-consuming manual cluster thinning technique, which is often used in Sangiovese vines to control crop. Yet, the quality of the machine work should be improved by modulating tractor speed or the rpm of the stripping system in relation to cultivars or environmental conditions.

Both defoliation treatments influenced not only berry skin anthocyanin concentration but also anthocyanin profile. These aspects need to be better investigated with particular reference to enological grape characteristics, although a recent experiment on Graciano and Carignan cultivars (Tardaguila *et al.*, 2010) have shown a positive effect of pre-bloom defoliation on wine quality.

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