Effects of Early Defoliation on Shoot Photosynthesis, Yield Components, and Grape Composition

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Abstract: The effectiveness of early leaf removal on high-yielding cultivars Sangiovese and Trebbiano (*Vitis vinifera* L.) was investigated as a tool for reducing crop potential and for inducing looser clusters that are less susceptible to rot. Fruit set, cluster weight, berry number per cluster, berry size, and cluster compactness were reduced by all defoliation treatments as compared to non-defoliated shoots. Physiological assessment performed in a one-year study on Sangiovese indicated that prebloom removal of the six basal leaves elicited no difference between treatments in mean seasonal assimilation (A) per shoot (2.91 µmol s^{-1} for control against 2.81 µmol s^{-1} for the defoliated), a fact due to the offsetting action of more vigorous lateral shoot formation and higher A rates for both main and lateral leaves after veraison in the defoliated shoots. Grape composition was improved by defoliation (higher Brix in both cultivars and higher anthocyanins and phenolics in Sangiovese) as a result of more assimilates being available per unit of cropping and smaller berries characterized by an increased skin-to-pulp ratio. The three-year-study on Trebbiano also showed no carryover effects of defoliation on the following year's bud differentiation and very few year x treatment interactions, suggesting the prevailing effects of leaf removal over variability because of climate. Overall, early defoliation may be an excellent tool for yield control, replacing time-consuming manual cluster thinning. A time-consistent response suggests that this practice may also improve grape composition.

Key words: *Vitis vinifera* L., leaf removal, gas exchange, fruit set, berry growth

One of the most frequently applied summer canopy management operations in winegrape growing is fruit-zone leaf removal, whether manual or mechanical (Bledsoe et al. 1988, Kliewer and Antcliff 1970, Koblet 1996, Percival et al. 1994, Reynolds et al. 1996, Smart 1985, Zoecklein et al. 1992). Although this practice may have different goals, it is usually employed from fruit set to veraison on high-density canopies to improve light exposure and air circulation around the clusters, with substantial benefits in terms of pigmentation and tolerance to rot (Bledsoe et al. 1988, Reynolds et al. 1996, Smart 1985). Yet, improved fruit composition is not a consistent result of leaf removal (Percival et al. 1994), and, when present, it often appears to be an indirect consequence of improved cluster microclimate. Indeed, excessive leaf removal, with resulting overly exposed clusters, has led to lower berry color in red varieties (Price et al. 1995). A recent study found that leaf removal from the lower quarter of the canopy during the lag phase of berry growth caused a significant decrease of whole-vine photosynthesis even on a per-unit leaf area basis, thus suggesting that the lower portion of the canopy contributed more than the upper portion to the whole-vine carbon budget (Petrie et al. 2003). A possible explanation of this finding is that although basal, and hence older, leaves are removed by defoliation, they are also the largest leaves along the shoot and their size can offset lower photosynthetic rates. Additionally, removal of all the leaves from the fruiting area, which thereby exposes the clusters to full sun, might lead in warm climates to compromised fruit composition because of excessive berry temperatures, which can hinder color formation and cause a sharp drop in malic acid concentrations (Bergqvist et al. 2001).

The effects of leaf removal on yield are quite variable depending upon timing and severity. Carbohydrate supply at flowering is a primary determinant of fruit set (Caspari and Lang 1996, Coombe 1959), and early leaf removal (e.g., within four weeks after flowering) typically reduces yield and the amount of total sugar per vine (Hunter and Visser 1990, Kliewer and Antcliff 1970, May et al. 1969). However, if leaf removal is performed later and/or with minimal severity, yield may not change (Bledsoe et al. 1988, Hunter and Visser 1990, Smith et al. 1988) or might even occasionally increase as compared with non-defoliated vines (Zoecklein et al. 1992). The variability of the impact that leaf removal has on yield and their components is likely dependent upon the negative effects on

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fruit set and berry growth in the current year and positive effects on bud induction and differentiation for the next year's crop via an improvement in canopy microclimate.

The functional relationship between source availability around bloom and yield (Caspari and Lang 1996, Coombe 1959, Kliewer 1970, May et al. 1969, Petrie et al. 2003) inherently implies that defoliation carried out around flowering can reduce fruit set, leading to looser clusters. This approach could potentially be very useful in high-yielding environments and cultivars marked by large, excessively tight clusters which increase the danger of infection by rot and even in obviating the need for time-consuming and expensive cluster thinning. While preliminary investigations have shown how powerful early defoliation can be in reducing yield while achieving better must composition and reduced Botrytis infection (Prior 2003, Poni et al. (2005), they did not consider the physiological changes triggered by this technique or provide long-term observations. Thus, our present effort was designed with the following objectives: (1) to investigate the seasonal modifications of shoot photosynthesis elicited by early defoliation as well as assess correlations with yield components and grape composition in pot-grown Sangiovese grapevines and (2) to determine the effects of several early defoliation treatments in field-grown Trebbiano grapevines over a relatively extended period.

Materials and Methods

Pot study. This trial was carried out in 2005 near Piacenza, Italy (lat: 44°55'N; long: 9°44'E), on five-year-old Sangiovese (*Vitis vinifera* L.) grapevines (clone 12T grafted to SO4) grown outside in 70-L pots wrapped with foil to limit root-system overheating. Sangiovese was chosen because it is the top red cultivar grown in Italy (current acreage stands at about 70,000 ha) and serves as the basis of such prestigious wines as Brunello and Chianti, and it is characterized by highly compact clusters that are very susceptible to rot. In addition, given its very high bud fruitfulness, Sangiovese easily tends to overcrop, especially when grown at vigorous sites. Specific aims of the pot study were to clarify the physiological bases of this early leaf removal and establish correlations with final grape composition.

Six vines trained to bilateral Guyot (total cane length ~2 m/vine) were arranged along a single SE-NW (35°) oriented row. Shoots were vertically positioned along catch wires up to a maximum canopy length of 1.8 m. To mimic a field situation, border rows were created with extra vines of the same variety 2 m from the test row. Vines were protected from hail damage by a white shading net (90% light transmission) and irrigated twice a day with microdrippers to deliver about 6 L of water per day. Pest treatments were applied as per local practices; no sprays against Botrytis were performed (Regione Emilia-Romagna 2006).

When shoots had reached stage G (separate clusters) (Baggiolini 1952), shoot thinning was applied to each vine so as to retain about 30 shoots per vine (≅15 per meter of cane length), and clusters were manually thinned to one inflorescence per shoot. On day 145 (25 May) of the year (DOY), corresponding to stage H (separate flower buttons) (Baggiolini 1952), three vines were assigned in a completely randomized design to a defoliated treatment which consisted in removing the first six basal main leaves of each shoot, whereas the remaining three vines were left non-defoliated. Concurrently, six shoots per vine were randomly chosen and tagged for subsequent detailed measurements. All shoots of each vine were trimmed to 16 main leaves on DOY 167 (16 June) to avoid shoot overhang of the fruiting area and to reproduce a condition frequently met under field conditions.

Fruit-set estimate, growth, yield, and grape composition. Each cluster per tagged shoot was photographed against a dark background with a digital camera held perpendicular to the inflorescence the day before defoliation. A regression between actual flower number and the number of flowers counted on photo prints was then established for 20 inflorescences taken from extra vines and the resulting linear relationship (Figure 1) was used to estimate initial flower number on tagged inflorescences.

Leaf area removed by defoliation and final total leaf area per shoot were estimated via a leaf area meter (LI-3000A, LI-COR Biosciences, Lincoln, NE), with main and lateral contributions being kept separate. At harvest (31 Aug; DOY 242) the tagged clusters were individually picked, immediately weighed, and the number of normal and shot berries counted. Cluster compactness was visually estimated using code OIV 204 (OIV 1983), which ranks "berries in grouped formation with many visible pedicels" as 1 and "berries out of shape" as 9.

From each test cluster, 10 berries were sampled and individually weighed. These berries were then sliced in half with a razor blade, seed and flesh were carefully removed

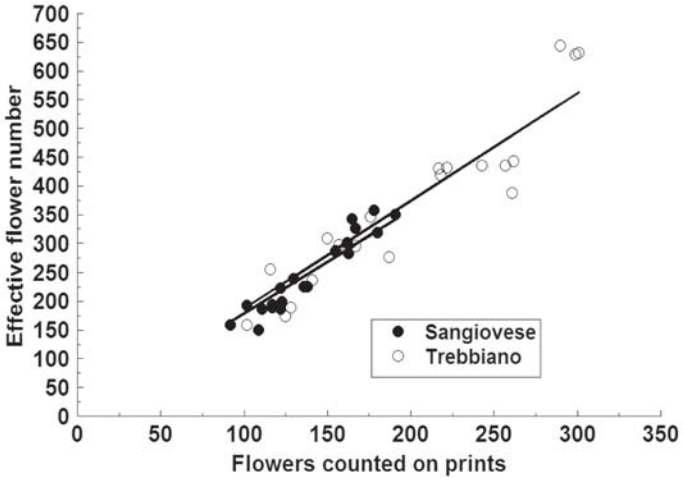

Figure 1 Relationship between actual flower number and flower number counted on photo prints of 20 inflorescences for pot-grown Sangiovese and field-grown Trebbiano grapevines (n = 20). Regression equations: $y = 1.7907x$, $r^2 = 0.88$ (Sangiovese); $y = 1.8721x$, $r^2 = 0.87$ (Trebbiano).

from each berry half using a small metal spatula, without rupturing any pigmented hypodermal cells, and seeds were then carefully separated by hand from the berry flesh. Both skins and seeds were rinsed in deionized water, blotted dry, and weighed.

Half of the remainder of each cluster was crushed and the concentration of total soluble solids (Brix) was determined by a temperature-compensating refractometer (RX-5000 ATAGO U.S.A., Bellevue, WA). Titratable acidity (TA) was measured by titration 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 on the second half of each cluster after Iland (1988). The cluster parts were homogenized at high speed (20000 rpm) with an Ultra-Turrax (Rose Scientific, Alberta, Canada) homogenizer for 1 min. Two grams of the homogenate were transferred to a pretared centrifuge tube, enriched with 10 mL aqueous ethanol (50%, pH 5.0), capped, and mixed periodically for one hour before centrifugation at 3500 rpm for 5 min. A portion of the extract (0.5 mL) was added to 10 mL 1 M HCL, mixed, and let stand for three hours; then the absorbance values were registered at 520 nm and 280 nm as taken on a Kontron spectrophotometer (Tri-M Systems and Engineering, Toronto, Canada). Total anthocyanins and phenolics were expressed as mg per berry and per g of fresh berry mass.

Gas exchange. Leaf assimilation (A) was measured the morning before defoliation (DOY 145), and then on DOY 162, 181, 200, 216, and 242 (i.e., 17, 36, 55, 71, and 97 days after defoliation) on three of the six tagged shoots per vine using an LCi ultracompact portable gas-exchange system (ADC, Hoddesdon, UK). The system featured a broad leaf chamber having a 6.5 cm^2 window and all readings were taken at ambient relative humidity with an air flow adjusted to 400 mL min^{-1}. Within each date, every other main leaf was measured by sampling acropetally, starting with the first basal, normally developed leaf (usually the one inserted at node 2). On each shoot, two lateral shoots inserted at basal (i.e., between nodes 1 and 8) and distal (i.e., between nodes 9 and 16) position on the main stem were selected and one every other lateral leaf was measured by sampling acropetally beginning with node 1 on the lateral shoot; because of negligible growth, no lateral leaves were measured on DOY 145. All leaves were measured in the morning (from 09.00 to 12.00 hr) under conditions of saturating light.

The lamina length of each main and lateral leaf inserted on the tagged shoots was measured on DOY 145, 162, 181, 200, and 216. This allowed estimation of the total leaf area per shoot using models relating actual leaf area (y) and squared lamina length (x) for main ($y = 0.9189x$; $r^2 = 0.89$) and lateral ($y = 1.0029x$; $r^2 = 0.93$) leaves. Regressions were built over 30-leaf samplings of each leaf type taken at the end of the season from the extra vines.

Field study. This trial was conducted over three years (2003 to 2005) in a 15-year-old Trebbiano (*Vitis vinifera* L.) vineyard grafted on Kober 5BB and located in Imola, Italy (lat: 44°20'N; long: 11°42'E). Vines were spur-pruned (~40 nodes per vines) and spaced 2 m in the row and 3.5 m between rows. Cordon height was 1.2 m aboveground and four catch wires were placed above to allow a total of about 3 m maximum canopy height. The extensive canopy wall meant that shoot trimming was not usually needed, except for a late trim around veraison in 2005.

Both Trebbiano and Sangiovese have high-yielding capability and compact clusters that are quite susceptible to rot. Four adjacent rows were selected from a uniform plot of 12 rows to build a complete randomized block design where each row was used as a block. Within each row, four vines with comparable node number were tagged and randomly assigned to the following treatments: (A) non-defoliated (control); (B) hand removal of the first eight basal leaves at stage H (treatment H-100%); (C) hand removal of the first eight basal leaves at the phenological stage of fruit set (stage J, defined as ovary diameter ~3 to 4 mm) (Baggiolini 1952) (treatment J-100%); and (D) hand removal of one every two leaves within the 1 to 8 node shoot zone at stage H and of the remaining four leaves at stage J (treatment H-J-50%). All shoots per vine were treated according to the experimental layout, and, at each removal date, any laterals developed within the 1 to 8 node shoot zone were also removed. Concurrently, four shoots per vine were randomly chosen and tagged for subsequent detailed measurements. Initial flower number on test clusters was estimated after the method described above for Sangiovese (Figure 1). Dates of leaf removal were 25 May, 4 June, and 26 May at stage H and 10, 14, and 10 June at stage J for 2003, 2004, and 2005, respectively. Harvest dates were 19, 29, and 9 Sept for 2003, 2004, 2005, respectively. Weather in the 2003 season was exceptionally dry with only 51 mm of rain in June to August, whereas rainfall for the same period in 2004 and 2005 was 90 and 143 mm, respectively, thus configuring a pattern of moderate and abundant water supply (Figure 2).

Leaf area removed by defoliation, final leaf area per shoot (main and lateral contributions), yield per shoot, cluster

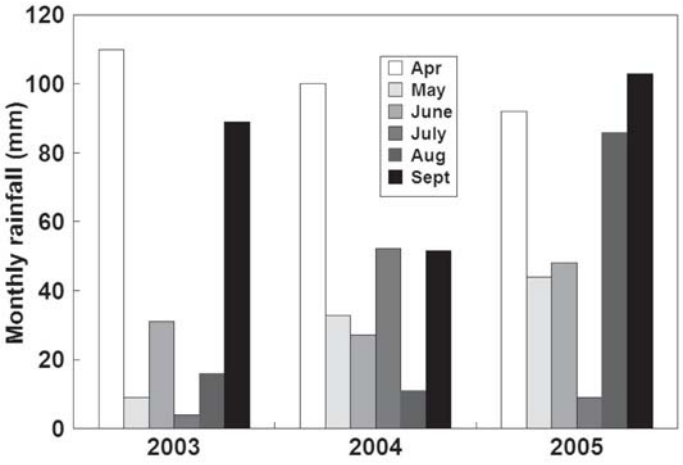

Figure 2 Monthly rainfall (April through September) recorded each year (2003 to 2005) at the Trebbiano experimental site.

compactness, soluble solids, pH, and TA were recorded as previously described for Sangiovese. Tartrate was assessed on must via the colorimetric method based on silver nitrate and ammonium vanadate reactions (Lipka and Tanner 1974). Malate was determined with a Boehringer kit (Boehringer, Mannheim, Germany), which uses L-malic dehydrogenase to catalyze the reaction between malate and NAD^+ to oxaloacetate and NADH. The reaction products were measured spectrophotometrically by the change in absorbance at 340 nm from the reduction of NAD^+ to NADH.

The weight of any distal clusters was also recorded separately and the severity of rot was estimated by calculating the ratio of berries with visual symptoms to total berries in each basal cluster. The incidence of shot berries was negligible for any year and treatment. The total number of clusters per vine at the 2004 and 2005 harvests was recorded, and the total number of primary canes was counted at leaf fall to calculate mean cane fruitfulness and to estimate carryover effects on bud differentiation induced by leaf removal.

Statistical treatment. Treatment comparison for the Sangiovese study was performed by *t*-test and by assessing the variation around means as determined by the standard error. A combined analysis of variance analysis over years (Gomez and Gomez 1984) was performed using the GLM procedure of the SAS statistical package (SAS Institute, Cary, NC) on the Trebbiano data. Year was considered as a random variable and the error term for the defoliation treatments was the year x treatment interaction mean square. Mean separation between defoliation levels was performed with the Student Newman-Keuls test. The year x treatment interaction was tested over the pooled error and considered only if significant.

Results

Sangiovese study. Defoliation reduced the amount of main leaf area as compared to control shoots on all dates thereafter and also triggered the strongest lateral regrowth, which after trimming proceeded slowly until about 40 days after removal and then remarkably hastened over the second part of the season, reaching a higher value than that recorded on the non-defoliated shoots at harvest (Figure 3). Defoliation at stage H removed about 30% of final total leaf area per shoot at the end of the trial, although it was not different from the value recorded in the control vines because of compensation related to stronger lateral formation (Table 1).

Fruit set (percent of total berries to total flower number), number of shot berries, cluster weight, and berry weight were all reduced by leaf removal, which also induced the related effect of looser clusters (Table 2). Non-defoliated vines had larger berries, more pulp, and higher seed numbers and weights per berry (Table 3). Since the total skin weight per berry was unaffected, leaf removal achieved a higher skin-to-pulp ratio, whereas the fraction of skin over total berry weight was 8% in defoliated shoots versus 6.4% in control.

Leaf removal improved must Brix and total anthocyanins (both concentration and per berry) (Table 4). Interestingly, defoliation also increased TA and total phenols when expressed on a concentration basis. Shoot efficiency evaluated as total sugar per shoot, per berry, and per unit leaf area was not affected by treatments nor was the leaf area-to-yield ratio (shoot basis), although it was slightly higher for the defoliated shoots (Table 1).

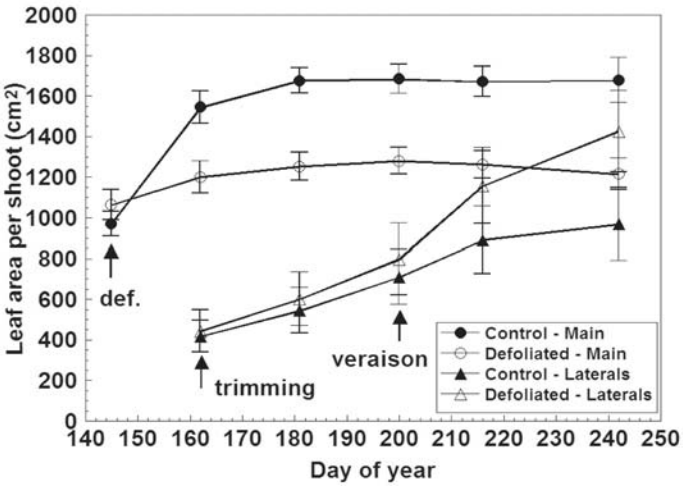

Figure 3 Seasonal variation of leaf area per shoot (main and laterals) in pot-grown Sangiovese grapevines subjected to early defoliation or non-defoliated (control). Vertical bars indicate SE.

Table 1 Influence of early defoliation on vegetative growth and source-sink balance of pot-grown Sangiovese grapevines as compared to a non-defoliated control.

Source of variation	Removed LA/shoot[a] (cm²)	Final LA/shoot (cm²)	Final main LA/shoot (cm²)	Final lateral LA/shoot (cm²)	Total sugar (g)			LA shoot/yield (cm²/g)
					per shoot	per berry	per cm² LA	
Control	0	2504.7	1577.4 a	927.3 b	55.9	0.39	0.0263	8.4
Defoliated[b]	777.5	2632.2	1193.1 b	1439.1 a	49.7	0.40	0.0254	11.4
Significance[c]	-	ns	**	*	ns	ns	ns	ns

[a]LA: leaf area.
[b]Removal of leaves from node 1 to 6 on main stems at stage H (separate flower buttons).
[c]Means separated within columns by *t*-test. *, **, ns: significant at $p \leq 0.05$, 0.01, or not significant, respectively.

Regression of A/leaf versus leaf position on main stem (data recorded before defoliation and pooled over treatments) showed maximum rates at node 4 and then a steady decline toward the apex (Figure 4). Pre-defoliation mean leaf A rates assessed in the morning over the gradient of leaf age of expanded main leaves along the shoot were very similar between treatments and close to values considered to be optimal for *V. vinifera* (Figure 5). Readings taken at fruit set (17 days post-defoliation) showed some photosynthetic compensation occurring on main leaves of the defoliated shoots, although this effect disappeared 36 days after defoliation when assimilation rates measured on main leaves of the non-defoliated shoots were higher than those recorded on defoliated shoots (Figure 5). However, this latter treatment closed this gap at veraison and showed higher photosynthesis over the last two measurement dates as compared to control. Assimilation measured seasonally on lateral leaves essentially showed similar

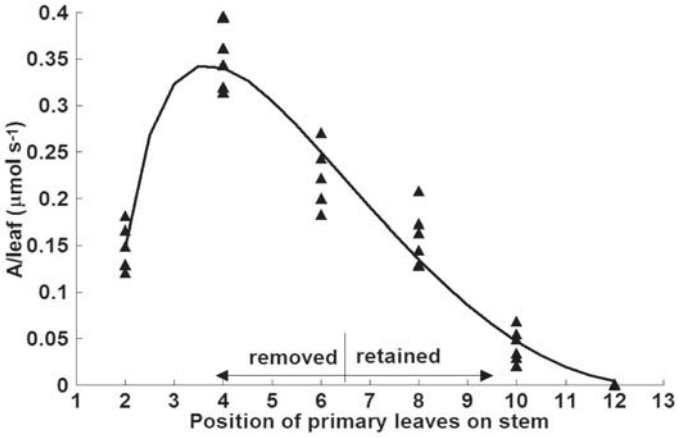

Figure 4 Relationship between assimilation rate/leaf and position of primary leaves on stem determined before defoliation in pot-grown Sangiovese grapevines (25 May, data pooled over treatments). Curvilinear regression equation: $y = 1.57\text{-}0.209x+0.0077x^2\text{-}2.070/x$, $r^2 = 0.93$.

Table 2 Influence of early defoliation on fruit-set traits and cluster components of pot-grown Sangiovese grapevines as compared to a non-defoliated control.

Source of variation	Flowers/ cluster	Fruit set (%)	Normal berries/ cluster	Shot berries/ cluster	Total berries/ cluster	Cluster wt (g)	Berry wt (g)	Cluster compactness (rating)[a]
Control	408	35.2 a	119.2	24.5 a	143.7	305 a	2.59 a	5.9 a
Defoliated[b]	419	29.5 b	112.9	10.9 b	123.8	245 b	2.22 b	4.1 b
Significance[c]	ns	*	ns	**	*	**	**	**

[a]Rated according to OIV 204 standard.
[b]Removal of leaves from node 1 to 6 on main stems at stage H (separate flower buttons).
[c]Means separated within columns by *t*-test. *, **, ns: significant at $p \leq 0.05$, 0.01, or not significant, respectively.

Table 3 Influence of early defoliation on berry components of pot-grown Sangiovese grapevines as compared to a non-defoliated control.

Source of variation	Berry wt[a] (g)	Pulp wt (g/berry)	Skin wt (g/berry)	Seed wt (g/berry)	Skin-to-pulp ratio (%)	Seed no./ berry
Control	3.00 a	2.68 a	0.195	0.129 a	7.4 b	3.4 a
Defoliated[b]	2.41 b	2.12 b	0.194	0.103 b	9.3 a	2.7 b
Significance[c]	**	**	ns	**	**	**

[a]Based on 10-berry subsamples per cluster.
[b]Removal of leaves from node 1 to 6 on main stems at stage H (separate flower buttons).
[c]Means separated within columns by *t*-test. *, **, ns: significant at $p \leq 0.05$, 0.01, or not significant, respectively.

Table 4 Influence of early defoliation on standard must composition variables, total phenolics, and anthocyanins of pot-grown Sangiovese grapevines as compared to a non-defoliated control.

Source of variation	Soluble solids (Brix)	pH	TA (g/L)	Total anthocyanins		Total phenolics	
				mg/berry	mg/g	mg/berry	mg/g
Control	18.3 b	3.39	5.7 b	0.637 b	0.787 b	1.744 a	2.154 b
Defoliated[a]	20.1 a	3.41	6.1 a	0.729 a	1.133 a	1.541 b	2.385 a
Significance[b]	**	ns	**	*	**	**	**

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

relative variations between treatments, although significance was reached only at DOY 181 (higher values for control) and at harvest (higher values for the defoliated shoots).

When A/shoot was calculated by combining leaf area and assimilation rates over the season, non-defoliated shoots had higher values 36 days after defoliation (Figure 6). However, A/shoot in the control vines showed a steady decline from veraison onward, whereas defoliated shoots had a rather constant total assimilation over the last three measurement dates. The difference between treatments broadened at harvest but without reaching significance, primarily because of intravine variability in lateral development.

Trebbiano study. Although the total leaf area removed on a three-year-basis in the H-100% treatment was lower with respect to the other defoliation levels (Table 5), because of compensating lateral regrowth, the same treatment showed at the end of season a residual total leaf area similar to the remaining leaf-removal treatments. Final total leaf area per shoot in H-100%, J-100%, and H-J-50% was 71, 65, and 70% of control, respectively, whereas in the same order the fraction of final leaf area contributed by laterals was 41, 23, and 27%. In general, early leaf removal promoted more lateral regrowth.

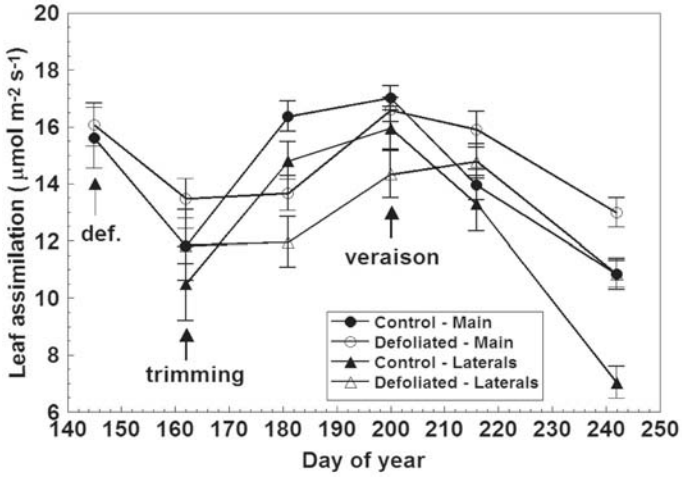

Figure 5 Seasonal variation of leaf assimilation (main and lateral leaves) in pot-grown Sangiovese grapevines subjected to early defoliation or non-defoliated (control). Vertical bars indicate SE.

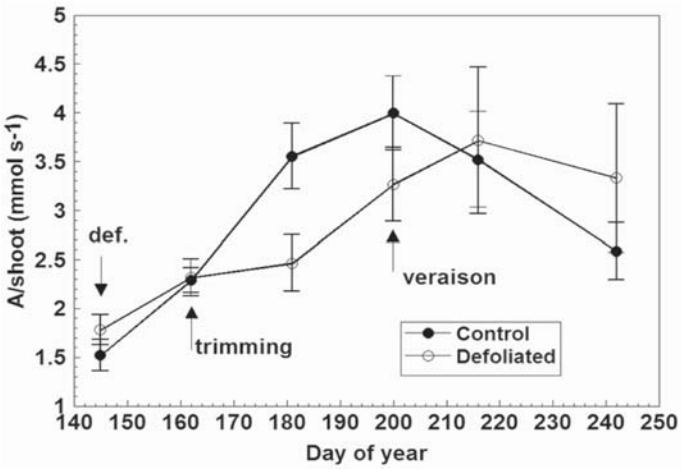

Figure 6 Seasonal variation of assimilation/shoot in pot-grown Sangiovese grapevines subjected to early defoliation or non-defoliated (control). Vertical bars indicate SE.

A significant year-by-treatment interaction was found for the total number of flowers per cluster and fruit-set percentage (Table 6). Partitioning of these interactions showed that, although the percent of fruit set was reduced each year by all defoliation treatments as compared to control, the extent of this reduction was lower in 2004 (Figure 7A). Conversely, while the number of flowers per cluster showed no differences among treatments in 2003, the same variable was lower in most defoliation treatments in 2004 and 2005 as compared to the counts on control shoots (Figure 7B). On a three-year basis, yield per shoot, cluster and berry weight, berry number per cluster, cluster compactness, and rot incidence were in all cases markedly reduced by defoliation as compared with the control (Table 6). The yield component that contributed most to lower the cropping potential was number of berries; berry weight was more negatively affected by the J-100% treatment compared with the other leaf-removal treatments. The final leaf area-to-yield ratio, calculated on a per shoot basis, increased in all defoliation treatments, reaching significance for H-100% and H-J-50% (Table 5). Total sugar produced per shoot was much higher in the non-defoliated shoots, although this response changed when sugar was given on a per berry basis, indicating either a decrease (J-100%) or even an increase (H-100%) as compared with the control plots. No significant carryover effects of the defoliation treatments were found on next season's bud fertility (Table 6).

Must composition variables were markedly affected by leaf removal. All defoliation treatments led to increases in must Brix (Table 7) and to a general tendency toward lower pH, higher tartrate, and lower malate concentrations. The significant year-by-treatment interaction found for soluble solids (Figure 8) indicated a year-by-year difference in the magnitude of the soluble solids gain because of defoliation; yet, the absolute values were invariably higher in the leaf-removal treatments.

Discussion

The decrease in fruit set found in both studies confirms that, even in nongirdled shoots, the source supply around anthesis is the primary determinant of fruit set, as shown in pioneering work (Coombe 1962) and in more recent contributions (Caspari and Lang 1996, Poni et al. 2005).

The fruit-set reduction in Sangiovese (5.7% less than control) was lower than the decrease observed in year one for Trebbiano (Figure 7A), which was 19% less than the control compared with the mean of all defoliation treatments. Besides differences related to genotype and growing conditions (pot versus field), the major reason for this gap resides in the severity of defoliation (six basal

leaves removed in Sangiovese versus eight in Trebbiano), emphasizing that the relationship between the number of mature leaves removed and the percent of fruit set might not be linear. In a detailed defoliation study conducted on girdled Sauvignon blanc shoots, the authors reported a drop in fruit set from 17 to 5% when the number of mature leaves removed at bloom increased from six to eight (Caspari and Lang 1996). In a different study, the number

Table 5 Influence of early defoliation treatments on vegetative growth (LA: leaf area) and source-sink balance of field-grown Trebbiano grapevines as compared to a non-defoliated control. Data averaged over 2003 to 2005.

Source of variation[a]	Removed LA/shoot (cm^2)	Final LA/shoot (cm^2)	Final main LA/shoot (cm^2)	Final lateral LA/shoot (cm^2)	Total sugar (g)			LA/yield (cm^2/g)
					per shoot	per berry	per cm^2 LA	
Control	–	3079 a	2287 a	792 a	95.7 a	0.375 b	0.034	6.2 b
H-100%	1357 b	2190 b	1554 b	635 ab	53.8 b	0.402 a	0.027	8.9 a
J-100%	1720 a	2011 b	1551 b	461 b	53.5 b	0.353 c	0.031	7.6 ab
H-J-50%	1738 a	2155 b	1569 b	586 ab	48.6 b	0.376 b	0.028	9.4 a
Significance[b]	*	**	**	**	**	**	ns	**
Defoliation x year interaction	ns	ns	ns	ns	ns	*	ns	ns

[a]H: stage of separate flower buttons; J: stage of fruit set; 100%: removal of leaves from node 1 to 8 on main stems; 50%: removal of one of every two leaves from node 1 to 8 on main stems.

[b]Means separated within columns by Student Newman-Keuls test. *, **, ns: significant at $p \leq 0.05$, 0.01, or not significant, respectively.

Table 6 Influence of early defoliation treatments on fruit set, yield components, and next season's bud fruitfulness of field-grown Trebbiano grapevines as compared to a non-defoliated control. Data averaged over 2003 to 2005, except as indicated.

Source of variation[a]	Flowers/ cluster	Fruit set (%)	Yield/ shoot (g)	Cluster wt (g)	Berries/ cluster	Berry wt (g)	Cluster compactness (rating)	Rot incidence (%)	Bud fruitfulness[b] (clusters/shoot)
Control	492 a	42.7 a	498 a	400 a	210 a	1.97 a	6.56 a	5.8 a	1.02
H-100%	404 b	27.5 b	247 b	210 b	111 b	1.86 b	4.04 b	1.4 b	0.82
J-100%	424 b	30.7 b	263 b	223 b	130 b	1.74 c	4.35 b	0.61 b	0.93
H-J-50%	390 b	29.2 b	228 b	199 b	114 b	1.78 bc	4.08 b	1.26 b	0.82
Significance[c]	**	**	**	**	**	**	**	**	ns
Defoliation x year interaction	**	**	ns	ns	ns	ns	ns	ns	ns

[a]H: stage of separate flower buttons; J: stage of fruit set; 100%: removal of leaves from node 1 to 8 on main stems; 50%: removal of one of every two leaves from node 1 to 8 on main stems.

[b]Averaged over 2004 to 2005.

[c]Means separated within columns by Student Newman-Keuls test. *, **, ns: significant at $p \leq 0.05$, 0.01, or not significant, respectively.

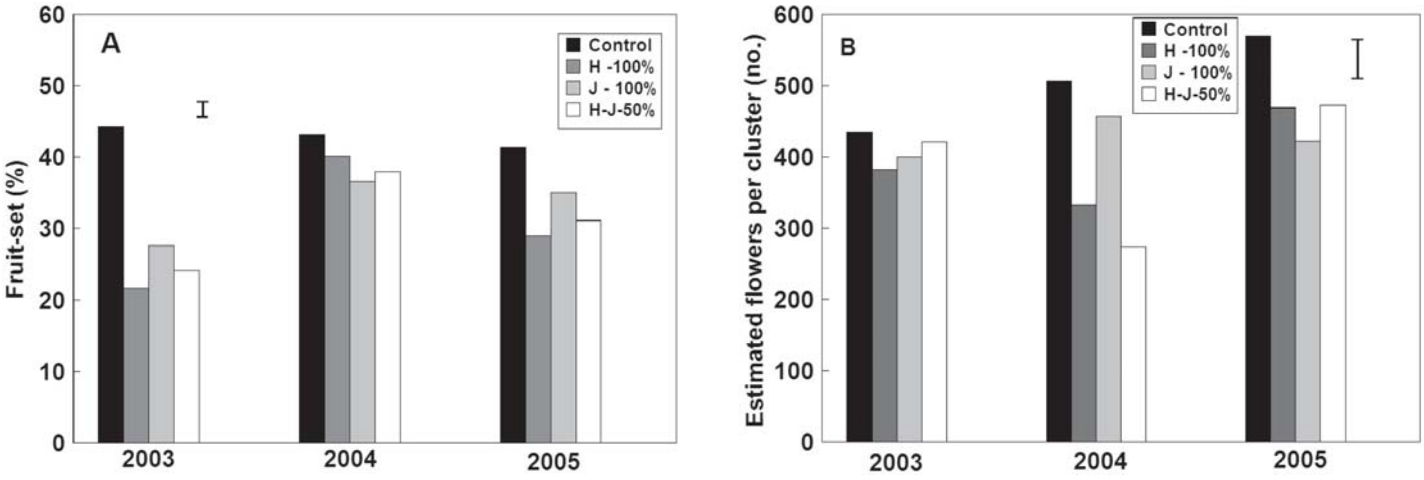

Figure 7 Variation over years for percentage of fruit set (**A**) and number of flowers per cluster (**B**) for the different defoliation treatments applied on the field-grown Trebbiano grapevines. H: stage of separate flower buttons; J: stage of fruit set. Vertical bar indicates interactive SE.

Table 7 Influence of early defoliation treatments on must composition variables parameters of field-grown Trebbiano grapevines as compared to a non-defoliated control. Data averaged over 2003 to 2005.

Source of variation[a]	Soluble solids (Brix)	pH	TA (g/L)	Tartrate (g/L)	Malate (g/L)
Control	19.0 c	3.30 a	5.8 ab	6.1 b	1.52 a
H-100%	21.4 a	3.30 a	5.6 b	6.3 b	1.45 a
J-100%	20.3 b	3.16 c	6.2 a	6.7 a	1.27 b
H-J-50%	21.0 a	3.20 b	6.2 a	7.1 a	1.23 b
Significance[b]	**	**	**	**	**
Defoliation x year interaction	*	ns	ns	ns	ns

[a]H: stage of separate flower buttons; J: stage of fruit set; 100%: removal of leaves from node 1 to 8 on main stems; 50%: removal of one of every two leaves from node 1 to 8 on main stems.

[b]Means separated within columns by Student Newman-Keuls test. *, **, ns: significant at $p \leq 0.05$, 0.01, or not significant, respectively.

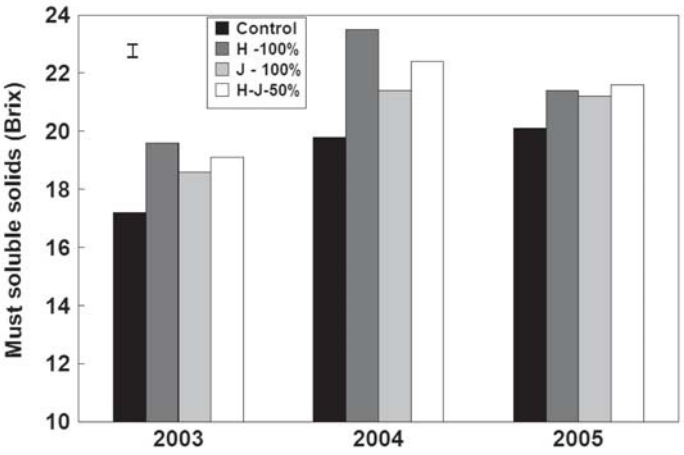

Figure 8 Must soluble solids (Brix) over years 2003 to 2005 for each defoliation treatment applied on field-grown Trebbiano grapevines. H: stage of separate flower buttons; J: stage of fruit set. Vertical bar indicates interactive SE.

of seeded Grenache berries almost tripled when the number of mature leaves left on the shoot at prebloom was raised from 2 to 4 (Coombe 1962). Interestingly, although the defoliation in Sangiovese eliminated about 73% of total shoot photosynthesis (Figure 4), the response in terms of reduced fruit set was moderate on the whole, thus confirming the high-yielding characteristic of this variety. On the other hand, it is notable that fruit set in Trebbiano was reduced in each year by any of the defoliation treatments, despite some variation in the initial flower number per cluster.

Generally speaking, both berry number per cluster and berry size were negatively affected by early leaf removal and, hence, contributed to the reduction in yield per shoot. In agreement with other studies (May et al. 1969, Kliewer 1970, Ollat and Gaudillère 1998), the Trebbiano study indicates that postflowering leaf removal appears to be more effective than an earlier removal when the goal is also to achieve some control over berry size, although as noted also by others (Petrie et al. 2003), even postbloom defoliations are quite effective in causing abortion or growth arrest of set berries. In the Sangiovese study, the prebloom defoliation led to a drastic drop in the number of shot berries, which is inherently a factor of quality improvement since uniformity of ripening within the same cluster might improve. However, the physiological basis of this response is uncertain. According to May (2004) shot berries undergo a regular fertilization process, but an early ovule abortion occurs and seeds are missing or present in traces. It is also known that a hierarchy among flowers exists in the grape inflorescence, where the main type of flower grouping on secondary or tertiary branches is the dichasium (or triad) featuring a central (king) flower and two lateral (secondary) flowers (May 2004). Thus, given a well-documented different potential for flowers and fertilized ovules within the same inflorescence, it can be postulated that the source limitation induced by early defoliation may favor a mechanism by which the plant gets rid of the weaker flowers (those more likely to develop into shot berries) while maximizing retention of the normal berries.

The fruit-set limitation recorded in both studies led to per shoot yield reduction of 20 and 48% in Sangiovese and Trebbiano as compared the controls. The extent and constancy of crop constraint in the field study strongly suggest that early leaf removal may be an excellent tool for limiting yield by replacing time-consuming cluster thinning and avoiding its negative side effects (for example, offsetting growth by the retained clusters, which might actually become more compact and also feature bigger berries with a lower skin-to-pulp ratio).

The second notably consistent feature in both studies was the increase in final grape composition, which in Sangiovese was expressed as a concurrent increase in Brix, total anthocyanins, and phenols (concentration basis) and matched previous findings for the cultivar Barbera (Poni et al. 2005). Moreover, the significant year-by-treatment interaction found in the Trebbiano study was attributable only to variation in the seasonal amount of sugar increase in the defoliation treatment versus control, thereby indicating that the treatment effects far outweighed environmental effects, which were marked in their own right (Figure 2). The removal of source leaves and increased Brix in grapes appears to be physiologically unsound, as previous work has shown that grape composition can even worsen when defoliation is performed between fruit set and veraison (Bledsoe et al. 1988). Yet our results evince at least four factors that might justify the improved grape composition.

First, the defoliation strategies we adopted generally led to final leaf-to-yield ratios (shoot basis) that never dropped below those of control and in some cases were much higher (Tables 1 and 5). This implies that the yield reduction induced by defoliation treatments through a fruit-set and berry size effect was equal or greater than

proportional to the leaf-removal constraint, thereby partly explaining the increased sugar and pigment concentration. Another mechanism acting in favor of nonlimiting final leaf-to-fruit ratios in the defoliated plots is the tendency to offset the loss of removed leaf area by promoting a stronger lateral shoot growth, which was more pronounced under earlier treatments according to our Trebbiano data.

Second, improved grape composition in the defoliated shoots also relates to the "quality" of the source. Removing source leaves around bloom causes dynamic changes to shoot photosynthesis and age as well as to source-sink balance. Calculated A/shoot at various dates integrated many of these effects, and trends clearly suggested that total photosynthesis in the defoliated shoots matched the control level after veraison (Figure 6). Mean A/shoot values for the two Sangiovese treatments pooled over the six measuring dates were very close (2.91 μmol s^{-1} for control versus 2.81 μmol s^{-1} for defoliated), and, when compared with yield per shoot (20% less in defoliated treatment), more carbohydrates were made available for ripening.

Partitioning of the A/shoot variable into the components of leaf area development and leaf assimilation rates indicated that leaf removal triggered a more sustained lateral formation over the season and that some photosynthetic compensation occurred in both main and lateral leaves (+14% and +12% for A rates as compared with control 17 days after defoliation). The capacity of photosynthetic compensation in retained grapevine leaves triggered by leaf removal is well-documented (Candolfi-Vasconcelos and Koblet 1991, Hofäcker 1978, Petrie et al. 2003, Poni and Giachino 2000). However, our A rates measured on main and lateral leaves about five weeks after defoliation were significantly lower in the defoliated shoots, an outcome that essentially implies the temporary nature of leaf assimilation compensation. This result was highlighted in a study where three levels of defoliation (3, 6, and 12 main leaves retained) performed one week after full bloom induced higher A in the fewer leaf treatments up to five weeks after stripping and thereafter their rates sharply declined below those of the 12-leaf treatment (Candolfi-Vasconcelos and Koblet 1991). In our study, however, the A-rate pattern recorded 36 days after defoliation might also reflect a response to the shoot trimming at 16 nodes carried out 17 days after defoliation, the result being that readings taken on DOY 181 represented only fully expanded main leaves. These leaves, because of previous compensation efforts, may have suffered from an excessive build-up of assimilates, leading in turn to suboptimal daily A rates (Flore and Lakso 1988).

The recovery of leaf assimilation rates on the following dates by the defoliation treatment might reflect either a less-pronounced sink limitation or, above all, changes in the shoot age. Higher A rates in main leaves over the last two measurement dates are linked to effects of leaf age on photosynthesis (Poni et al. 1994), suggesting that basal leaves (not present in the defoliated shoots) might undergo a substantial loss of assimilation capacity after veraison; on the other hand, the likewise higher A rates measured on lateral shoots that were produced on shoots subjected to leaf removal are dependent upon their higher vigor and, hence, maturity (Poni and Giachino 2000). Overall, it appears that the "younger" canopies that the defoliated vines exhibit at veraison (median and apical shoot leaves at this time are now mature and more lateral leaves can be present as a compensating reaction to early main leaf removal) may lead to higher photosynthesis late in the season, thereby helping to explain the better grape composition.

A third factor that may account for improved grape composition in the defoliated shoots is linked to changes in source-sink balance. Evidence exists that early defoliation applied at the cluster-zone level hastens translocation of assimilates toward the cluster. Quinlan and Weaver (1970) fed $^{14}CO_2$ at fruit set to an upper fully expanded leaf and at the same time either darkened or defoliated the shoot zone below it. Auto-radiographs taken 24 hours later showed that darkening or defoliation caused a reversal (i.e., basipetal) of photosynthate movement. Another study reported that, over the three weeks after veraison, 12% of ^{14}C reserves was translocated to the fruit of defoliated plants compared with 1.6% found in the clusters from untreated control vines, whereas the respective fraction of ^{14}C remobilized from trunk and roots was 32 and 0.7% (Candolfi-Vasconcelos et al. 1994).

Finally, improved grape composition has been achieved in the defoliated treatments by a change in berry size, hence skin-to-pulp ratio. It has been suggested that the proportion of a grape berry represented by skin and seed tissues can vary with respect to both mass and volume (Roby and Matthews 2004). In other words, berries having the same mass might differ substantially in terms of relative skin and seed mass depending upon the source involved in the limitation of berry growth (e.g., crop load, water stress). In the present study, our Sangiovese data indicated that the restricted berry growth induced by defoliation did not affect the absolute values of skin tissue per berry and, consequently, resulted in an increase in relative skin-to-pulp and skin-to-total berry weight ratios. Although applied early in the season, the effect of the defoliation on the formation of the exocarp appeared to be nonsignificant, and the final skin-to-pulp ratio registered by this treatment seemed to reflect more closely the effect of lower seed number per berry, which in turn links to reduced mesocarp growth (May 2004).

Specific berry composition changes induced by defoliation in our study also included higher TA and higher Brix in Sangiovese (Table 4) and a marked tendency toward higher tartaric acid and lower malic acid in Trebbiano, although TA was unaffected (Table 7). While the response of lower malic acid can derive from increased cluster exposure (Kliewer and Smart 1989), hence berry temperature, the increase in tartaric acid might link to work by Kliewer

and Schultz (1964), who reported higher amounts of $^{14}CO_2$ incorporated into tartaric acid for berries held in full sun as compared with amounts recovered in shaded berries.

Finally, an expected, but nevertheless crucial, effect achieved by defoliation is the decrease in cluster compactness, which in turn had a positive impact on the incidence of rot in Trebbiano. This feature may be of utmost importance for cultivars grown in humid climates.

Conclusions

Early leaf removal in winegrape has received attention from a basic physiological basis related to the investigation of mechanisms of assimilation compensation, variation in source-balance, and carryover effects. On a practical basis, it has been traditionally emphasized that leaf removal around flowering should be avoided because of its negative effects on yield. We investigated the possibility that early leaf removal used on high-yielding cultivars with large, compact clusters may achieve yield control through a reduction in fruit set and berry size and, at the same time, may lead to grape composition improvement. This hypothesis held true for two different cultivars (pot-grown Sangiovese and Trebbiano). The mechanisms involved in improved quality in defoliated shoots relied on higher leaf-to-fruit ratios (shoot basis), no difference in the seasonal assimilation per shoot as compared to control, higher skin-to-pulp fractions, and looser clusters less susceptible to rot. In our conditions, early defoliation can fully replace the costly and time-consuming cluster thinning as a tool of yield control. Work is in progress to verify if early leaf removal can be performed by machine and be integrated with trellis design.

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