A THREE-YEAR SURVEY ON THE IMPACT OF PRE-FLOWERING LEAF REMOVAL ON BERRY GROWTH COMPONENTS AND GRAPE COMPOSITION IN CV. BARBERA VINES

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Abstract

Aims: Competitiveness in modern viticulture requires that vineyard management techniques used for crop regulation, besides being economically viable, would assure stable grape composition improvement across variability of genotypes and seasons. Pre-flowering basal leaf removal has previously been shown as an effective tool in controlling yield primarily through a reduced fruit-set. This study aims to evaluate the impact of early leaf removal on the differential growth of the various berry organs (skin, flesh and seeds) as well as the constancy of such effects across season variability and their impact on final grape composition.

Methods and results: Pre-bloom defoliation (D) of the first six basal leaves on main shoots was applied to the field-grown Barbera cultivar (*Vitis vinifera* L.) from 2006 to 2008 and compared with non-defoliated (ND) controls. Over a three-year period, defoliation induced a 34% average reduction in berry number as compared to ND, which led to a notable decrease in fruit-set (-7.6 %), cluster mass (-34%) and yield per shoot (-30%). The berry mass showed a significant relationship with the year factor, as berry size in D shoots was larger, similar and smaller than ND in 2006, 2007 and 2008, respectively. In both treatments, skin mass was closely correlated with berry mass and, within each season and berry mass category, D had relative skin mass values higher than ND. Although the effects were milder in 2007, D induced a significant increase in soluble solids, anthocyanins and phenolics, whereas total acidity was unaffected.

Conclusion: These results indicate that, regardless of final berry size, a pre-flowering defoliation favors skin development, which results in higher relative skin mass than ND vines. This effect appears to predominate over variations due to specific weather and cultural conditions along the season and leads to a marked improvement in final grape composition.

Significance and impact of study: Repeatability of results implies a strong physiological regulation of this technique and encourages extension of its use in areas where the occurrence of high cropping and large clusters with a high degree of compactness does exist. In a global warming scenario, it is also relevant that the yield decrease and the improvement in overall grape composition are not obtained at the expense of a further reduction in berry acidity.

Keywords: berry growth, fruit-set, skin mass, soluble solids, leaf-to-fruit ratio

Résumé

Objectif : La compétitivité dans la viticulture moderne nécessite des techniques culturales économiquement viables qui assurent une amélioration continue de la composition des raisins, malgré la variabilité des cépages et des millésimes. L'effeuillage des feuilles basales en préfloraison est un outil efficace dans le contrôle du rendement principalement grâce à une nouaison réduite. L'étude vise à évaluer l'impact de l'effeuillage précoce sur la croissance de la baie (pellicule, pulpe et pépin), la stabilité du traitement entre les millésimes et l'impact sur la composition finale du raisin.

Méthodes et résultats : L'effeuillage (D) des six premières feuilles basales des rameaux principaux a été appliqué sur des cépages de Barbera (*Vitis vinifera* L.) cultivés en plein champ entre 2006 et 2008 et comparés avec des témoins non effeuillés (ND). Sur une période de trois ans, l'effeuillage a réduit le nombre des baies d'environ 34 % par rapport au témoin ND, la nouaison (-7.6 %), le poids de la grappe (-34 %) et la production par rameau (-30 %). Le poids de la baie a montré une relation significative avec le facteur millésime, car la taille des baies de D était supérieure, similaire et inférieure à ND en 2006, 2007 et 2008 respectivement. Pour les deux traitements, le poids des pellicules était étroitement corrélé au poids de la baie. D a montré des valeurs de poids de pellicules plus importantes que ND. Bien que l'effet a été plus modéré en 2007, D a produit une augmentation significative des solides solubles, des anthocyanes et des phénols, tandis que l'acidité totale n'a pas été affectée.

Conclusion : Les résultats obtenus montrent que l'effeuillage en préfloraison favorise le développement des pellicules indépendamment de la taille finale de la baie, qui se traduit par un poids des pellicules supérieur au témoin. Durant la saison, cet effet semble prédominer les variations dues aux conditions météorologiques et culturales et conduit vers une forte amélioration de la composition finale du raisin.

Signification et impact de l'étude : La répétabilité des résultats implique une forte régulation physiologique de cette technique et encourage l'extension de son usage dans les zones viticoles caractérisées par des rendements élevés et par des grosses grappes serrées. Dans un scénario de réchauffement, il est également important que la réduction du rendement et l'amélioration qualitative du raisin soient poursuivis, en conservant l'acidité de la baie.

Mots-clés : croissance de la baie, nouaison, poids des pellicules, solides solubles, rapport surface foliaire et poids de récolte

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INTRODUCTION

Crop control in vineyards is primarily achieved through regulation of node number per vine retained at winter pruning (Winkler *et al.*, 1974). Then, depending upon bud break rate, shoot fruitfulness and extent of cluster development, a finer tuning might be needed such as hand shoot and/or cluster thinning. To be effective, both operations need to be accurately timed and executed; thus they are usually resulting in high costs. Besides, the crop reduction brought about by cluster thinning does not always achieve the expected, significant grape composition improvement in the retained clusters as compensating growth might lead to thicker clusters having also bigger berries with a less favorable skin-to-flesh mass ratio (Bravdo *et al.*, 1984; Morris *et al.* 1987; Keller *et al.*, 2005).

More recently, it has been shown that a leaf removal carried out around flowering by pulling out a variable number of basal primary leaves (e.g. from 4 to 8) is very effective in reducing yield primarily through a drastic drop in fruit-set, hence reducing the final number of berries per cluster (Poni *et al.*, 2006). Findings were especially encouraging as large yield constraint was obtained across a wide variability of genotypes, environmental conditions and cultural practices (Poni *et al.*, 2006; Diago *et al.*, 2009). Further appealing to such a technique has been recently added by Intrieri *et al.* (2008) showing that pre- or post flowering defoliation can also be quickly performed by machine while maintaining effectiveness in regulating yield.

Regardless of modality of intervention (manual vs. mechanical), this early leaf removal has led to a significant grape composition improvement in both white and red cultivars coupled with an increased leaf-to-fruit ratio in the defoliated shoots as yield was reduced more than total leaf area (Poni *et al.*, 2006, Diago *et al.*, 2009).

However, the impact of early leaf removal on final berry size is not so straightforward. Although a tendency towards final lower berry size in the defoliated shoots is the general expectation (May *et al.*, 1969; Caspari and Lang, 1996), cases have also been reported where berry mass showed no variation or even increased at harvest in the defoliated vines as compared to non-defoliated (Poni *et al.*, 2009). This variability might be related to different processes acting together and triggering complex interactions: on one side a reduction in berry size would be expected simply on the basis of the heavy source limitation which should hinder active cell division in flowers and young berries; on the other side, strongly reduced berry number in the defoliated shoots is conducive to compensatory growth, whereas improved light and temperature microclimate in the fruiting zone might partially counterbalance the negative impact related to the removal of source leaves.

The relationship between early leaf removal, berry growth and final grape composition becomes especially challenging when berry development is also monitored as relative growth of single berry organs (skin, flesh and seeds). In a recent paper, Poni *et al.*, (2009) have shown that early defoliated Barbera vines had decidedly higher relative skin mass in spite of a significantly higher total berry mass, suggesting that this precocious source limitation is able to exert a strong control of the differential growth rates of the various berry organs. As this previous work was limited to a single season data set, the present study was conducted on a three-year basis to determine the consistency of relative skin mass variation in defoliated

Table 1. Monthly mean, maximum and minimum temperature (T) and monthly summation (Σ) of rainfall recorded from April to September during the three seasons (2006-2008) of the trial.

Month	2006				2007				2008			
	T mean (°C)	Tmax (°C)	Tmin (°C)	Rainfall (∑, mm)	T mean (°C)	Tmax (°C)	Tmin (°C)	Rainfall (∑, mm)	T mean (°C)	Tmax (°C)	Tmin (°C)	Rainfall (∑, mm)
April	13.1	18.6	8.3	48.4	16.7	22.6	11.1	15.2	13.2	17.3	9.7	92.8
May	17.6	23.7	12.3	24.6	18.9	25.1	13.6	97	18.3	22.4	14.6	132.4
June	22.0	28.7	15.4	63.6	21.4	27.4	16.0	80	22.2	26.5	18.3	169.4
July	26.1	32.5	19.9	91.2	24.9	31.6	17.8	14.4	24.5	29.6	19.6	7.2
August	21.4	27.6	16.2	105.6	22.3	28.6	16.7	84.8	24.8	30.2	20	40.6
September	20.2	25.9	15.3	187.2	18.3	24.7	12.5	45.8	19.5	24.0	15.7	37
Mean or summation	20.1	26.2	14.6	521	20.4	26.7	14.6	337	20.4	25	16.3	479

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Barbera vines as related to absolute values of berry mass as well as its effect on final grape composition.

MATERIALS AND METHODS

1. Plant material and experimental layout

The 2006-2008 trial was carried out in Piacenza (West Po Valley, 44° 55' N, 9° 44' E, silty clay-loam soil), Italy, using a 10-year-old planting of *Vitis vinifera* L. cv. Barbera (clone AT 84 grafted to 420A rootstock) with a vertically shoot-positioned (VSP) trellising system, spur-pruned cordon with a bud-load of about 10 nodes per meter of row length. Vine spacing was 2.5 m x 1.2 m (inter- and intra-row) and the cordon was raised to 90 cm from the ground with three pairs of surmonting catch wires for a canopy extending 1.4 m above the cordon. Pest management in both vineyards was run according to local standard practices (Regione Emilia-Romagna, 2006) and shoots were mechanically trimmed when most started outgrowing the top wire. Harvest dates were 19 September [Julian Day (JD)] 262], 20 September (JD 263) and 13 September (JD 253) in 2006, 2007 and 2008, respectively. The monthly weather records for mean, maximum and minimum daily air temperatures and daily rainfall from April to September at the experimental site are reported in table 1.

Three adjacent rows were selected to set-up a completely randomized-block design with each row as a block. Within each row, two panels of 3-post spaces (18 m row length, 15 vines per panel) were tagged and randomly assigned to the treatments non-defoliated (ND) or defoliated (D), the latter carried out by manually removing the first six basal primary leaves at stage H (« flowers separated », Baggiolini, 1952). Removal dates were May 24 (JD 144) in 2006 and 2008 and May 17 (JD 137) in 2007. All shoots were treated according to the experimental layout and any laterals that had developed within the 1-6 node shoot zone were removed as well. Ten shoots per row and treatment combination growing at apical position on a spur were then randomly chosen and tagged for subsequent measurements.

2. Fruit-set estimate, berry growth, yield and fruit composition

Each basal cluster per tagged shoot was photographed against a dark background with a digital camera held perpendicular to the inflorescence on the day of defoliation. A regression between the actual flower number and the number of flowers counted on photo prints was then established for 20 inflorescences taken in 2006 from extra shoots chosen within the same row-panel vines and the resulting linear relationship ($y = 1.934x$, $R^2 = 0.95$) was used to estimate initial flower number on tagged inflorescences for the two treatments.

Defoliated leaf area and final total leaf area per shoot were determined upon measuring the surface of each lamina via a leaf area meter (LI-3000A, LI-COR Biosciences, Lincoln, Nebraska, USA), with main and lateral contributions being kept separate. At harvest, the tagged clusters were individually picked, immediately weighed and the number of berries counted. The weight of any distal cluster was also recorded separately. Compactness was visually estimated on each basal test cluster using code OIV 204 (Organisation international de la vigne et du vin, 1983), which ranks from 1 « berries in grouped formation with many visible pedicels » to 9 « misshapen berries ».

Ten berries were taken from each test cluster and individually weighted. The sampled berries were then sliced in half with a razor blade, the seeds and flesh carefully removed from each half-berry using a small metal spatula without rupturing any pigmented hypodermal cells and the seeds then carefully separated by hand from the flesh. Both skins and seeds were rinsed in deionized water, blotted dry and weighted. The remaining flesh was used to make a juice sample for measurement of soluble solids (°Brix), pH and titratable acidity (TA). Total soluble solids were determined by a temperature-compensating refractometer (RX-5000 Atago-Co Ltd, Tokyo, Japan). TA was measured by a Crison Compact Titrator (Crison, Barcelona, Spain) with 0.1 N NaOH to a pH 8.2 end point and was expressed as g/L of tartaric acid equivalents. Total anthocyanins and phenolics were determined according to Iland (1988) on 20-berry samples taken from the remainder of the basal cluster. The berry samples were homogenized at high speed (20,000 rpm) with an Ultra-Turrax (Rose Scientific Ltd, Alberta, Edmonton, Canada) homogenizer for 1 min. Two grams of the homogenate were transferred to a pre-tared centrifuge tube, enriched with 10 mL aqueous ethanol (50%, pH 5.0), capped and mixed periodically for an hour before centrifugation at 959 x g for 5 min. A portion of the extract (0.5 mL) was added to 10 mL 1M HCL, mixed and let stand for three hours; then the absorbance values were recorded at 520 nm and 280 nm on a Kontron (Tri-M Systems and Engineering Inc., Toronto, Ontario, Canada) spectrophotometer. Total anthocyanins and phenolics were expressed as mg per berry and per g of fresh berry mass.

3. Statistical treatment

Data were subjected to a two-way analysis of variance using the SigmaStat software package (Systat Software, Inc. San Jose, CA, USA). Treatment comparison was performed by t-test. Each season, berry mass data were grouped into equally spaced (0.3 g) berry size categories. The number of berry mass categories was seven in 2006 (1.2 – 3.3 g range), eleven in 2007 (0.6 – 3.9 g range) and

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nine in 2008 (1.2 – 3.9 g range). Visual ratings of cluster compactness were subjected to square root transformation prior to analysis.

RESULTS

Total rainfall was the most abundant (521 mm) and fairly well distributed from April through September in 2006, whereas 2007 was the driest season with only 337 mm of rain from April to September and unseasonally warm temperatures in April and May. Conversely, 2008 showed exceptionally high rainfall in early summer and a very dry month of July (table 1).

Based on the three-year survey, the final total leaf area per shoot was lower in D vines, an effect primarily resulting from the pulling of the primary leaves at the timing of defoliation and insufficient lateral re-growth compensation upon defoliation (table 2).

While the two treatments had a very similar flower number per cluster (table 3), defoliation induced a three-year averaged 34% reduction in berry number as compared to ND which, in turn, led to a notable decrease in fruit-set (-7.6%), cluster mass (-34%) and yield per shoot (-30%). Berry mass showed a significant interaction with the year factor, as berry size in D shoots was larger, similar and smaller than ND in 2006, 2007 and 2008, respectively (figure 1A). Conversely, the nature of the year x treatment interaction for cluster compactness was different as D vines always displayed looser clusters, with less pronounced differences in 2007 (figure 1B).

Except for seed number, all components of berry growth (skin, flesh and seeds) showed a significant interaction with season regardless if expressed on an absolute or relative growth basis (table 4). The nature of the interaction was similar to the one previously reported for cluster compactness, as relative skin and seed weight

Table 2. Influence of pre-bloom defoliation on vegetative growth of field grown Barbera grapevines as compared to a non defoliated control. Data are means over 2006-2008.

Source of variation	Removed leaf area/shoot (cm^2)	Final leaf area /shoot (cm^2)	Final primary leaf area/shoot (cm^2)	Final lateral leaf area/shoot (cm^2)
Control	0	4080	2374	1706
Defoliated[a]	1018	3164	1633	1531
Significance[b]	-	*	*	ns
Defoliation x year int.	ns	ns	ns	ns

[a] Removal of leaves from node 1 to 6 on main stem at stage H (flowers separated).
[b] Means separated within columns by t test. *, ns: Significant at $p \leq 0.05$, or not significant, respectively.

Table 3. Influence of pre-bloom defoliation on fruit set, yield per shoot, cluster components and leaf-to-fruit ratios of field grown Barbera grapevines as compared to a non-defoliated control. Data are means over 2006-2008.

Source of variation	Flowers /cluster	Fruit- set (%)	Yield/ Shoot (g)	Total berries/ cluster	Cluster mass (g)	Berry mass (g)	Cluster compactness[a] (OIV rating)	Leaf area shoot/yield (cm^2/g)
Control	547	23.7	553	130	310	2.39	5.2	7.4
Defoliated[b]	534	16.1	387	86	205	2.38	4.1	8.2
Significance[c]	ns	*	*	**	*	ns	*	ns
Defoliation x year int.	ns	ns	ns	ns	ns	**	**	ns

[a] Data transformed into square root prior to analysis.
[b] Removal of leaves from node 1 to 6 on main stem at stage H (flowers separated).
[c] Means separated within columns by t test. *,**, ns: Significant at $p \leq 0.05$, 0.01, or not significant, respectively.

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was always higher in D vines, albeit with attenuated differences in 2007. Conversely, relative flesh weight had an opposite, yet mirror-like pattern (figures 2A,C).

The frequency distribution of fresh berry mass among equally-spaced (0.3 g) categories in all cases fit to normality; yet frequencies of larger size berry categories were higher in D vines in 2006 and 2007, whereas an opposite trend was found in 2008 (figures 3A-C).

Skin mass was closely and positively correlated to berry mass in all seasons (figures 4A-C). However, slope

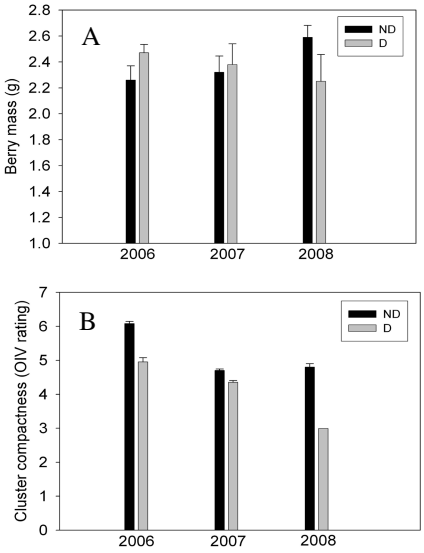


Figure 1. Variation of berry mass (A) and cluster compactness (B) within each year for defoliated and non-defoliated vines.

Vertical bars show 2*standard error (SE) for each treatment combination. Interactive SE = 0.062 for berry mass and 0.211 for cluster compactness, respectively (n = 6). Visual ratings of cluster compactness were subjected to square root transformation prior to analysis.

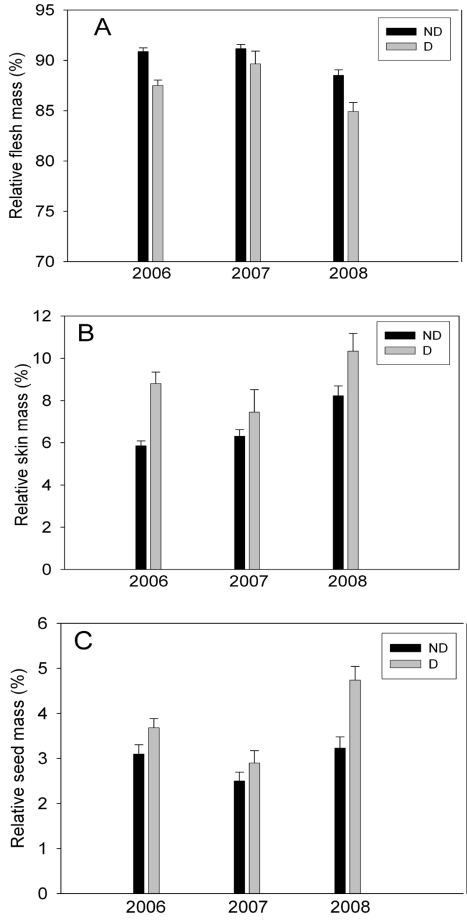


Figure 2. Variation of relative flesh (A), skin (B) and seed (C) mass within each year for defoliated vs. non-defoliated vines.

Vertical bars show 2*standard error (SE) for each treatment combination. Interactive SE = 0.387 for relative flesh mass, 0.662 for relative skin mass, 0.124 for relative seed mass (n = 6). Relative organ mass = 100 (organ mass/total fresh berry mass).

Table 4. Influence of pre-bloom defoliation on berry components and their relative contributions to berry weight of field grown Barbera grapevines as compared to a non-defoliated control. Data are means over 2006-2008.

Source of variation	Flesh mass (g/berry)	Skin mass (g/berry)	Seed mass (g/berry)	Seed number/ berry	Flesh to berry ratio (%)	Skin to berry ratio (%)	Seed to berry ratio (%)
Control	2.16	0.158	0.069	2.12	90.4	6.7	2.9
Defoliated[a]	2.07	0.208	0.088	2.10	87.5	8.8	3.7
Significance[b]	*	*	*	ns	*	*	ns
Defoliation x year int.	*	**	*	ns	**	**	**

[a] Removal of leaves from node 1 to 6 on main stem at stage H (flowers separated).
[b] Means separated within columns by t test. *, ns: Significant at $p \leq 0.05$, or not significant, respectively.

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coefficients of linear regressions were higher in the D treatment which, in almost all cases, also had higher fresh mass than ND within any given category of berry mass.

In both treatments, the relative skin mass decreased as berry mass increased and, except for the ND treatment in 2008, the relationship fit to the data was non linear indicating a faster drop of relative berry mass along the gradient from the smallest berry size class to the 1.2-1.5 g category. In 2006, the change was very small (< 1%) in both treatments over the five highest mass categories (figure 5A). In 2007, a < 1% change was seen in D going from 2.1 to 3.9 g of berry mass (figure 5B), while in 2008 relative skin mass in the defoliated vines decreased by only 0.74% within the berry mass range between 2.1 and 3.6 g (figure 5C). Regardless of berry size, the mean values calculated for relative skin mass were 8.8%, 7.4% and 10.3% in the defoliation treatment as compared to 5.8%, 6.3% and 8.2% in the ND vines for 2006, 2007 and 2008, respectively.

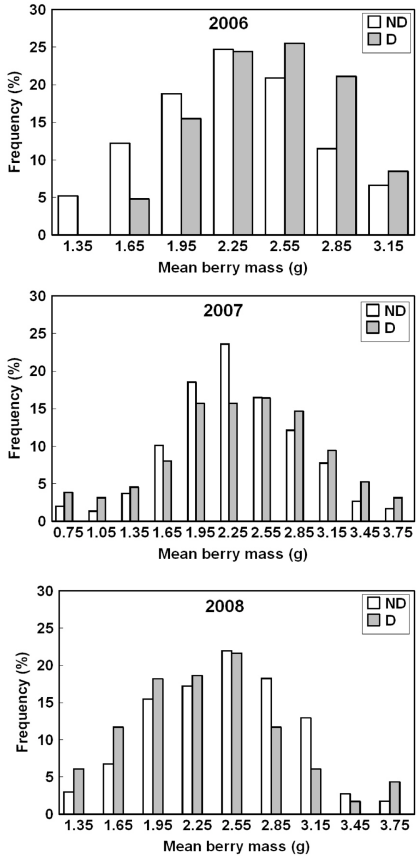


Figure 3. Frequency distribution (% of sampled population) of berry mass in clusters from non-defoliated and defoliated Barbera grapevines in 2006, 2007 and 2008.

Category size was set at 0.3 g. Each treatment population comprises ≈ 300 all seeded, non-dehydrated berries. Normal distribution of each berry population was assessed through the Kolmogorov-Smirnov normality test (P > 0.200).

Leaf removal increased the must pH and total phenols (both concentration and content) while having no effect on total acidity (Table 5). Soluble solids concentration (°Brix) and color accumulation displayed a significant interaction with year as gains in °Brix and anthocyanins in D vines was of greater magnitude in 2006 and 2008, as compared to the 2007 season (figures 6A-C).

Total sugar per shoot was higher in ND vines, while no effects were seen on a per unit of leaf area basis (table 5). Sugar accumulated per berry varied between the two treatments according to season; it was higher in D vines in 2006, whereas no statistical differences were found between treatments in 2007 and 2008 (figure 6D).

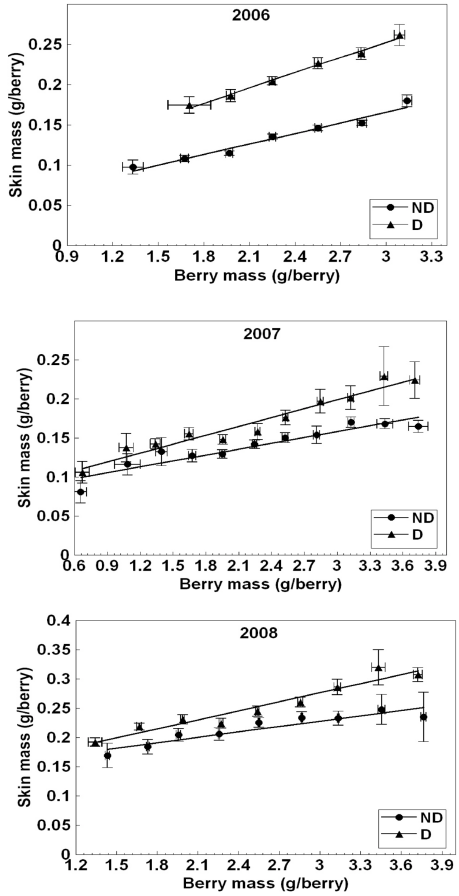


Figure 4. Correlation between skin and berry mass in 2006 (top), 2007 (middle) and 2008 (bottom) for non-defoliated and defoliated Barbera grapevines.

Plotted values represent means of data falling within each category. Error bars represent standard error in both x and y directions. In 2006, the smallest berry category in D had too few berries to be represented. Linear regression equations were: $y = 0.044x + 0.0342$, $R^2 = 0.97$ (ND-2006); $y=0.0626x + 0.0648$, $R^2 = 0.99$ (D-2006); $y=0.025x + 0.0832$, $R^2=0.88$ (ND-2007), $y = 0.0317x – 0.0168$, $R^2 = 0.96$ (D-2007); $y=0.0306x + 0.1363$, $R^2=0.87$ (ND-2008); $y = 0.0515x + 0.1222$, $R^2 = 0.93$ (D-2008).

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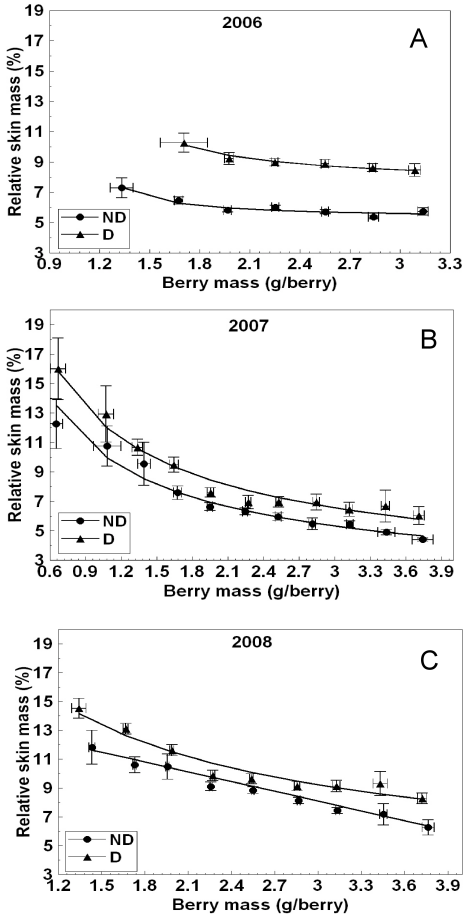


Figure 5. Correlation between relative skin and berry mass in 2006 (top), 2007 (middle) and 2008 (bottom) for non-defoliated and defoliated Barbera grapevines.
Plotted values represent means of data within each category. Error bars represent standard error in both x and y directions. In 2006, the smallest berry category in D had too few berries to be represented. Regression equations were: $y = 7.59x^{-0.3004}$, $R^2 = 0.82$ (ND-2006); $y = 11.58x^{-0.2885}$, $R^2 = 0.88$ (D-2006); $y=10.44x^{-0.6127}$, $R^2=0.97$ (ND-2007); $y=12.53x^{-0.5881}$, $R^2 = 0.96$ (D-2007); $y = -2.264x + 14.688$, $R^2=0.98$ (ND-2008); $y=16.607x^{-0.5352}$, $R^2=0.93$ (D-2008).

DISCUSSION

Data reported in the present study confirm, on a long-term basis, that a pre-flowering leaf defoliation is a very reliable technique for crop control and improved berry composition for field-grown Barbera vines, as also previously shown for cv. Trebbiano (Poni *et al.*, 2006) and cv. Sangiovese (Intrieri *et al.*, 2008). Lack of significant interaction with the year factor for all major yield components implies that the physiological principle by which removing source leaves from a shoot at pre-bloom induces a reduction in the number of set berries (Coombe, 1962; May *et al.*, 1969; Caspari and Lang, 1996) was overwhelming vs. the effects and interferences due to weather conditions which, under our case study, were extremely variable especially in spring and early summer. Yet, partitioning the yield/shoot parameters into its components suggests that the primary mechanism regulating crop is berry number per cluster, whereas berry size displays a response which is mainly driven by other factors (i.e. rate of fruit-set, weather conditions during berry development, etc.). Under such circumstances, the yield response observed in 2006 is self-explanatory: larger berries in D vines (figure 1A) did not prevent a strong decrease in yield per shoot (700 g vs 476 g in ND) and a significant reduction in cluster compactness (figure 1B). In general, in spite of the same intensity of defoliation used in the present study, the effects of crop reduction were milder in 2007. Accumulated heat in spring 2007 (April and May) was much higher [466 Degree Days (DD) according to Winkler *et al.*, 1974] than the values recorded in 2006 (337 DD) and 2008 (351 DD) and resulted in a significant earliness in the grapevine growing cycle. As defoliation was decided to be applied at the onset of the « separate flowers » stage (Baggiolini, 1952), which was reached with considerable advance in 2007 (17 May), it cannot be ruled out that the basal leaves targeted for removal and located in the upper nodes (namely 4th to 6th) were still actively growing and thus exerted sink rather than source activity.

Similarities in the final leaf area per gram of fruit ratios confirm that leaf area curtailment due to defoliation was fully offset from the correlated reduction on fruit-set, hence the yield per shoot; yet, on the other hand, the absence of differences contrasts with the remarkable soluble solids improvement recorded in the defoliation treatment. Very recently (2009), Lakso and Sacks have discussed the inadequacy of this index ratio which is typically static (calculated at harvest) and ignores seasonal dynamics which, especially in a study like ours where a very early and drastic modification of the source-to-sink balance is undertaken, might play a primary role. Therefore, the excellent performance of the D treatment in sugar accumulation is likely to be attributed to both temporary leaf photosynthetic compensation after leaf removal (Candolfi-Vasconcelos and Koblet, 1991) and younger leaf age after veraison (Poni and Giachino, 2000). Furthermore, Poni *et al.* (2008) have conducted a seasonal evaluation of whole-canopy net CO_2 exchange rates (NCER) in pre-flowering defoliated and non-defoliated Sangiovese vines concluding that NCER/yield (shoot basis) was 38% higher in the D vines, thus resulting in increased carbohydrate availability for ripening. Evidence also exists that early defoliation applied at the cluster-zone level hastens translocation of assimilates towards the cluster (Quinlan and Weaver, 1970).

Another challenging outcome in fruit composition was that the °Brix increase was not coupled with a somewhat expected decrease in TA. Such a decrease

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might be expected based on the increase in sun exposure and temperature of clusters following defoliation which should foster the well-documented process of temperature-driven malic acid degradation (Kliewer and Schultz, 1964). Although malic and tartaric acid contributions were not determined in the present study, the absence of reduction in the total acidity might actually be due to enhanced synthesis of both these acids during the green phase of berry growth. Lakso and Kliewer (1975) have shown that malic acid accumulation is favoured by moderate temperatures (20-25 °C) which, however, during the cell division stage of berry growth are more easily reached in the defoliation plots as a result of the abrupt change in cluster microclimate; moreover Kliewer and Schultz (1964) reported for berries exposed to direct sunlight higher amounts of $^{14}CO_2$ incorporated into tartaric acid as compared to the shaded berries.

As previously shown for single-season data from Poni *et al.* (2009), our three-year survey strongly reinforces that, regardless of the treatment imposed on the vine, a very close linear and positive correlation between skin

Table 5. Influence of pre-bloom defoliation on standard must parameters, total berry anthocyanins and phenols, and sugar accumulation efficiency of field grown Barbera grapevines as compared to a non-defoliated control. LA = leaf area. Data are means over 2006-2008.

Source of variation	Soluble solids (°Brix)	pH	TA (g/L)	Total anthocyanins		Total phenols		Total sugar/		
				mg/berry	mg/g	mg/berry	mg/g	shoot (g)	berry (g)	cm^2 LA(mg)
Control	21.9	3.04	10.4	2.98	1.27	4.30	1.83	121	0.523	34.6
Defoliated[a]	24.1	3.10	9.9	3.90	1.68	5.55	2.39	93	0.573	33.4
Significance[b]	*	*	ns	*	*	*	**	*	ns	ns
Defoliation x year int.	**	*	ns	*	*	ns	ns	ns	**	ns

[a] Removal of leaves from node 1 to 6 on main stem at stage H (flowers separated).
[b] Means separated within columns by t test. *,**, ns: Significant at $p \leq 0.05$, 0.01, or not significant, respectively.

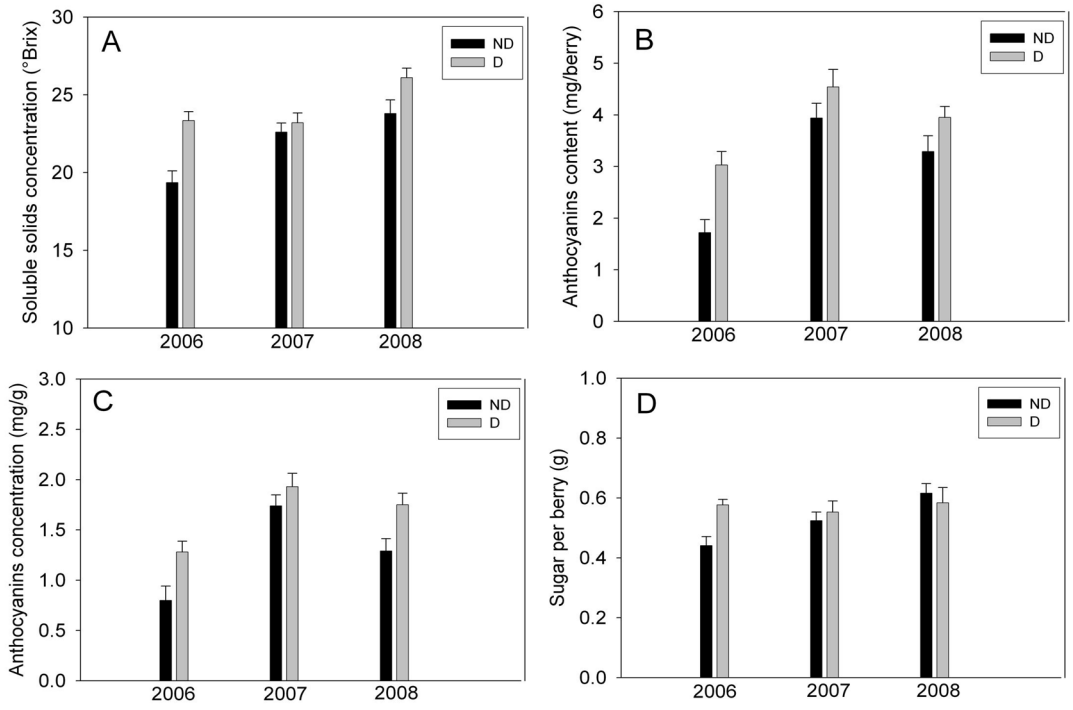


Figure 6. Variation of must soluble solids (A), anthocyanins content (B), anthocyanins concentration (C) and sugar per berry (D) within each year for defoliated and non-defoliated vines.
Vertical bars show 2*standard error (SE) for each treatment combination. Interactive SE = 0.337 for must soluble solids, 0.145 for anthocyanins content, 0.060 for anthocyanins concentration, 0.0167 for sugar per berry (n = 6).

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and berry mass in Barbera does exist. This indicates that changes in the skin-to-berry ratios with increasing berry mass follows a pattern which cannot be simply explained from the expected dilution calculated using the surface area-to-volume ratio of a sphere. Most remarkably, in the D vines, there was little or no variation in relative skin mass for the berry categories c, d and e in 2006 (1.8 – 2.7 berry mass range), categories e, f, g , h in 2007 (1.8 – 3.0 g berry mass range) and categories d, e, f and g in 2008 (2.1 – 3.3 g berry mass range) which were representative, within each season, of approximately 60% of the total berry population. Roby and Matthews (2004) showed essentially that there were no changes in relative skin mass for Cabernet Sauvignon berries ranging from 0.6 to 1.6 g of fresh mass, whereas Walker *et al.* (2005) reported no significant modifications in the ratio of skin fresh weight across seven mass categories of both own-rooted and Ramsey-grafted Shiraz berries.

For all berry size categories, D berries had more absolute and relative skin mass than ND berries, the effects being particularly important in 2006 and 2008 (figures 4, 5, A-C). This outcome provides strong evidence that early defoliation is predominant at leading to a higher relative skin mass regardless of specific climate trends, vigor conditions and, most noticeably, final berry size, which was similar to, smaller or larger than ND control, in 2006, 2007 and 2008, respectively. It is therefore suggested that skin growth in the D treatment greatly benefits from the improved microclimate regime following defoliation and, in the short term, increased cluster temperature might have created local conditions more favourable to cell division, hence, skin deposition. Earlier work (Harris, 1968; Kliewer, 1977) had shown that cell division at either the pericarp or exocarp level seems to be quite sensitive to temperature. Then, a more recent and focused contribution from Caporali *et al.* (2005) has shown that Barbera berries sampled from vines defoliated at the beginning of fruit-set developed more cell layers in the skin than non-defoliated vines. Indirect support to this hypothesis is also provided from Mescalchin *et al.* (2008) who showed that leaf removal performed at bloom drastically reduced sunburn damage in Pinot Gris berries as compared to later defoliations, suggesting that the early defoliated vines had thicker berry skin. In our study, higher relative skin mass gave a significant increase in berry anthocyanin concentration (mg/g fresh mass), thereby matching the significant relationship ($R^2 = 0.70$) between the same two variables found for Cabernet Sauvignon by Roby *et al.* (2004).

CONCLUSION

Our three-year survey on the effects of a pre-flowering leaf removal on vine performance of cv. Barbera fortifies its effectiveness as a technique to decrease cropping, primarily through reduced fruit-set effect, and improve grape composition. Yet, the consistent improvement observed in berry pigmentation (anthocyanins concentration and content) appeared to be closely related to a local effect on skin growth which, on a relative basis, was invariably enhanced by the early defoliation. A related significant outcome was that higher relative skin mass in defoliated shoots occurred regardless of final berry size, suggesting that berry size itself is not an inherent quality factor and that, rather, the causal factors that change the relative growth of different berry organs are the main actors for final grape berry composition. Then, due to the projection of future global warming and increasing need of adapting pattern of growth and ripening to the changing climatic scenarios, it is encouraging to note that this technique does maintain acidity levels while fostering tolerance to berry scorching and sunburn through a higher relative skin mass. Overall, we feel that findings presented in this study might provide a good example of how an intervention on summer pruning (i.e. defoliation) might actually be "used" by the growers to promote desirable features in the final product rather than being seen like something which « has to be done » simply based on tradition or rules of thumb with no physiological bases for forecasting late season effects and ultimate impact on grape composition.

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