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Effect of pre-bloom anti-transpirant treatments and leaf removal on 'Sangiovese' (*Vitis vinifera* L.) winegrapes

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Summary

Recent trials have shown that basal shoot leaf removal at pre-bloom is effective in reducing fruit-set and yield, leading to better grape quality composition. The present trial was designed to determine whether similar results can be achieved with 'Sangiovese' vines by testing a pre-bloom spray of film-forming anti-transpirant (P) against pre-bloom hand defoliation of basal shoots (HD) and no defoliation (C). The results of our three-year experiment show that, compared to C, P reduced net assimilation of treated leaves for 20-40 days and photosynthetic compensation was not found after treatments in the upper untreated leaves of P and in the remaining upper HD leaves; berry-set, cluster weight and yield were significantly reduced in P and HD; bunch compactness decreased in HD, with P clusters registering an intermediate value; must soluble solids content (°Brix) and pH of P and HD were higher; no differences were found among treatments for titratable acidity. Berry and skin weight and anthocyanin content of P and C berries were similar, whereas HD berries, which were fully exposed to ambient light and temperature throughout each season, showed higher skin weight and skin weight-to-berry weight ratio, but a decreasing of total skin anthocyanins content compared to C and P.

K e y w o r d s : anti-transpirant, gas exchange, berry-set, anthocyanins, bunch compactness, bunch rot.

Introduction

Changing the source-sink ratio by pre-bloom basal-shoot leaf removal, a practice which can be carried out by hand or by machine in commercial vineyards (Intrieri *et al.* 2008, Filippetti *et al.* 2009), has shown promising results in cultivars marked by tight clusters and high cropping because the reduction of source leaves limits potential photo-assimilate uptake by flower clusters and reduces berry set, which is highly dependent on carbohydrate supply (Coombe 1959, Caspary and Lang 1996). It follows that clusters are less compact, and hence less subject to rot, berries are smaller, and the reduction in yield load improves grape ripening and quality (Poni *et al*. 2006, Intrieri *et al.* 2008, Filippetti *et al.* 2009). Evidence has also been found that pre-bloom basal leaf removal leaves clusters exposed to full light from their initial formation stage while influencing at the same time berry anatomical and biochemical structure by boosting skin thickening and increasing its phenol content (Poni *et al.* 2008). On the other hand, if the best light exposure can increase skin phenol synthesis of flavonol compounds like quercetin (Price *et al.* 1995, Haselgrove *et al***.** 2000, Spayd *et al*. 2002), then hot weather may well cause a temperature rise in exposed clusters to such an extent as to inhibit the synthesis or boost the degradation of these anthocyanins, thereby lowering color formation in red varieties (Bergqvist *et al.* 2001, Mori *et al.* 2007, Movahed *et al.* 2011).

It is thus of considerable interest to test whether analogous effects can be achieved by anti-transpirant treatments applied in pre-bloom without actual leaf removal and, hence, without changes to cluster exposure, as achieved in part by Palliotti *et al.* (2010). These treatments reduce the photosynthetic capacity of basal shoot leaves because the compounds form an impermeable layer on foliar laminas that temporarily lower gas exchange between stoma and atmosphere and limits the amount of photo-assimilate supply to flower clusters. The decision to investigate the pre-bloom effect of anti-transpirant treatment in comparison to leaf removal is also predicated on the weather changes that in the last few years have affected several growing areas in northern Italy, where spring-summer temperatures have reached peaks much higher than seasonal averages (Jones *et al.* 2005).

Material and Methods

The trial was run over the three-year span 2008-2010 in a N-S oriented 'Sangiovese' vineyard planted in 2004 at the Bologna University experiment station (44°.30' N and 11°.24' E). The vines were grafted to SO4, trained to Guyot with 1 m intrarow and 3 m interrow spacing and pruned to a 12-bud cane per vine. In winter 2008 the central rows of the vineyard were used for three treatments under an experimental design of four randomised blocks of three vines treated as follows: C, untreated control; P, pre-bloom application of anti-transpirant Pinolene (a water emulsion of a terpenic polymer di-1-*p* menthene, Intrachem Bio Italia), on the first 8 basal main and lateral shoot leaves; and HD, pre-bloom hand removal of the main and laterals leaves at the first 8 basal shoot nodes. Pruning to a 12-bud cane per vine was maintained every winter and the treatments were repeated every year on the same vines. Each spring after sprouting, when inflorescences were clearly visible

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(Baggiolini stage G, 1952), a shoot with two well-formed flower clusters on each vine was tagged. The basal inflorescence of each tagged shoot was also tagged and photographed each year against a white background at a distance of 20 cm when the single flower buttons were completely formed but still unopened (Baggiolini stage H, 1952). At the same stage, 20 basal inflorescences from extra vines were also photographed and then removed and transferred to the lab, where the flower buttons of each inflorescence were counted and their real number was compared to the number of flower buttons visible in the corresponding photo print. As reported by Poni *et al.* (2006) and by Intrieri *et al.* (2008), the regression between the two numbers was then used to estimate non-destructively via the pre-bloom photo print the initial flower number on the basal inflorescences of the tagged shoots. The yearly regression coordinates were y = 1.8137 x (R2 = 0.9299) in 2008, y = 1.5137 x (R2 = 0.9135) in 2009 and y = 1.7723 x (R2 = 0.9690) in 2010.

The leaf area of each tagged shoot was estimated at the onset of bloom each year by removing 20 shoots from extra vines and measuring their length and real main leaf area with an LI-3000 leaf area meter (Li-Cor Biosciences, Lincoln, Nebraska, USA); the same procedure was used for their laterals. The x and y coordinates for the recorded parameters of removed shoots (data not reported) were used to estimate non-destructively the leaf area of individual tagged shoots by measuring the length of each shoot and of their laterals at that growth stage. Immediately thereafter, when the first open flowers appeared on the inflorescences (27 May in 2008, 26 May in 2009 and 25 May in 2010), the primary and lateral leaves were removed at the first 8 basal nodes in all shoots of HD vines and the area of the removed leaves from the tagged HD shoots was measured in each vine by a leaf area meter for comparison against the corresponding leaf area estimated in pre-defoliation.

At the same dates of defoliation, the anti-transpirant Pinolene, supplemented with a non-ionic surfactant, was sprayed at 2 % with a portable pump on the first 8 main and lateral basal leaves of all shoots of the P-treated vines. Note that in 2008 the P spray of 27 May was repeated on 5 June following heavy rains on 29 and 30 May (Fig. 1, A and Fig. 2, A). Every year immediately before P treatment, and then at about ten days intervals through August, a portable CIRAS 1 PP System was employed to measure the assimilation rate under saturating light of two leaves at nodes 5 and 6 of each tagged shoot on C and P plants (24 leaves per treatment). The assimilation of HD, C and P leaves at nodes 11 and 12 of tagged shoots (24 leaves per treatment) was also measured under saturating light at about 10-d intervals as soon as the leaves reached full size (approximately 15-20 d after treatments). Every year the daily max and mean air temperature and rainfall data from 1 April to 30 September were logged by a weather station near the trial site. At harvest, 24 and 25 September in 2008 and 2009 and 6 October in 2010, the basal clusters of each tagged shoot of C, P and HD vines were collected and weighed, the berries of each cluster detached and weighed and their number counted for comparison to the corresponding flower number per inflorescence estimated via photo prints in pre-bloom in order to determine the extent of fruit set per treatment. Thereafter the C, P and HD vines were fully harvested and the following data taken in each vine: total yield and cluster number and weight; the extent of botrytis attack, assessed as percent of surface

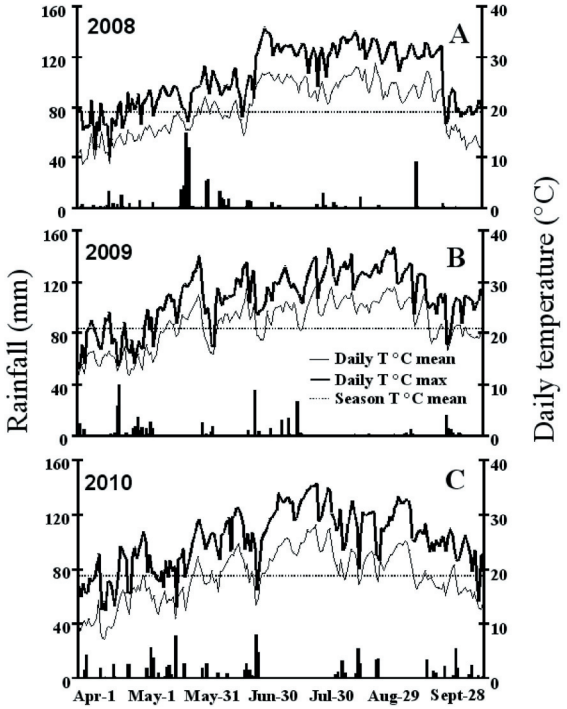

Fig. 1: Daily mean and max air temperature (°C) and rainfall (mm) recorded in 2008 (**A**), 2009 (**B**) and 2010 (**C**) from 1 April to 30 September. The dotted lines indicate the seasonal mean temperature (°C). Data were taken from a weather station close to vineyard site.

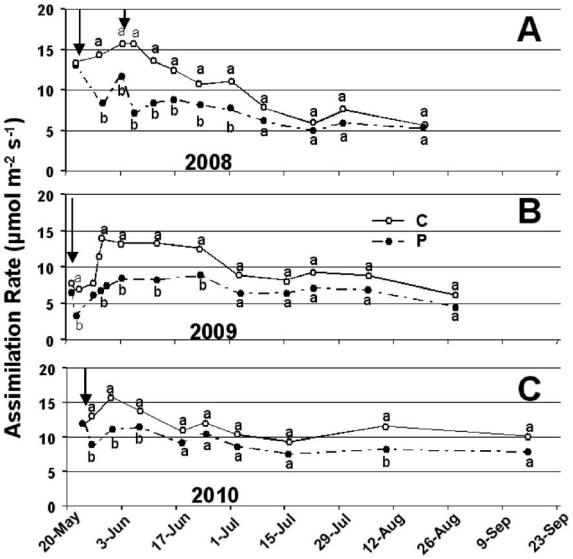

Fig. 2: Seasonal trends of assimilation rate recorded in 2008 (**A**), 2009 (**B**) and 2010 (**C**) on leaves at nodes 5 and 6 of each tagged shoot on C and P vines. Data were taken under saturating light with a portable CIRAS 1 PP System immediately before P treatment and then at about 10-d intervals through August-September. Vertical arrows indicate the days of treatments. At each date values assigned different letters are significantly different at $P \leq 0.05$.

area infected per cluster; and the index of cluster compactness evaluated on a 1 to 9 scale ("berries with many visible pedicels" as 1 and "misshapen berries" as 9) after code 204 of the descriptor list of the Office International de la Vigne et du Vin (OIV, 1983), as well as via the ratio of cluster weight (g) and the summation (cm) of main cluster length and any cluster wings, the ratio ranging from 9-10 for loose clusters and 12-13 for compact ones.

A random 30-berry sample was collected from clusters of each vine, the berries were crushed and juice sugar concentration (°Brix), pH and titratable acidity were analyzed. A second random sample of 20 berries per vine was picked, the berries were immediately frozen at -20 °C, weighed and carefully peeled: skin weight was recorded and the skin weight-to-berry weight ratio was calculated. The skins were then used for the preparation of anthocyanin extract, which was analyzed by HPLC after Mattivi *et al.* (2006). The anthocyanin content was expressed as per g of skin and per kilo of berries. Shoot number per vine was counted in post-harvest and a procedure similar to that applied in pre-bloom was used every year to estimate final main and lateral leaf area per vine, the 12 tagged shoots per treatment being removed and their main and lateral shoot length and the corresponding main and lateral leaf area measured with a leaf area meter. The resulting regressions (unreported data) were then used to calculate, via the number and length of all shoots and of their individual laterals, the total main and lateral leaf area per vine at the end of each season. We also tested any residual effect of treatments on flower bud differentiation in the spring of each following season (2009, 2010 and 2011) by counting the number of clusters emerging from the shoots of the fruiting canes.

Analysis of variance was carried out between years after Gomez and Gomez (1984) using the Mixed procedure in the SAS software package (SAS Institute, Cary, North Carolina, USA). Cluster compactness ratings and percentage of bunch rot were respectively subjected to arc sine and square root transformation prior to statistical analysis and then back-transformed.

Results

Climate data: The yearly recorded rainfall, daily mean and max °C temperature (T) from 1 April to 30 September and the T °C mean of the same period are reported in Fig. 1. The T°C mean from April to September was 19.8 in 2008, 20.9 in 2009 and 19.6 in 2010 (Fig. 1, A, B and C). Growing Degree Days (GDD, 10 °C baseline) from 1 April to 30 September were 1800 in 2008, 1997 in 2009 and 1766 in 2010 (Tab. 1), and the max air temperatures during veraison (August) were 35.1 °C in 2008, 36.8 in 2009 and 33.4 in 2010 (Tab. 1). The rainfall summations from July trough September were 76 mm in 2008, 97 in 2009 and 162 in 2010 (Tab. 1).

Tagged shoots: assimilation rate: The P treatments significantly reduced photosynthetic capacity of the tagged leaves at nodes 5 and 6 compared to C plants for nearly a month, from late May to early July in 2008 and 2009 and from late May to mid-June in 2010 (Fig. 2, A, B and C). The assimilation rate of the upper main expanded leaves of C, P and HD at nodes 11 and 12, tested every year from June through August-early September showed no significant differences among treatments (Fig. 3, A, B and C).

Tagged shoots: berry set, cluster traits and leaf area: At harvest, the weight of P and HD basal clusters on tagged shoots was 24 and 35 % less than that

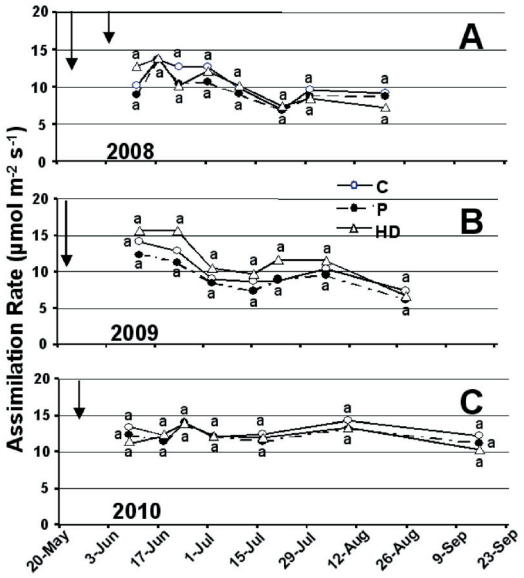

Fig. 3: Seasonal trends of assimilation rate recorded in 2008 (**A**), 2009 (**B**) and 2010 (**C**) on adult leaves at nodes 11 and 12 of each tagged shoot on C, P and HD vines. Data were taken under saturating light with a portable CIRAS 1 PP System starting as soon as the leaves reached full size (approximately 15-20 d after treatments) and then at about 10-d intervals through August-September. Vertical arrows indicate the days of treatments. At each date values assigned different letters are significantly different at $P \leq 0.05$.

Table 1

Mean and maximum air temperature (T °C) and rainfall summation (mm) registered at the experimental site in July, August and September 2008, 2009, 2010 (*)

	2008			2009			2010		
	July	August	Sept.	July	August	Sept.	July	August	Sept.
T mean (°C)	24.4	24.7	18.3	24.6	25.3	20.6	25.1	29.9	17.9
T max (°C)	34.2	35.1	32.9	36.8	36.8	31.7	35.8	33.4	28.2
Rainfall (mm)	24.0	10.0	42.0	55.0	13.0	29.0	23.0	63.0	76.0

(*) The growing degree days (GDD, 10°C baseline) from April 1st to September 30th were 1800 in 2008, 1997 in 2009 and 1766 in 2010.

of C clusters, and P and HD berry number per cluster was 32 and 40 % less than C's; no difference was found among treatments for single-berry weight (Tab. 2). Berry set fell from an average 38 % in C to 26.5 and 24.5 % in P and HD (Tab. 2). Total leaf area per tagged shoot, which was the same in all treatments before defoliation (Tab. 3), was reduced by about 70 % in pre-bloom HD vines, but proved to be the same as C and P at the end of each season, because of the significant 47 % increase of HD laterals (Tab. 3).

Whole test vines: yield, yield components and cluster traits. The experimental vines, individually picked, showed that average cluster number per vine was similar among treatments but yield per vine was reduced in P and HD, their average cluster weight being lower than C's (Tab. 4). Note that year x treatment interaction was found in yield per vine, which was significantly lower in P than C in 2008 and significantly lower in HD than C in 2008 and 2010 (Fig. 4). Cluster compactness

Table 2

Tagged shoots: effects of treatments on basal cluster weight, berry per cluster, berry weight and berry set. No year x treatment interactions. Data taken at harvest and averaged over 2008-2010 (*)

Treatments	C	P	HD
Cluster weight (g)	582 a	441 b	371 b
Berries per cluster (n.)	261 a	176 b	157 b
Berry weight (g)	2.1 a	2.4 a	2.3 a
Pre-treatment flower buttons per inflorescence (n.)	687 a	671 a	642 a
Estimated berry-set (%)	38.0 a	26.5 b	24.5 b

(*) Within rows, values assigned different letters are significantly different at $P \leq 0.05$.

Table 3

Tagged shoots: effects of treatment on leaf area. No year x treatment interactions. Data averaged over 2008-2010 (*)

Treatments		C	P	HD
Pre-treatments leaf area	Primary leaf area per shoot (cm^2)	1138 a	1102 a	1234 a
	Lateral leaf area per shoot (cm^2)	88 a	78 a	128 a
	Total leaf area per shoot (cm^2)	1226 a	1180 a	1362 a
Removed leaf area	Primary leaf area per shoot (cm^2)	0	0	911
	Lateral leaf area per shoot (cm^2)	0	0	83
	Total leaf area per shoot (cm^2)	0	0	994
Post-treatments leaf area	Primary leaf area per shoot (cm^2)	1138 a	1102 a	323 b
	Lateral leaf area per shoot (cm^2)	88 a	78 a	45 b
	Total leaf area per shoot (cm^2)	1226 a	1180 a	368 b
Leaf area at harvest	Primary leaf area per shoot (cm^2)	1760 a	1818 a	1283 b
	Lateral leaf area per shoot (cm^2)	605 b	529 b	893 a
	Total leaf area per shoot (cm^2)	2365 a	2347 a	2176 a

(*) Within rows, values assigned different letters are significantly different at $P \leq 0.05$.

Table 4

Whole test vines: effects of treatments on yield, yield components and cluster morphology. Year x treatment interaction found on yield per vine (*). Data taken at harvest and averaged over 2008-2010 (**)

Treatments	C	P	HD
Cluster per vine (n)	15.6 a	16.4 a	15.6 a
(*)Yield per vine (kg)	6.5 a	5.8 b	4.3 c
Cluster weight (g)	417 a	354 b	276 c
Cluster compactness index (OIV classes 1 to 9)	6.4 a	5.2 ab	4.8 b
Cluster compactness index (Cluster weight / $\sum$ rachis lengths 9 to 13)	12.6 a	11.3 ab	9.8 b
Botrytis (% of surface area / cluster infected)	3.91 a	0.97 a	0.36 a
(***) Fertility (n. of cluster per shoot)	1.52 a	1.54 a	1.51 a

(**) Within rows, values assigned different letters are significantly different at $P \leq 0.05$.
(***) Evaluated in spring 2009, 2010 and 2011.

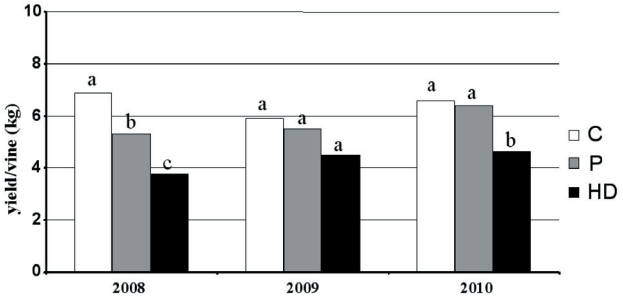

Fig. 4: Variation over years (2008 to 2010) of yield (kg·vine^{-1}) of C, P and HD vines. At each year values assigned different letters are significantly different at $P \leq 0.05$.

indices were also reduced in HD compared to C, whereas intermediate values were recorded for P vines (Tab. 4). No differences in treatments were found for botrytis attack nor in the residual effect of P and HD on flower-bud differentiation in the following years since their average level of fertility, evaluated as cluster per shoot, was the same as in C vines (Tab. 4).

Whole test vines: grape quality: The average juice soluble solids concentration (°Brix) and pH in P and HD fresh crushed berry samples were higher compared to C, but no differences in must acidity was found among treatments (Tab. 5). While the frozen berry samples showed no differences among treatments in berry weight or in skin weight per berry in P compared to C, skin weight per berry in HD was higher compared to C and P, giving rise to higher HD skin weight-to-berry weight ratio (Tab. 6). Although skin weight per berry was higher in HD, their total anthocyanin count per g of skin was significantly lower compared to C and P, whereas non-significant differences were found among treatments in total anthocyanins evaluated as per kg of berries (Tab. 6).

Table 5

Whole test wine: must composition of fresh crushed berry samples. No year x treatment interactions. Data taken at harvest and averaged over 2008-2010 (*)

Treatments	C	P	HD
Soluble solids (°Brix)	21.5 b	22.4 a	22.9 a
pH	3.40 c	3.46 b	3.48 a
Titratable acidity (g·L^{-1})	6.62 a	6.52 a	6.59 a

(*) Within rows, values assigned different letters are significantly different at $P \leq 0.05$.

Whole test vines: vegetative growth: At the end of growth the primary leaf area per vine was lower for HD compared to C and P, but no differences in total leaf area per vine were found among treatments because lateral leaf recovery was higher in HD compared to C and P (Tab. 7). The higher HD leaf area-to-yield ratio compared to C (Tab. 7) was mainly due to the consistent HD crop reduction (Tab. 4), while the leaf area-to yield-ratio of P (Tab. 7), in which crop reduction was more limited (Tab. 4), showed an intermediate value and no significant differences compared to C and HD (Tab. 7).

Table 6

Whole test wine: morphological berry traits and anthocyanins concentration on frozen berry samples. No year x treatment interactions. Data taken at harvest and averaged over 2008-2010 (*)

Treatments	C	P	HD
Berry weight (g)	2.4 a	2.5 a	2.3 a
Skin weight per berry (g)	0.31 b	0.32 b	0.34 a
Skin weight to berry weight ratio	0.13 b	0.13 b	0.15 a
Total anthocyanins (mg·g^{-1} of skin)	6.51 a	6.28 a	4.99 b
Total anthocyanins (mg·kg^{-1} of berries)	823 a	805 a	765 a

(*) Within rows, values assigned different letters are significantly different at $P \leq 0.05$.

Table 7

Whole test wine: effect of treatments on leaf area and crop load index. No year x treatment interactions. Data taken at harvest and averaged over 2008-2010 (*)

Treatments	C	P	HD
Primary leaf area per vine (m^2)	2.68 a	2.64 a	1.87 b
Lateral leaf area per vine (m^2)	0.96 b	1.13 b	1.53 a
Total leaf area per vine (m^2)	3.64 a	3.77 a	3.40 a
Total leaf area / yield (m^2·kg^{-1})	0.56 b	0.65 ab	0.79 a

(*) Within rows, values assigned different letters are significantly different at $P \leq 0.05$.

Discussion

Our overall results confirm that Sangiovese is responsive to pre-bloom anti-transpirant spray, as already reported by Palliotti *et al.* (2010), and very responsive to early leaf removal, although the present findings are not fully comparable to those reported for the same cultivar by Intrieri *et al*. (2008), Filippetti *et al.* (2009) and Gatti *et al.* (2012) and to those reported for other *Vitis vinifera* cvs. by Poni *et al*. (2006 and 2008) Tardaguila *et al.* (2010) and Diago *et al.* (2012).

Tagged shoots: assimilation rate: The window of assimilation reduction of basal leaves of our pre-bloom P-treated tagged Sangiovese shoots lasted about 20-30 d (Fig. 2). In the study conducted by Palliotti *et al.* (2010) using 'Sangiovese' and 'Ciliegiolo', where pre-bloom P was sprayed at the higher concentration of 3 %, compared to our 2 % rate, and the sprays were repeated 15 d later, the period of source limitation of treated leaves extended to 70-80 d. Accordingly, 20-30 d can be a good estimate of the persistence of a single treatment in the absence of heavy rains. However, unlike the results recorded by Palliotti *et al.* (2010), no assimilation compensation was found in P-treated leaves after product decay (Fig. 2). Nor was compensation found on the main expanded leaves at nodes 11 and 12 of P and HD shoots compared to control (Fig. 3), a result which agrees with other findings by Palliotti *et al.* (2011). Moreover, the total photosynthesis of pre-bloom defoliated shoots in 'Sangiovese' potted

vines proved to be lower than control up to post-veraison despite a partial compensation effect around 20 d after leaf removal (Poni *et al.* 2006). Thus, although an increase in photosynthesis of remaining leaves has often been reported after partial leaf stripping (Candolfi-Vasconcelos and Koblet 1991), it seems that assimilation recovery is subject to many different factors and not always triggered by source limitation.

Tagged shoots: berry set, cluster traits and leaf area: The source limitation produced by pre-bloom P treatment and leaf removal can be held responsible for the significant reduction in basal cluster weight recorded in HD and in P shoots at harvest, in which berry number per cluster was lower because berry set decreased by some 35-40 % compared to C. The lower fruit-set rate was thus the main contributing factor to cluster weight constraints because berry weight was unaffected by treatments (Tab. 2). The failure of early source limitation to reduce berry size has already been reported (Intrieri *et al.* 2008, Poni *et al.* 2008, Tardaguila *et al.* 2010) and seems to indicate that carbohydrate supply is never a limiting factor during subsequent stages of berry development in which other physiological mechanisms come into play to allow full berry size, a compensatory growth triggered by the reduction of berry number being one such instance. According to the literature, it seems that the contribution of reduced berry size to the lowering of cluster weight after early source limitation may vary depending on season and cultivar (Poni *et al.* 2006, 2008, Palliotti *et al.* 2010, 2011).

A look at leaf area indicates that while the P tagged shoots did not exhibit adverse effects on shoot growth, nor did they react to P sprays by boosting the growth of laterals, early defoliation of HD shoots was offset by higher growth of laterals (Tab. 3) on the upper non-defoliated nodes. Although main and lateral leaf area development in C, P, and HD shoots was not recorded throughout each season, it is likely that at veraison the total leaf area of HD-tagged shoots was similar to that of C and P shoots.

Whole vines: yield, yield components and cluster traits: Consistent yield reduction per whole vine induced by P and HD compared to C was due to lower single-cluster weight, as indicated *supra* for clusters of tagged shoots, because cluster number per vine was similar among C, P and HD vines, thus confirming the lack of carry-over effect of P and HD on bud fertility (inflorescences per shoot) in the years after treatments (Tab. 4). Average cluster weight per whole vine was obviously lower compared to the average weight of the basal cluster of tagged shoots (Tab. 2), since cluster weight in the full harvested vines included the clusters in shoot's distal position, which are usually smaller than basal ones. Average cluster weight per vine was 417 g in C, against 354 g in P and 276 g in HD (Tab. 4), and, although berry number per cluster was not recorded at the whole-vine level, the lower P and HD cluster weight can be attributed to berry set reduction, as shown on tagged clusters. The year x treatment interaction on yield per vine showed higher yearly alternate bearing in C vines (Fig. 4), whose crop was 7 kg·vine^{-1} in 2008, 5.8 in 2009 and 6.8 in 2010, compared to a more limited alternate bearing in P (5.5, 5.7 and 6.2 kg·vine^{-1} in 2008, 2009 and 2010) and in HD vines (3.9, 4.5 and 4.6 in 2008, 2009 and 2010). The lowest 2009 C cropping (Fig. 4), presumably due to natural alternate bearing, may at least partially explain why the yields of P and HD were not statistically lower than C in that year. However, while the repeatability of reduced yield per vine after pre-bloom source limitation has been generally confirmed (Palliotti *et al.* 2010, Poni *et al.* 2006), different levels of response have also been reported across seasons (Poni *et al.* 2006) and may be linked to a different number of flower buttons formed year after year on the inflorescences, which in turn can modify the level of berry set induced by treatments. Despite the fact that bunch compactness tended to be reduced in P and was significantly lower in HD compared to C (Tab. 4), no difference in average botrytis infection was found among treatments (Tab. 4), a finding due mostly to the dry weather conditions during the last period of ripening (September) when rainfall summation was limited (Tab. 1).

Whole vines: grape quality and vine growth. As reported in Tab. 5, the analysed fresh berry samples showed higher sugar concentration in P and HD must compared to C's. The increase in P soluble solids mainly seems to be due to lower vine yield, while the higher HD soluble solids may be due not only to crop reduction but also to vine reaction, their offsetting of early defoliation by increasing the growth of laterals and thereby resetting the ratio of active leaf area to cropping (Tab. 7) at peak carbohydrate demand from ripening clusters. The effectiveness of P and leaf removal in enhancing berry sugar content in the treated vines of a high-yielding variety like 'Sangiovese' reflects the level of crop reduction and indicates the advanced maturity of P and HD clusters, as also shown by the higher pH of P and HD must compared to C's (Tab. 5). However, total acidity was little affected since its decreasing in P and HD musts was not significant compared to C's, a result also reported elsewhere (Intrieri *et al.* 2008, Palliotti *et al.* 2010 and 2011, Tardaguila *et al.* 2010). The berry samples for anthocyanin analysis showed no differences in berry weight among treatments (Tab. 6), thus matching the data for berries of tagged clusters and confirming that the effect of early source limitation on final berry size can be erratic. Another interesting finding was the lack of difference between P and C in skin weight per berry and in the total anthocyanin count (Tab. 6), thus confirming that these parameters may not always respond to anti-transpirants sprays, as found by Palliotti *et al.* (2010). By contrast, leaf removal consistently affected both berry skin growth and anthocyanin synthesis. Berry skin weight was higher in HD compared to C and P (Tab. 6) and it is likely that the HD treatment, which removed the primary and lateral basal leaves prior to fruit set, exposed the young cluster to more favourable light and/or temperature during late spring, thus promoting adaptive mechanisms of the growing epidermal cells, a finding also reported by Poni *et al.* (2008) and Gatti *et al.* (2012). As a result, a greater skin weight-to-berry weight ratio was registered in HD berries compared to C and P (Tab. 6). Our data also show a negative effect of leaf removal on anthocyanin storage

in the skin of HD berries, where their count, measured as mg per g of skin, was significantly lower than that in C and P (Tab. 6). Total HD anthocyanin accumulation also tended to be lower than that in C and P, albeit not significantly when measured as mg per kg of berries, in spite of the higher HD skin weight-to-berry weight ratio (Tab. 6). This is a contrasting result compared with some previous studies, in which a consistent feature of early defoliation involved an increase not only in °Brix but also in total anthocyanins per berry in *Vitis vinifera* cvs. like 'Sangiovese' (Poni *et al.* 2006, Intrieri *et al.* 2008, Palliotti *et al.* 2011, Gatti *et al.* 2012), 'Barbera' and 'Lambrusco Salamino' (Poni *et al.* 2008), 'Carignan' (Tardaguila *et al.* 2010) and 'Tempranillo' (Diago *et al.* 2012).

Note that in many of the leaf stripping tests cited *supra*, while the primary leaves were completely eliminated, the laterals were partially or not removed at all (Intrieri *et al.* 2008, Poni *et al.* 2008, Gatti *et al.* 2012, Tardaguila *et al.* 2010, Diago *et al.* 2012). A new cluster leaf cover thus grew after fruit set due to the development of laterals from basal nodes and higher content of anthocyanins per berry was registered at harvest (Intrieri *et al.* 2008, Tardaguila *et al.* 2010, Palliotti *et al.* 2011). In the present trial the primary leaves and the laterals were removed up to node eight and the clusters exposed to full light throughout the season since the new laterals mainly formed above the clusters. A possible explanation for the lack of a positive HD effect on anthocyanin accumulation in our experiment may thus be linked to the excessive exposure of bunches during ripening. Despite no monitoring of cluster light and temperature, the recorded climate data show very hot weather conditions every year (Fig. 1 and Tab. 1), with a T°C max exceeding 30 degrees registered for many days in July, August and September. It is well known that the effect of continuous cluster light exposure on flavonoid biosynthesis is complicated by the difficulty in distinguishing between the effects of light and temperature, which can act in a concerted and complex way and yield contradictory results (Spayd *et al.* 2010, Tarara *et al.* 2008). However, as noted in several papers, the decrease of anthocyanin accumulation in the skin of berries under high temperature, with critical T identified around 30 °C, appears to be due to both anthocyanin synthesis inhibition and degradation increase (Dokoozlian and Kliewer 2006, Mori *et al.* 2007, Mohaved *et al.* 2011). These reports add further support to the effects of high temperature, emphasising that excessive cluster exposure in very hot weather is one of the key factor that can reduce final anthocyanin berry count. The same reports may also explain why our pre-bloom P sprays did not change berry skin growth and anthocyanin synthesis, since it is unlikely that the microclimate around the inflorescences was modified by the treatments and clusters remained naturally shaded by the main and laterals leaves throughout the season.

Conclusions

Our trial data show the importance that crop management based on vine physiology plays in controlling a given cultivar's yield and upgrading grape quality. These findings are of particular importance in the field management of 'Sangiovese', a high-fertility and -cropping cultivar whose vines often need hand cluster thinning to keep yield within the limits set by management protocols and to upgrade grape quality. Its clusters, however, are naturally very compact and, although they are usually thinned around veraison to reduce the offset effect of the larger size of the berries on the remaining clusters, post-thinning clusters become more compact and, hence, more susceptible to rot. Thus, unlike traditional and time-consuming manual cluster thinning, pre-bloom anti-transpirant treatment or defoliation, which may act in effect like berry thinning, can be used to control cropping by reducing fruit set and, hence, the "density" of each cluster, thereby also reducing the risk of botrytis infection.

If we look at crop quality, we see that the anti-transpirant treatments reduced yield and exerted a positive effect on berry sugar. Nor did they interfer with the anatomical structure of growing berries or with anthocyanin synthesis in berry skin because treatment does not modify the natural state of clusters vis à vis light and heat exposure. While early leaf removal also reduced cropping and increased sugar accumulation, the enhancing of anthocyanin synthesis and grape quality because the bunches are better exposed to light remains a controversial question, since it is linked to defoliation severity and weather, conditions which may expose clusters to excessively high temperatures along the season and depress anthocyanin synthesis and/or increase their degradation, as seemed to have occurred in our trial.

A practical guideline drawn from our and other studies is that pre-bloom basal leaf removal performed by hand should be used quite cautiously and limited to primary leaves and eventually to the fully expanded laterals leaves near the inflorescences. Note too that hand defoliation demands an hourly work load similar to that for cluster thinning and is mainly advisable for small, high-quality vineyards. However, early leaf removal can also be carried out by machine, which removes only 40-50 % of the leaf area pulled by hand, the results being positive but to a lesser extent than manual leaf removal.

Our pre-bloom Pinolene treatment, which temporarily depresses the photosynthesis of shoot basal leaves, proved to be promising and advisable in vineyards where leaf removal is routinely performed by hand, thereby exacting a high work load and requiring skilled labour, and especially in growing districts where summer temperatures can reach very high peaks. From the practical point of view Pinolene treatment is easy and low-cost (2 % is corresponding to 2-4 kg of product·ha^{-1}) since the pre-bloom target leaf area is very limited.

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