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cycle XXVII

S.S.D: AGR/03

Manipulation of ripening in V*itis vinifera* L.: leaf-to-fruit ratio and cultural practices interactions

Candidate: **Natalia Bobeica**
Matr. n.: 4012085

Academic Year 2013/2014

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Manipulation of ripening in V*itis vinifera* L.: leaf to fruit ratio and cultural practices interactions

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To my daughter

Acknowledgements

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1. Introduction

Vine phenology and harvest dates are strongly related to climate. The effects of climate change are already visible in many viticultural regions (Schultz 2000; Soar et al. 2008; Battaglini et al. 2008; Fraga et al. 2012; Fraga et al. 2013). Vines are generally sensitive to climate variability and temperature influences berry composition and grape quality (Coombe 1987); therefore climate change has the potential to bring changes in wine styles and affect the sustainability of the wine industry (Jones et al. 2005, Hannah et al. 2013). Higher temperatures may inhibit the formation of anthocyanin (Mori et al. 2007, Mira de Orduña 2010, Sadras and Moran 2012), amino acids (Schultz 2000, Camps and Ramos 2012), sugars (Bergqvist et al. 2001; Sadras et al. 2013) and influence the organic acid concentration (Sweetman et al. 2014).

Managing source to sink balance is an essential aspect of vineyard management, aimed to achieve a high quality of berry grapes. Nowadays vine growers and winemakers are becoming more conscious about the changing climatic traits and are adapting cultural practices (Gaal et al. 2012, Galbreath 2012, Ruml et al. 2012, Camps and Ramos 2012). Canopy management practices, such as pruning, thinning, training systems, trellis system, leaf removal, irrigation, crop load influence grape growth and quality (Jogaiah et al. 2013).

2. Literature review

2.1. Climate change impact on vine development and berry quality

The timing of phenological stages in grapevine is fundamentally temperature driven, therefore grape production and quality depends greatly on climate. In the last years, the study of effects of climate change became important, and working groups were created in order to try to limit climate change and build a sustainable future. According to IPCC (IPCC 2014), since 1950 changes in extreme weather and climate events have been observed, and some of these changes are linked to human influences. According to all scenarios recerched by IPCC, it is very likely that temperatures will continue to increase, and higher temperature will last longer, as well as extreme precipitation will become more intense and frequent in many regions. It was also stated that more than 90% of energy accumulation in the climate system between 1971 and 2010 has accumulated in the ocean, according to IPCC (2014), land temperatures remain at historic hights, while ocean temperatures continue to increase (Figure 1).

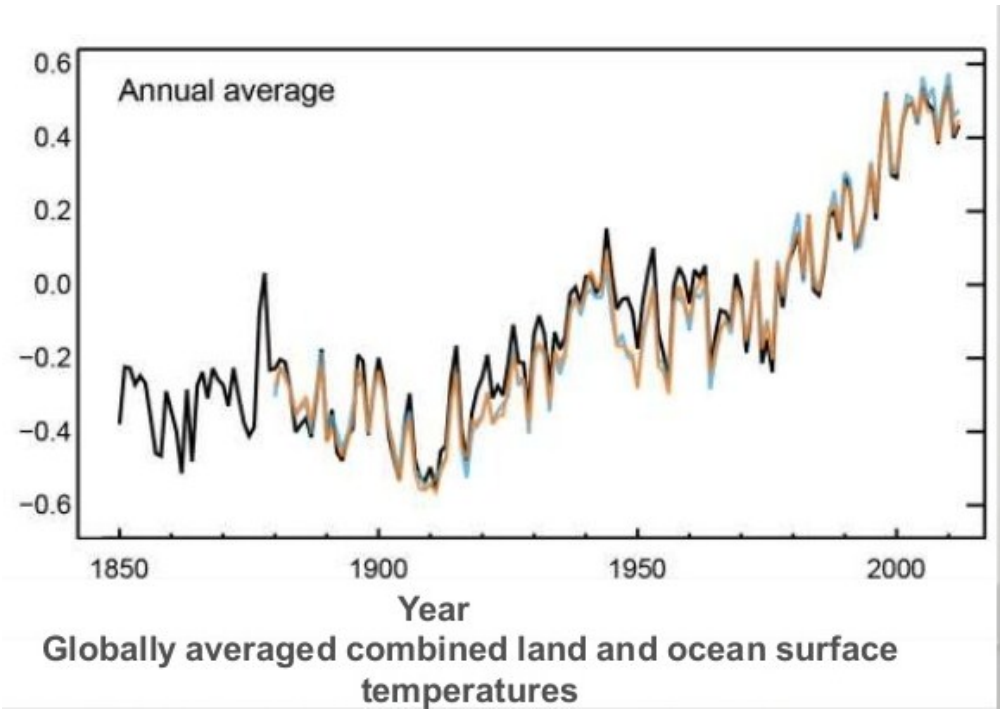

Figure 1. Annually and global averaged combined land and ocean surface temperature anomalies relative to the average over the period 1986 to 2005. Colours indicate different data sets (IPCC, 2014)

Temperatures in most vinegrowing areas began to increase after 1950, which can be resulted in tresspasing the optimum temperature for berry ripening, especially for the european varieties (Jones et al. 2005). The increase in temperatures has caused an accelerated vine phenology. Several researches have predicted that an advancement of harvest date occur in several countries (Duchêne and Schneider 2005, Keller 2010, Jones and Alves 2012). High temperature will advance bud break, shoot growth and development of leaf area (Ferguson et al. 2011). A high temperature during flowering will inhibit véraison, and higher temperatures near véraison will slow the cell expansion and sugar accumulation (Greer and Weston 2010). An increase of 1 °C in temperature, will result in an advance of 5 to 10 days in vegetative development of grapevine (Schultz and Jones 2010, Jones and Alves 2012). Another important factor in grape berry development is the effect of high temperatures on berry quality. During the accumulation of sugars, the optimal temperature is from 8 to 33 °C (Coombe 1987). Consequently a higher temperature near grape berries will inhibit sugar accumulation (Kliewer 1977, Greer and Weston 2010). The optimum net assimilation rate by photosynthesis of grapevines is between 18°C and 33°C, above or beyond this values, the efficiency is decreasing significantly (Kliewer 1973, Iland et al. 2011). Moreover, color accumulation in grape berries is temperature and light dependent, in that case low or high temperature are associated with less colour, the optimum temperature for anthocyanin synthesis is from 17°C to 26°C (Coombe 1987). High temperatures reduce the anthocyanin content and accumulation in grape berries (Kliewer and Ough 1970, Mori et al. 2007, Sadras et al. 2012). Also a higher global temperature will cause a decrease in berry acidity and inequality in aromatic compounds (Jones et al. 2005).

As nowadays, the issues of climate change on world viticulture and wine production are known, a series of actions can be done in order to adapt to new climate. Different studies have presented an increase in alcohol content of wines, because of the excessive sugar accumulation (Duchêne and Schneider 2005, Keller 2010, Santos et al. 2012). Specifically, the wine consumption shows a downward trend, because of change in lifestyle and tastes, anti-alcohol drinking campaigns and health concerns (Figure 2). In the European Union, there is a limit in alcohol content concentration of wines (8.5 and 15 %

vol.), and with higher temperatures, it will be difficult to maintain this limit without affecting other wine components, such as color, astringency, acidity and aroma.

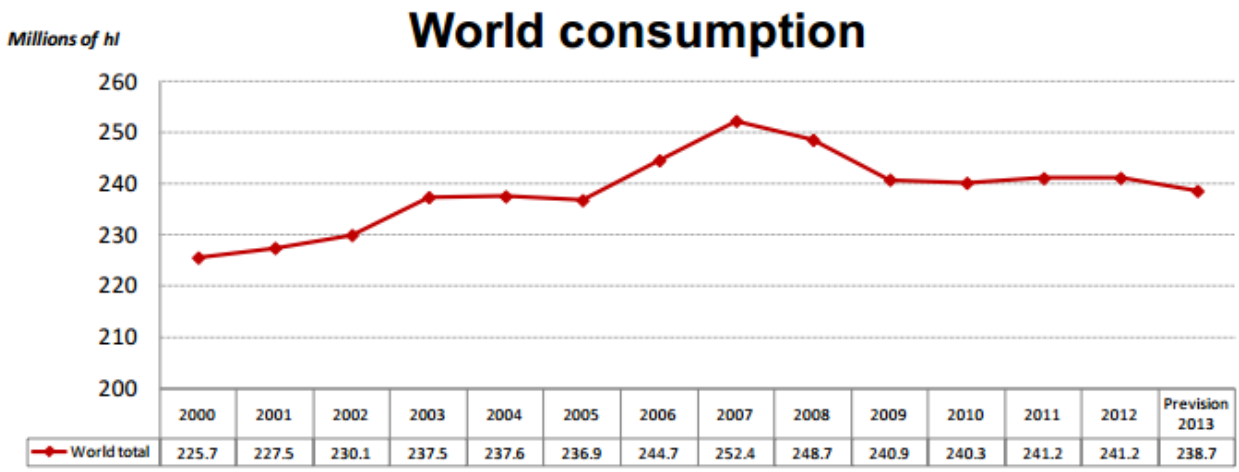

Figure 2. World wine consumption (OIV 2014)

2.2. Manipulation of ripening

There are some techniques effective in modulating the maturation and in particular the accumulation of sugars in the grapes, whose level contributes frequently to determine the time of harvesting. Winter late pruning in Merlot berries from New Zealand delayed grape maturity after a delay in vine phenology, according to Friend and Trought (2007). Traditionally, techniques based on the change in the source : sink balance can be able to delay grape maturity. A low leaf area to yield ratio (less than 0.8 m2/Kg) can produce less sugar in grape berries (Kliewer and Dokoozlian 2005). There are a series of cultural techniques that change the leaf area to yield ratio.

Shoot trimming can be successful at delaying berry ripening, although its postponing effects depend upon timing and severity (Stoll et al. 2010). According to (Palliotti et al. 2014) other techniques that can elicit a delayed berry maturation are:

- late irrigation combined with shoot trimming which can promote the outburst of lateral shoots;

- using of shading nets that will limit photosynthetic assimilation (Kliewer et al. 1967, Smart et al. 1985, Morrison and Noble 1990);

- using of anti-transpirant sprays to limit photosynthesis (Palliotti et al. 2013); use of growth regulators which will change the reproductive cycle of vine (Jeong et al. 2004, Böttcher et al. 2011).

One of the most used techniques in vine management to achieve high fruit quality is leaf removal. Depending on the timing and severity of leaf removal, this operation can also delay ripening.

2.3. Source and sink

Vine leaves (source) directly affect the changes in the cluster microclimate, and canopy photosynthesis is responsible for the synthesis of carbohydrates and their export to the clusters (sinks); therefore, the balance between leaves and clusters is crucial for grape quality. During grapevine development, an organ can be a source or a sink (Iland et al. 2011).

Source : sink relationship is the capacity of vine to undergo photosynthesis, by fixing CO_2 in the source organs, and to transport the fixed carbon to various sink tissues, and also the ability of sink organs to assimilate the fixed CO_2, which depends on genotype, environment and viticultural management practices. Source tissues are those organs that are capable of exporting solutes, and where these solutes are produced or stored. Sink organs is where the solutes are sent and used for growth and metabolic processes (like sugar accumulation, anthocyanin synthesis) (Iland et al. 2011). According to Ollat and Gaudillere (1998), during their first stage of berry growth, berries can be considered as utilization sinks, but after véraison an higher carbon import makes the berry a storage sink. During photosynthesis, the carbohydrates that are produced are used in metabolism and growth or are stored as reserves in the woody structures of the vine.

Farrar and Jones (2000) established that carbon allocation to various sink is controlled both by sink demand (activity and size) and by source control of photosynthate production. It was demonstrated that carbohydrate levels affect gene regulation and offer a mechanism for control of resource distribution among various sources and sinks (Koch 1996, Sheen et al. 1999). The carbohydrate consumption up-regulates genes responsible for photosynthesis, mobilization and export, while abundance of carbohydrate, up-regulates

genes responsible for the storage and use (Figure 3). Eventually through modification in gene expression, source and sink activities can be altered in order to adjust growth patterns in response to carbon availability and acquisition (Koch 1996, Farrar and Jones 2000).

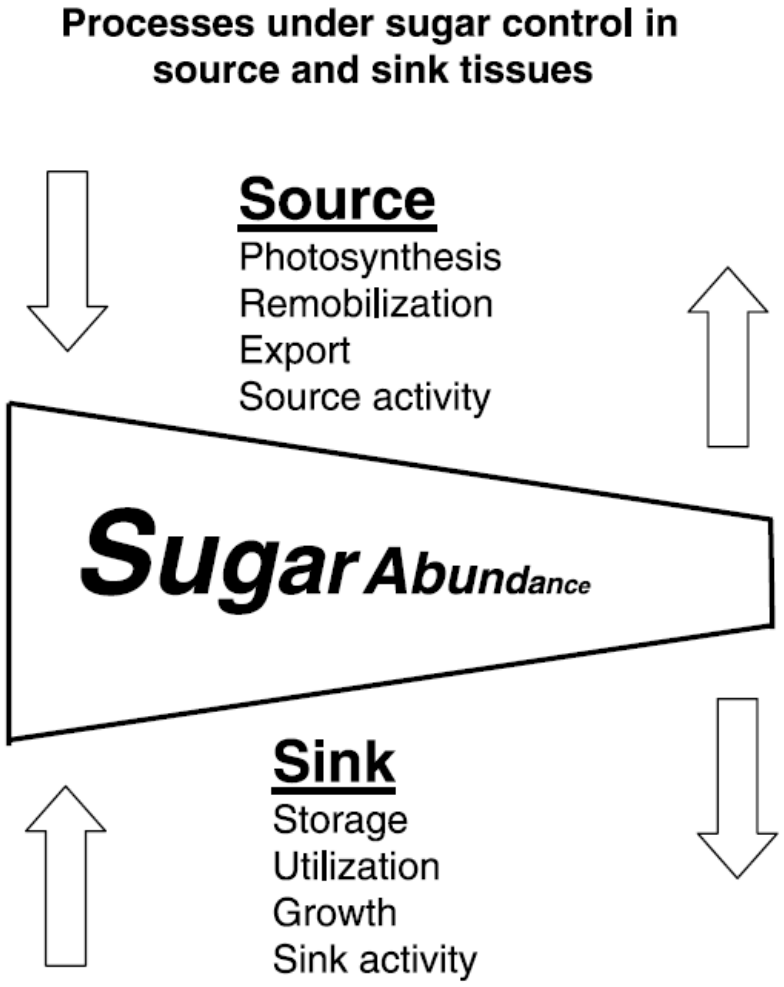

Figure 2. Processes in source and sink tissues that are under carbohydrate control in plants. A downward arrow indicates gene down regulation in response to high or low sugar abundance, while an upward arrow indicates up regulation in response to high or low sugar abundance (Andersen 2003).

2.4. Vine balance

Grapevine balance is important for production of quality grape berries. Vine balance can be defined as the balance between vegetative and reproductive growth which will achieve desired fruit quality and production, while preserving vine health (Skinkis and Vance 2013). One of the many approaches for calculating vine balance is the equilibrium between vegetative growth and crop load. Howell (2001) has reported that this balance must range from 0.7 to 1.4 m^2 leaf area per kg of fruit. Moreover, Kliewer and Dokoozlian (2005) stated that a value of 0.8 to 1.2 m^2 leaf area per kg fruit is required for a good maturation of grape berries. Bellow this values, vines are considered out of balance with under ripe berries or large canopies. However there are other indices that are used, like the Ravaz index of Smart and Robinson (1991), which is calculated using fruit yields at harvest and dormant vine pruning weights during winter following harvest. A Ravaz index of 5 to 10 is optimum for vine balance in warm climates, whereas a value of 3 to 6 may be good for cool climates (Kliewer and Dokoozlian 2005).

2.5. Grapevine source : sink modifications

The maximization of the quality of the grapes is achieved by balancing the vegetative and reproductive growth, achieved by optimizing vine training system. There are some vineyard management practices which can be used to achieve vine balance, but there are variations linked with locations (climate and soil) and vine variety, and can be more or less efficient like: rootstock, training system, irrigation, pruning system, and shoot thinning, cluster thinning, hedging, leaf removal, cover cropping (Kliewer and Dokoozlian 2005).

2.5.1. Leaf removal

Leaf removal is traditionally used to increase bunch exposure to sunlight and airflow, thus leading to improved berry composition and disease control (Smart et al. 1990, Jackson and Lombard 1993). This canopy management method is a must in cool and wet climates, where the incidence of Botrytis is higher (English et al. 1989).

However, over the last years it has been reported that not only leaf removal can modify cluster microclimate but also change yield, fruit composition, cluster morphology, whole vine photosynthesis capacity, and vine/root carbohydrate reserves (Staff et al. 1997, Poni et al. 2006, Intrieri et al. 2008). Leaf removal can be done at different stages of vine growth: pre-bloom, post-bloom, berry set, pre-véraison, véraison, depending on the needed result; manually or mechanically, apical or basal.

2.5.1.1. Early leaf removal

The most studied leaf removal treatment is the one done at early stages, mainly in cool and humid areas for improving air circulation, spray penetration, bud fruitfulness and fruit health and composition (Zoecklein et al. 1992). This practice is based on the functional relationship between yield and availability of carbohydrates at pre-bloom stage (Caspari et al. 1998). Leaves are the main carbohydrate source for the inflorescence development during flowering (Lebon et al. 2008), and the removal of leaves before flowering will affect the source-sink balance significantly. Some recent research has shown that early defoliation lead to smaller cluster with fewer and smaller berries, that will control the incidence of Botrytis (Poni et al. 2006, Diago et al. 2010, Tardaguila et al. 2010, Palliotti et al. 2011), the regulation of grape yield and improvement of grape and wine composition (Intrieri et al. 2008, Poni et al. 2009, Tardaguila et al. 2012).

Leaf removal can also change grape berry composition. For example in some research it was found that early leaf removal increased soluble solids and total anthocyanin content (Poni et al. 2006, Intrieri et al. 2008, Tardaguila et al. 2010), decreased malic acid in Carignan and Graciano berries (Tardaguila et al. 2010); additionally it was seen an increase in wine color, higher alcohol content and greater amount of hydroxycinnamic acids, flavonols and anthocyanins (Kemp et al. 2011, Diago et al. 2012); modification of wine aroma quality in Tempranillo wines was reported by Vilanova et al. 2012. Leaf removal can modify anthocyanin content in grape berries. Lee and Skinkis (2013) and Sternad Lemut et al. (2011) found that early leaf removal, compared to no defoliated Pinot noir vines, produced higher anthocyanin accumulation. The same results on berry phenolics were found in Tempranillo grapes (Risco et al. 2014), in Merlot grapes (Spayd et al. 2002, Tarara et al. 2008, Di Profio et al. 2011), in Cabernet Sauvignon berries

(Hunter et al. 1991, Di Profio et al. 2011), in Cabernet Franc berries (Di Profio et al. 2011), in Graciano and Carignan grape berries (Tardaguila et al. 2010). This is due, most likely, because of the increase in light and temperature in the fruit environment, that will affect the enzymes involved in the synthesis of phenolic compounds (Di Profio et al. 2011).

The photosynthetic activity of a leaf gradually increases until it reaches its maximum size, significantly after 40 days. In contrast, some leaf removal studies have revealed increased photosynthetic activity in the remaining leaves, as an effect of compensation of source to sink ratio (Candolfi-Vasconcelos and Koblet 1990, Hunter and Visser 1990, Petrie et al. 2000a). A defoliation near flowering caused a great reduction in source, because of the removed basal leaves, which at this stage have high photosynthetic rates (Petrie et al. 2003, Poni et al. 2006). By removing the most active leaves, there is the possibility to cause various changes in vine physiology and berry quality. The age of leaves is very important, since they act as sinks until reaching 50 to 80% of final size (Vasconcelos and Castagnoli 2000). In the early stages of the vegetative cycle of vine growth, basal leaves are the main responsible for the photosynthetic activity of the plant, according to (Hunter and Visser 1990, Petrie et al. 2003). Moreover, it was demonstrated that after a loss in main leaf area, it is possible to have an increase in secondary shoots (Poni et al. 2006).

2.5.1.2. Late leaf removal

Stoll et al. (2010) showed that ripening in Riesling vines can be postponed. The same results were found in Cabernet Sauvignon and Merlot berries, leaf removal delayed berry maturity, with no changes in anthocyanin and total phenol concentrations, although this was not seen in Cabernet franc grapes (Spayd et al. 2002, Joscelyne et al. 2007, Di Profio et al. 2011). In Pinot Noir grapes, when cluster leaf removal is done after bloom, juice soluble solids were reduced, without changes on yield components (Vasconcelos and Castagnoli 2000).

If defoliation treatment is done around véraison in the fruit zone this will increase cluster exposure to light (King et al. 2012), and as result it will enhance anthocyanin biosynthesis, but there is a point at which the temperature will have a negative impact,

and can decrease eventually the anthocyanin concentration. The temperature which cause lower pigmentation in red grapes is around 32°C (Price et al. 1995, Downey et al. 2006, Yamane et al. 2006, Mori et al. 2007). Therefore, excessive sunlight exposure can lead to berry color reduction (Haselgrove et al. 2000, Bergqvist et al. 2001) or even to berry sunburn (Bergqvist et al. 2001, Spayd et al. 2002), especially in warm climate regions.

2.6. Grape berry development

Grape berries are non-climateric fruits that have a double sigmoid pattern of development, with two distinct phases of growth (Figure 4). The two phases are separated by a lag phase, which leads to véraison, when the onset of ripening take place (Coombe 1992, Ollat et al. 2002, Conde et al. 2007). The growth of berries in this stage firstly occurs by cell division when the volume and weight are increased, and afterward by cell expansion. In the first phase the berry is firm and with high titratable acidity, because of the accumulation of tartaric and malic acid. At the same time, a high development of shoots take place in the vine. The first stage usually persists for 35 to 40 days (Mullins et al. 1992).

The lag phase begins with the decreasing of berry growth, the highest concentration of organic acids and reducing of chlorophyll concentration that lead to translucent color of berry. In this stage, the berry reduces photosynthesis and respiration. This phase generally last for 4 to 30 days (Fregoni 1998).

The second developmental phase begins with rapid berry growth and fruit ripening, namely véraison. During this stage, berries soften and lose chlorophyll, and the changing of color takes place. Berry volume, sugar, aroma components, polyphenols and anthocyanins begin to accumulate, whereas the concentration of organic acids declines (Mullins et al. 1992, Dokoozlian 2000). This phase last for 35 to 55 days on average.

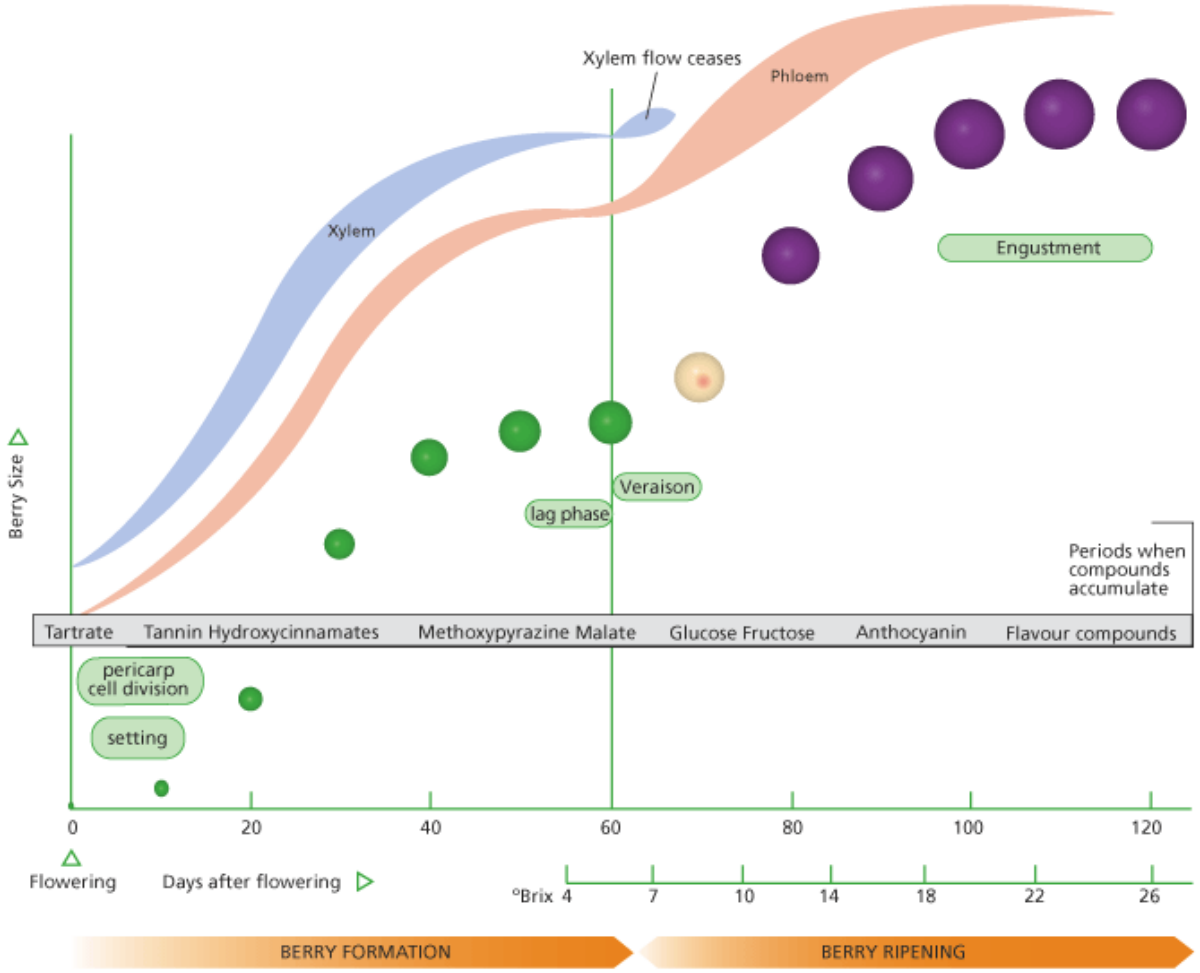

Figure 4. Timing and pattern of grape berry development from fruit set to harvest from Kennedy, (2002).

2.7. Berry ripening

Ripening in grape berries consist of fruit softening, the accumulation of sugars and synthesis of anthocyanins, metabolism of organic acids, accumulation of flavor compounds and the continuation of berry growth. During ripening, a series of physiological and biochemical changes simultaneously occur in the grape berry (Coombe 1992). However, many factors impact this developmental stage and harvest date, like grape variety, climate and crop level (Dokoozlian 2000). The leaf area to yield ratio can influence berry development and composition (Jackson and Lombard 1993), because a value of 1.5 m2/kg of fruit is the optimum for berry maturation (Kliewer and Dokoozlian 2005). Vine phenology and harvest dates are strongly related to climate and the effects of

climate change are already visible in many viticultural regions (Schultz 2000; Soar et al. 2008; Battaglini et al. 2008; Fraga et al. 2012; Fraga et al. 2013).

2.8. Berry composition

Grape berry quality is very important for winemaking, which depends mostly of berry composition, which changes during berry development. The berry has three important parts: pulp, skin and seeds.

2.8.1. Sugars

Sugar, which is imported in berry (sink organ) from the leaves (source organs), is important for ripening and fruit growth. During berry development, sugar concentration in berries, in the first phase is very low, behaving like chlorophyll organs with high photosynthesis (Conde et al. 2007). Then after véraison a massive accumulation of glucose and fructose occurs, as a consequence of leaf photosynthesis. In small amounts, there are other sugars present in berries. Sugars are the basis for many compounds, such as organic and amino acids, anthocyanins, and very important for trunk and roots growth (Rolland et al. 2006).

2.8.2. Organic acids

Organic acids are accumulated in grape berries from the beginning, and have an important role in berry quality. There are more than twenty organic acids in berries, with highest concentration of tartaric acid in the first phase of berry development and the highest concentration of malic acids at lag phase (Kliewer 1966). After véraison, organic acids are consumed during berry respiration, or are transformed in sugars (malic acid) and tartaric acid decreases by dilution, linked with berry growth (Lakso and Kliewer 1975, Conde et al. 2007). Kliewer and Weaver (1971) showed that temperature is very important for tartrate and malate concentration in grape berries, but relatively independent for light intensity. The optimum temperature for acids synthesis is between 20 and 25 °C, higher temperatures decrease synthesis of malic acid (Kliewer 1966).

2.8.3. Phenolic compounds

Phenolic compounds in grapes are important secondary metabolites. The most important phenolic in berries are phenolic acids, flavonols, flavanols and anthocyanins. Anthocyanins are red pigments present in berry skin and sometimes in berry pulp as well as in the flesh of some varieties (He et al. 2010). In addition anthocyanins are important for winemaking, because of their color and astringency (Kennedy et al. 2006). The common anthocyanins found in grape are: delphinidin, cyanindin, petunidin, peonidin and malvidin -3-O-glucosides, 3-/6-acetyl) glucosides and 3-(6-p-coumaroyl)-glucosides. Usually malvidin-3-O-glucoside is the most abundant anthocyanin present in berries. Anthocyanin synthesis is parallel with carbohydrate metabolism (Pirie and Mullins 1977), therefore the accumulation of anthocyanins is enhanced by increased sugar content (Cormier et al. 1997). There is a variation of anthocyanin composition among varieties according to Mattivi et al. (2006). Years of research have shown that vineyard location, climate, cultivar, rootstock, cultural practices, growing season, training system, vine spacing and root reserves are all factors that will influence grape anthocyanin accumulation (Hunter et al. 1991, Mazza et al. 1999, Petrie et al. 2000b, Joscelyne et al. 2007, Poni et al. 2008, 2009, Guidoni et al. 2008, Intrieri et al. 2008, Chorti et al. 2010, Tardaguila et al. 2010, Lohitnavy et al. 2010, Di Profio et al. 2011, Kemp et al. 2011, Sternad Lemut et al. 2011, Kotseridis et al. 2012).

2.8.4. Nitrogen compounds

Nitrogen compounds are important for must fermentation, because they are the metabolic factor that decides the rate of fermentation. Amino acids account for 50 to 90% of the total nitrogenous compounds in grape berries (Kliewer 1968, Ough and Stashak 1974). The most important free amino acids found in grape berries are proline and arginine, followed by alanine, aspartic acid and glutamic acid (Kliewer 1968, Kliewer and Ough 1970). Responsible for the type and concentration of amino acids in grape berries are berry maturity (Kliewer 1968, Kliewer and Ough 1970, Stines et al. 1999) and cultivar (Kliewer 1968, Stines et al. 2000, Lee and Schreiner 2010). At the beginning of berry growth, amino acids have a low concentration in berries, after véraison, the synthesis of amino acids begin to increase (Dokoozlian 2000).

3. Aim of study

The literature review shows that many authors studied the modifications of source to fruit ratio on berry composition and vine photosynthesis. However, most of the studies have sought the improvement of berry microclimate, and the increase in berry color and sugar content. In view of the global warming, higher temperatures in the vine regions lead to high level of sugar in grape berries, eventually with excess in wine alcohol, low acidity and unbalanced phenolic ripening in red varieties. Therefore, the major problem in the wine producing countries is the delaying of ripening in grape berries without changing the wine quality and finding the ideal balance between leaf area and fruit growth.

The aim of this study was to evaluate the effects of limiting the carbohydrate availability by manipulating the leaf-to-fruit ratio on two cultivars, aimed at delaying ripening. Analysis of berry composition and whole-vine net photosynthesis during ripening provided further insights into the complex relationship between source (leaves) and sink (fruit).

In the first year of research, we evaluated in Sangiovese the possibility that ripening might get delayed by an apical to cluster leaf removal done at pre-véraison and post-véraison, compared to a non-defoliated treatment. Readings of the whole canopy net CO_2 exchange rate and single leaf gas exchange were compared to see how they are modified during vine development.

In the second year, a study on low carbon availability (3 leaves per shoot) compared to sufficient carbon availability (12 leaves per shoot) on fruiting cuttings of Cabernet Sauvignon, in Bordeaux was instigated. The purpose of this research was aimed to understand the effects of the leaf removal on berry composition during ripening, and the connection between sugar accumulation and anthocyanin synthesis.

The third year, a replicate of the second year treatment was further evaluated on Sangiovese vines. The latter research provided a clearer answer on how vine photosynthesis and carbohydrate availability is related to berry composition, mainly sugar accumulation, anthocyanin accumulation and aminoacid composition during ripening.

This three year research was designated to verify if the source to sink modifications done on two red cultivars (Sangiovese and Cabernet Sauvignon), in two regions (Piacenza, Italy and Bordeaux, France) could possible delay grape berry maturation, in order to make good quality and healthy grapes for high quality wines in the new climate.

This thesis is based upon two self-standing manuscripts published or submitted in academic journals:

Poni S., Gatti M., Bernizzoni F., Civardi S., **Bobeica N**., Magnanini E., Palliotti A. (2013). Late leaf removal aimed at delaying ripening in cv. Sangiovese: physiological assessment and vine performance. Australian Journal of Grape and Wine Research 19, 378-387.

Bobeica N., Poni S., Hilbert G., Renaud C., Gomès E., Delrot S., Dai Z. (2015). Differential responses of sugar, organic acids and anthocyanins to source-sink modulation in Cabernet Sauvignon and Sangiovese grapevines. (submitted on 14.03.2015 to Frontiers of Plant Science).

A third paper is in preparation for Frontiers of Plant Science:

Bobeica N., Poni S., Hilbert G., Renaud C., Gomès E., Delrot S., Dai Z. (2015). Changes in amino acid composition of Cabernet Sauvignon and Sangiovese grape berries after source-sink modulation.

4. Late leaf removal aimed at delaying ripening in cv. Sangiovese: physiological assessment and vine performance

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Abstract

Background and Aims: Global warming is inducing a general earliness in the onset of grapevine phenological stages including ripening, a phenomenon that occurs often in the hottest seasons and which leads to unbalanced wines. Our aim was to assess the physiological basis of late leaf removal applied above the bunch area as a tool for delaying ripening.

Methods and Results: Potted cv. Sangiovese grapevines were subjected to leaf removal treatments applied pre-veraison (DEF-I) and post-veraison (DEF-II) by pulling out six to seven primary leaves and laterals, if any, above the bunch zone; untouched vines served as the control. Whole-canopy net CO_2 gas exchange was monitored seasonally from 9 days before DEF-I to 35 days after DEF-II. Concurrently, single-leaf gas exchange was assessed, and at harvest yield components, grape composition and the leaf-to-fruit ratio were determined. The seasonal carbon/yield ratio did not differ between treatments because of the high capacity for photosynthetic compensation shown by the DEF treatments and quantified as about a 35% higher net CO_2 gas exchange per unit of leaf area per day. While ripening was temporarily retarded in both DEF treatments, with sugar content being lower and titratable acidity higher, a week later both treatments had fully or partially recovered; phenolic ripening was unaffected at either harvest date.

Conclusions: Defoliation above the bunch zone applied at lag-phase and post-veraison (average 12°Brix) was effective in temporarily delaying technological ripeness without affecting colour and phenolics. This result depended upon the high compensation capacity for photosynthesis shown by vines in both treatments.

Significance of the Study: The data provide a preliminary yet robust physiological background for targeting better field application of the technique.

Keywords: *berry composition, gas exchange, summer pruning, yield*

4.1. Introduction

The effects of global warming are evident in viticulture everywhere. Indeed, observed changes in 27 premium wine regions across the globe have shown an average 1.3°C warming of the growing season from 1950 to 2000, and the outlook over the next 50 years projects a 2°C average warming (Jones 2012). The growing season temperature of Europe has increased by 1.7°C from 1950 to 2004 (Jones et al. 2005), thus inducing increased heat summations, reduced frost damage, altered ripening profiles, earlier phenology, altered pest outbreaks and severity, changes in soil fertility and erosion, as well as in water supply and irrigation demand. Duchêne and Schneider (2005) report a 2.5° increase in the alcohol content of wines produced in Alsace over the 30-year time span from 1970 to 2000. While the earlier onset of grapevine phenological stages is likely to be the change most clearly perceived by growers, phenology relationships over several cultivars and locations show a 5–10 day advancement for 1°C of warming, effects that appear to be quite well documented in the literature (Schultz and Jones 2010, Jones 2012). For instance, Ganichot (2002) highlighted that harvest date has been earlier by more than a month from 1945 to 2005 in the Châteauneuf du Pape region of France. For Germany's Rheingau region, Stoll et al. (2010) report a 10–17 day earlier bud-break and fruitset in cv. Riesling over the last 30 years, with veraison occurring 14–21 days earlier. A recent climate simulation of cv. Chardonnay phenology in Italy's Trentino region indicates that several mountain areas located at about 1000 m above sea level may become suitable for viticulture before the end of the century (Caffarra and Eccel 2011).

While global warming is indeed implicated in causing earlier vintages, factors of a different nature cannot be ruled out. For instance, policy guidelines in Italy now strictly limit yield per hectare in appellation areas, and recent trends are towards cultivars featuring low-to-medium cropping. Additionally, vineyard efficiency relative to berry sugar-storage capacity has increased because of both environmental concerns, such as the progressive rise in atmospheric CO_2 concentration that leads to a higher leaf assimilation rate, and vineyard management issues, such as better insight into physiology and cultural practices to upgrade overall canopy performance. These factors interact with climate and quite often result in a pending crop that at a notably early stage (i.e. mid-August) shows a

high potential alcohol content and low acidity, a fruit composition that would suggest immediate harvesting. At the same time, however, pH is usually unsuitably high, thereby favouring microbial instability of wines and constraining the expression of full grape aroma potential (Jackson 2008). According to Coombe and McCarthy (2000), a distinctive feature of berry ripening is that the increase in the concentration of free and glycosylated aroma compounds occurs in the advanced stages of ripening when sugar increase per berry has already slowed. Moreover, there is also a problem for phenolic ripeness and, especially, berry pigmentation in red cultivars because it is likewise well known that sugar storage commences in the soft, yet still uncoloured berries and that the initial signs of pigmentation follow several days later (Coombe 1992). This implies that berries might soon reach a high sugar concentration while full colour has not completely developed. Moreover, especially for white cultivars and sparkling wines, two more concerns are encountered. First, if the last stage of ripening occurs during the hottest part of the season, at peak maximum daily temperature (T) and/or minimal day/night thermal difference, aroma profiles can be adversely affected (Marais et al. 1999, Tomasi et al. 2006). Second, the main reason for forced early picking is the flattening of total acidity resulting primarily from temperature-driven, malic acid degradation. Thus, although management tools are available at the microclimate level to condition bunch exposure so as to preserve malic acid, the effect of its degradation appears primarily due to a high nocturnal temperature, which is clearly non-negotiable (Lakso and Kliewer 1975).

Along with altered or atypical phenolic and aroma profiles, extra-early seasonal ripening and the subsequent compression of the growing season strongly challenge vineyard organisation and winery facilities in relation to the feasibility of night harvesting, operational opening dates and the handling practices of warm grapes that are more prone to oxidation. Thus crop management demands for delaying ripening are more pressing and driven not only by the pressures of global warming but also by emerging social attitudes towards "light and more responsible" drinking that tend to prefer wines of moderate alcohol content (Seccia and Maggi 2011).

Yet there are several field practices, both traditional and innovative, available to control ripening date. Traditional approaches rely on techniques aimed at inducing effects

that can run contrary to received wisdom. For instance, there are several traditional ways of achieving delayed ripening: by a calibrated yield increase, late-season vine growth through scheduled shoot trimming and water supply, or even by imposing a source limitation on the vine canopy at specific dates via the use of shading nets, leaf removal or shoot trimming and anti-transpirant sprays (Filippetti et al. 2011). In effect, recent work by Palliotti et al. (2010), although aimed at a different goal, shows that the anti-transpirant Vapor Gard (Miller Chemical & Fertilizer Corporation, Hannover, PA, USA) applied twice at a 10-day interval on a portion of Sangiovese and Ciliegiolo canopies limits gas exchange [i.e. net photosynthesis (Pn) and stomatal conductance (gs)] by 40–70% as compared with that of the unsprayed control, and that this effect typically lasts 40–45 days after first spraying. Interestingly, once the spray is washed off, leaves show the ability to completely recover their function. This implies that a late-season anti-transpirant treatment could prove to be a powerful tool for controlling ripening. Another alternative is the application of growth regulators. Böttcher et al. (2011) recently reported that a pre-veraison treatment with 1-naphthaleneacetic acid was effective in both delaying ripening and ameliorating synchronicity in sugar accumulation in Shiraz. In addition, it has been shown that the application of brassinazole and 1-methylcyclopropene (inhibitors of brassinosteroids and ethylene perception, respectively) can postpone ripening (Symons et al. 2006). A somewhat newer approach to desirable delayed ripening is the use of unripe grapes harvested during bunch thinning as a method for reducing wine alcohol content and pH (Kontoudakis et al. 2011).

Based on well-established leaf age versus Pn relationships (Kriedemann et al. 1970, Poni et al. 1994), which attribute highest functionality to leaves located in the median shoot zone for the period between fruitset and veraison and best Pn performance to apical leaves in the postveraison period, our study focused on late-season leaf removal as a tool for delaying the ripening of cv. Sangiovese. The trial was specifically designed to: (i) establish how the whole-canopy net CO2 exchange rate (NCER) is seasonally modified in spur-pruned Sangiovese grapevines subjected to late-season leaf removal applied in the upper two thirds of the canopy as compared with non-defoliated control; and (ii) investigate whether and how seasonal NCER modifications are linked to single-leaf gas exchange rates, final vine balance and grape composition.

4.2. Material and methods

4.2.1. Plant material and experimental layout

The trial was run in 2012 at Piacenza (44°55'N, 9°44'E), Italy, using 4-year-old fruiting Sangiovese (*Vitis vinifera*) grapevines of the low-yielding VCR clone 23 grafted to SO4 rootstock and grown outdoors in 90-L pots. The pots were filled with a mixture of sand, loam and clay (65, 20 and 15% by volume, respectively) and kept well watered throughout the trial season. The vines were hedgerow-trained and pruned to a 100-cm long unilateral spur-pruned cordon raised 90 cm from the ground with three pairs of surmounting catch wires for a canopy wall extending about 1.3 m above the main wire. Winter pruning left an average of seven two-count bud spurs per vine.

Twenty-two uniform vines arranged along two single, NE–SW-oriented (35°) rows were randomly assigned in a completely randomised design to the following treatments: (i) defoliation at pre-veraison when berries were still hard and green, and no sign of pigmentation was apparent (DEF-I); (ii) defoliation postveraison at an average sugar concentration of about 12°Brix (DEF-II); and (iii) non-defoliated control (C). The two end vines of each row were used as borders. Leaf removal was manually applied on day-of-year (DOY) 195 (DEF-I) and DOY 207 (DEF-II) by stripping off six to seven main leaves and any laterals from the upper two thirds of the canopy. The stripping usually started underneath the fifth leaf below the shoot tip and proceeded downwards (Figure 1a). Severity of leaf removal was set at six to seven leaves to yield a window of about 40 cm in height in order to simulate the action of most commercial models of leaf plucking machines. As a few leaves above the distal bunches were retained, bunch microclimate was not directly modified by the treatments. Shoots were vertically positioned during elongation, and shoot trimming was applied above the top wire on DOY 172 to retain an average 17–18 bud number per shoot across treatments.


(a)

(b)

Figure 1. (a) The window opened in the canopies by removing on DOY 195 (DEF-II) and 207 (DEF-II) six to seven main leaves and any laterals from the medial-apical shoot zone and (b) the layout of the multichamber system in place.

4.2.2. Single-leaf gas exchange

Net photosynthesis (Pn) and stomatal conductance (gs) rates of well-exposed, mature primary leaves of vines in the first of the two test rows were measured on DOYs 186, 206, 218 and 240 using a CIRAS-2 portable photosynthesis system (PP Systems, Amesbury, MA, USA). At each measurement, four leaves per vine were chosen on two shoots and measured for each treatment in the morning hours (10:00–12:00) under constant saturating light [≅ 1500 μmol/(m^2•s)] imposed with an additional external lamp mounted on top of the leaf chamber. Measurements were taken at ambient relative humidity and, the flow fed to the broad-leaf chamber (2.5 cm^2 window size) was 200 mL/min. To ensure stability of the inlet reference CO_2 con centration [CO_2], a mini CO_2 cartridge was used to provide automatic control of inlet (CO_2) at 370 mmol/L. Three primary leaves inserted on the basal, median and apical portions on each shoot were tagged and sampled. In addition, the first or second fully expanded basal leaf of the lateral that had developed underneath the trimming cut was measured as well.

4.2.3. Whole-canopy gas exchange

Whole-canopy NCER measurements were taken using a custom-built, flow-through system adapted by Poni et al. (1997) to run automated NCER readings on grapevine canopies. Under the current configuration, the system features ten flexible, inflated, transparent polyethylene chambers air-fed by centrifugal blowers, a Ciras-2 SC single-channel infrared gas analyser (IRGA) (PP Systems) and a CR1000 data logger and control module (Campbell Scientific Inc., Logan, UT, USA) for system programming and data storing and processing (Figure 1b). Reference inlet and outlet CO_2 air streams were switched at 2-min intervals to the CIRAS-SC by a solenoid valve and the absolute CO_2 concentration (μmol/mol) was recorded automatically before switching. The system was remotely connected to an external PC, and data checking was performed via Team Viewer software (TeamViewer GmbH, Uhingen, Germany).

A BF2 sunshine sensor (Delta-T Devices, Ltd, Cambridge, England), which uses an array of cosine-corrected photodiodes and a shading pattern to produce two analogue outputs corresponding to the diffuse and total light components, both converted into

photosynthetically active radiation (PAR) quanta (≈400–700 nm), was placed horizontally on top of a support stake next to the chambers enclosing the canopies. The BF2 was wired to the CR10 data logger, and PAR data were recorded simultaneously with [CO_2]. Chamber inlet and outlet temperature was also recorded at the same frequency with copper- constantan thermocouples, respectively, placed within the duct of the inlet airflow stream and the ducts streaming air outside the chambers.

The chambers were set up on the second row and continuously operated from DOY 186 (4 July, 9 days before first leaf removal) to DOY 242 (29 August, 35 days after second leaf removal). Between 4 and 7 August, the system was temporarily halted for maintenance; a gap also occurred from 18 to 20 August because of failure of the IRGA light source, which had to be ordered and replaced. Three chambers were established on the C, DEF-I and DEF-II treatments; the remaining chamber was left empty to provide assessment for gas exchange alteration because of the physical presence of the plastic enclosure itself.

The flow rate fed to the chambers was initially set at 9.96 L/s and then raised to 12.6 L/s on DOY 216 to take into account a significant surge in diurnal mean air temperature. Because the polyethylene chambers had a volume of 1.25 m^3 ± 0.12, a complete volume air change occurred at an interval varying from about 120 to 80 s. Whole-canopy NCER per vine (µmol CO_2/s) and per leaf area (LA) unit (µmol $CO_2/m^2 \bullet s$) was calculated from differential CO2 and flow rates after Long and Hallgren (1985).

4.2.4. Vegetative growth, yield and grape composition

Total LA per vine defoliated at both dates was estimated via the linear relationship between leaf fresh mass (FM) (x) and leaf blade surface (y) as determined on a sample of 32 leaves (24 main and 8 lateral) pulled from the non-chambered vines and yielding the following linear equation: y = 39.026x, R2 = 0.964. The LA of this sample was quantified by measuring the surface of each lamina with a LA meter (LI-3000A, LI-COR Biosciences, Lincoln, NB, USA). The onset of veraison was visually assessed by berry colour appearance, which took place on 20 July (DOY 202). To allow assessment of recovery capacity for ripeness in the defoliated treatment, and having established a minimum

threshold of 18°Brix for acceptable ripeness, the control vines were harvested on 4 September (DOY 248), whereas the defoliated vines were harvested a week later on 11 September (DOY 255).

Each chambered vine was individually picked at harvest, and all bunches were counted and weighed. A 50-berry sample per vine was taken to ensure that the positions within the bunch (top, mid, bottom) and exposures (internal or external berries) were represented. These samples were then weighed and stored at −20°C for subsequent colour and phenolic analysis. All the remaining crop per vine was crushed and must soluble solids concentration (°Brix) determined by a temperature-compensating Atago refractometer (RX-5000 Atago Co., Ltd, Tokyo, Japan). Titratable acidity (TA) was measured by a Crison compact titrator (Crison, Barcelona, Spain) with NaOH 0.1 N to the end point of pH 8.2 and expressed as g/L of tartaric acid equivalent.

Anthocyanins and phenolic substances were determined after Iland (1988). The frozen 50-berry samples were thawed and then homogenised at high speed (7602 *g*) with an Ultra-Turrax (Rose Scientific Ltd, Edmonton, AB, Canada) homogeniser 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 1 h before centrifugation at 959 g for 5 min. A portion of the extract (0.5 mL) was added to 10 mL 1 M HCL, mixed and let stand for 3 h; then the absorbance values were measured at 520 nm and 280 nm on a Kontron spectrophotometer (Tri-M Systems and Engineering, Inc., Port Coquitlam, BC, Canada). Anthocyanins and phenolic substances were expressed as mg/g of FM and mg/berry.

Soon after harvest, the vines were entirely defoliated and the FM of main and lateral leaf fractions recorded separately. Total LA was then estimated from the relationship of leaf FM to LA. At leaf fall, total cane number per vine was recorded as well as the total number of primary buds per cane.

4.2.5. Statistical treatment

One-way analysis of variance was carried out and, in case of significance of F test, mean separation was performed by the Student-Newman-Keuls test at $P < 0.05$ and 0.01. Degree of variation around means is given as standard error.

4.3. Results

4.3.1. Climate trends and whole-canopy NCER

The 60-day measuring period (DOY 186–246) was marked by mostly clear days [average direct PAR from dawn to dusk ≥ 800 µmol/(m²•s)] and by a generally increasing trend of daily air vapour pressure deficit, which peaked at 2.99 kPa on DOY 235 (Figure 2b) when mean daily ambient temperature reached 35°C (Figure 2a). Although the summer at the trial site was one of the hottest in the last 50 years, the system was effective at controlling overheating because maximum ΔT (outlet-inlet) was 1.4°C recorded on control vines on DOY 241 (Figure 2b). Notably, the air circulating around the leaf removed canopies was occasionally even slightly cooler than ambient T.

The seasonal trend of canopy NCER (Figure 2c) plotted for all days logging the threshold of mean daily PAR ≥ 800 µmol/(m²•s) shows that the C vines reached their maximum just before veraison (estimated at DOY 201) and maintained that level for about 15 days before displaying a slight yet progressive decline. The baseline NCER/vine measured in the empty chamber averaged over the entire recording period was −0.0923 µmol/s, thus indicating negligible physical interference of the chamber itself.

Based on mean vine NCER calculated over the 3 clear days immediately preceding and following defoliation, the fractional reduction of NCER was 22.5 and 20.0% for DEF-I and DEF II, respectively, as compared with pretreatment NCER/vine (Figure 2c). The diurnal trend of canopy NCER plotted from dawn to dusk 2 days before and after DEF-I (DOY 193 vs DOY 197) (Figure 3b) and 2 days before and after DEF-II (DOY 205 vs DOY 209) (Figure 4b) shows that defoliation on both dates started to reduce pretreatment rates early in the morning and recorded maximum reduction during the late morning hours. Defoliation did not appear to affect CO2 assimilation from 15:00 onwards, i.e. when daily temperature peaked and PAR started to decline below the 1000 µmol/(m2•s) threshold (Figures 3a,4a).

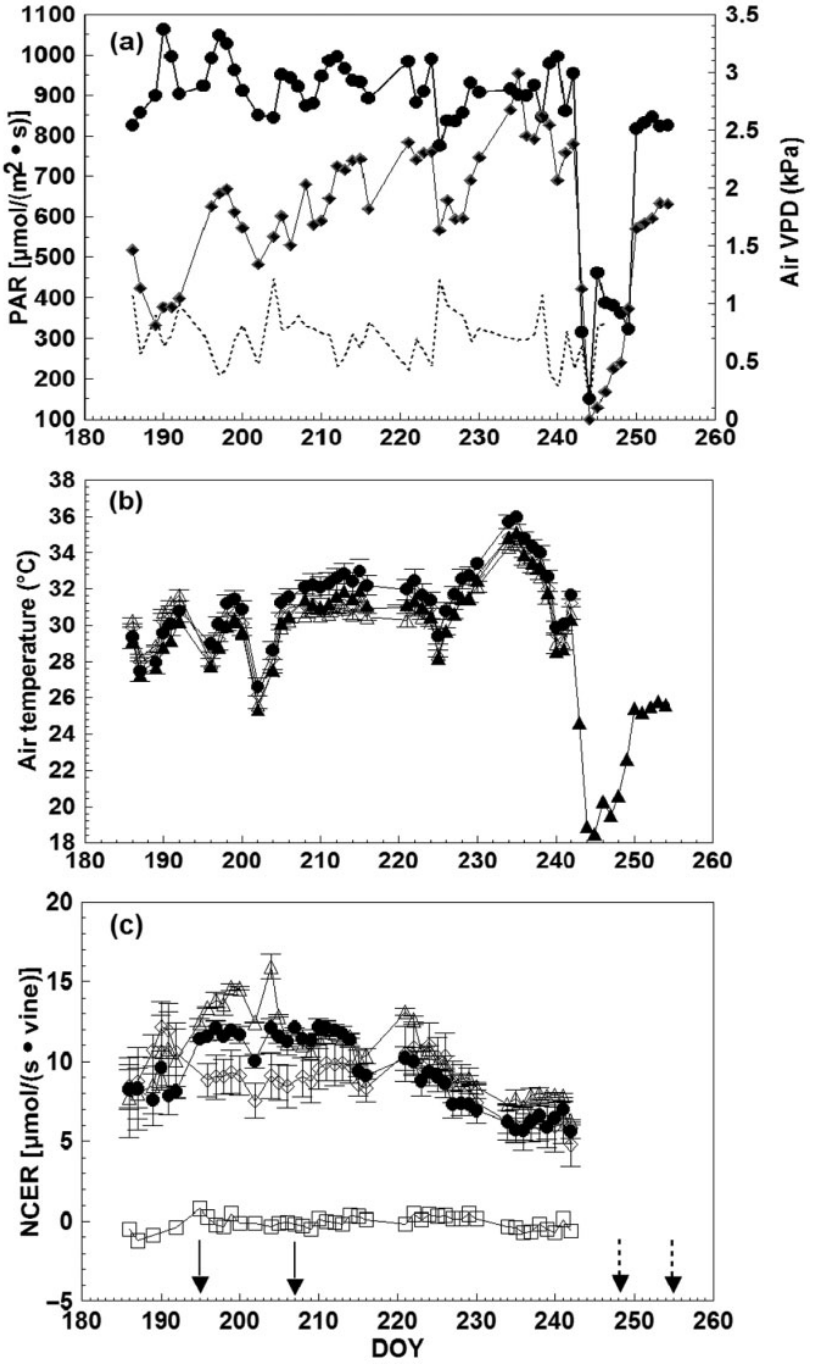

Figure 2. Seasonal trends of (a) air vapour pressure deficit (VPD) (◆), direct (●) and diffuse (---) photosynthetically active radiation (PAR), of (b) inlet and outlet chamber air T and of (c) whole-vine net CO_2 exchange rate (NCER) measured on Sangiovese grapevines: non-defoliated (C) (●), defoliated in preveraison (DEF-I) (◇) and postveraison (DEF-II) (△) and empty chamber (□). In (c), the solid arrows indicate dates of defoliation, and the dotted arrows the dates of harvest. Vertical bars indicate standard error (SE) (n = 3).

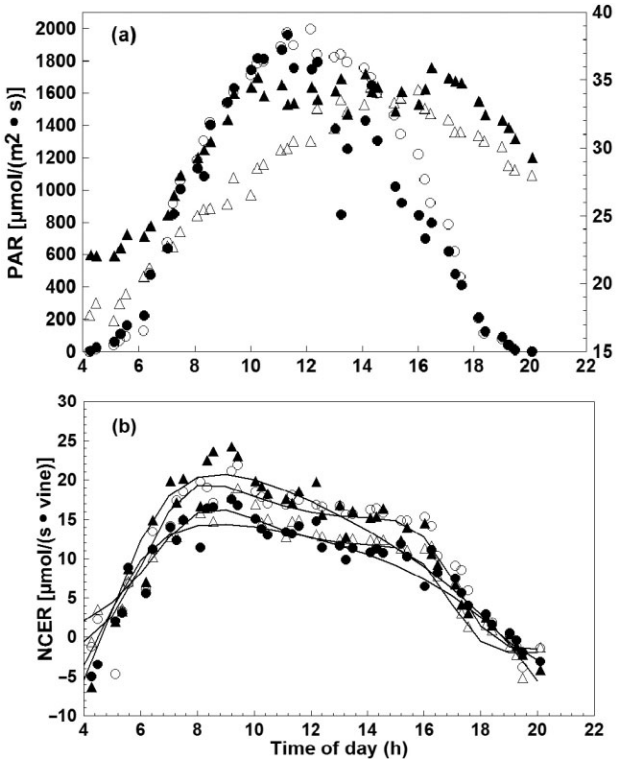

Figure 3. Diurnal trends of (a) ambient T (▲,△), direct and diffuse photosynthetically active radiation (PAR) (●, ○) and of (b) whole-vine net CO_2 exchange rate (NCER) measured two days before (DOY 193) (●) and after (DOY 197) (○) the preveraison defoliation carried out on DOY 195. Data reported for C and DEF-I treatments. Each data point is the mean of three vine replicates. Curves fitted to 5th or 6th order polynomial equations.

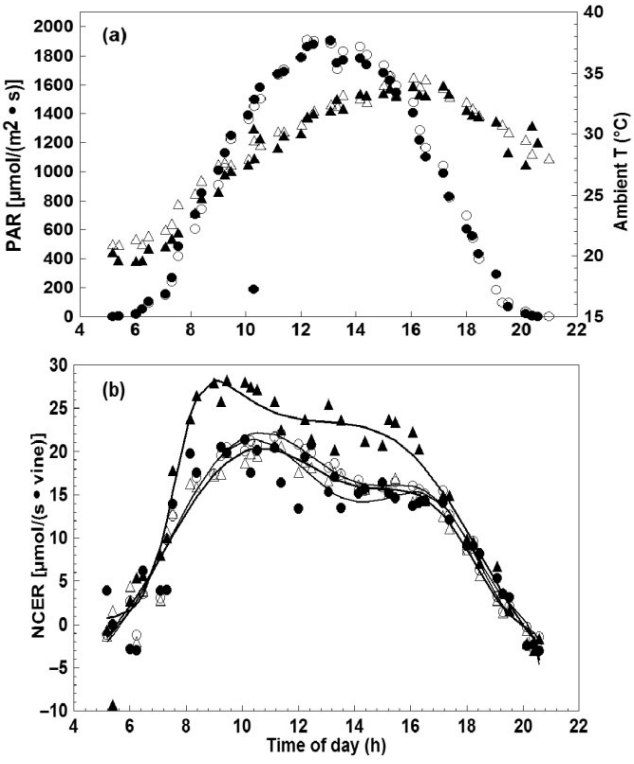

Figure 4. Diurnal trends of (a) ambient T (▲,△), direct and diffuse photosynthetically active radiation (PAR) (●, ○) and of (b) whole- vine net CO2 exchange rate (NCER) measured 2 days before (DOY 205) (●) and after (DOY 209) (○) the postveraison defoliation carried out on DOY 207. Data reported for C and DEF-II treatments. Each data point is the mean of three vine replicates. Curves fitted to 5th or 6th order polynomial equations.

When canopy/NCER is given on a per unit leaf basis (Figure 5) to homogenise within-treatment differences in LA development, it clearly shows significant NCER compensation in both treatments. In DEF-I, the maximum gain was reached on DOY 224, i.e. 29 days after treatment when NCER per unit LA increased by 1.98 µmol/(m2•s) as compared with that of C vines; at the end of the measurement season DEF-I still retained 21.7% higher NCER than did C vines. In DEF-II, maximum efficiency as NCER compensation was reached earlier, at DOY 221 [i.e. 14 days after treatment, scoring + 2.24 µmol/(m2•s) vs C vines], and at chamber dismantling DEF-II still showed a 35.3% higher NCER per unit LA than C.

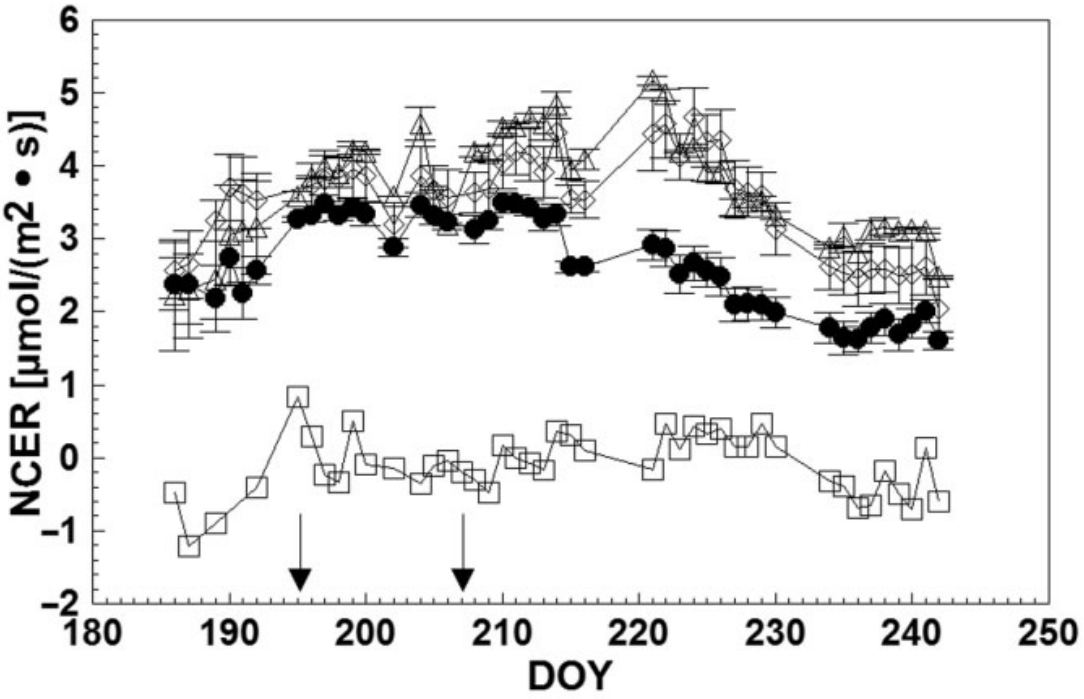

Figure 5. Whole-vine net CO_2 exchange rate (NCER) per unit leaf area measured on Sangiovese grapevines commencing on day-of-the-year (DOY) 180: non-defoliated (C) (●), defoliated in preveraison (DEF-I) (◇) and postveraison (DEF-II) (△) and empty chamber (□). Solid arrows indicate the dates of defoliation. Vertical bars indicate standard error (SE) (n = 3).

4.3.2. Single-leaf gas exchange

Net photosynthesis rates averaged over different leaf positions showed that DEF-I had much higher Pn [≅ +3 µmol/(m²•s)] than C at 12 and 26 days after treatment, whereas DEF-II scored higher Pn than C on DOY 221 [+1.3 µmol/(m²•s)], i.e. 14 days after treatment (Figure 6). At preharvest, DEF-I approached the Pn rates measured on C; however, DEF-II had maximum Pn compensation quantified as 2 µmol/(m²•s) higher than that of C.

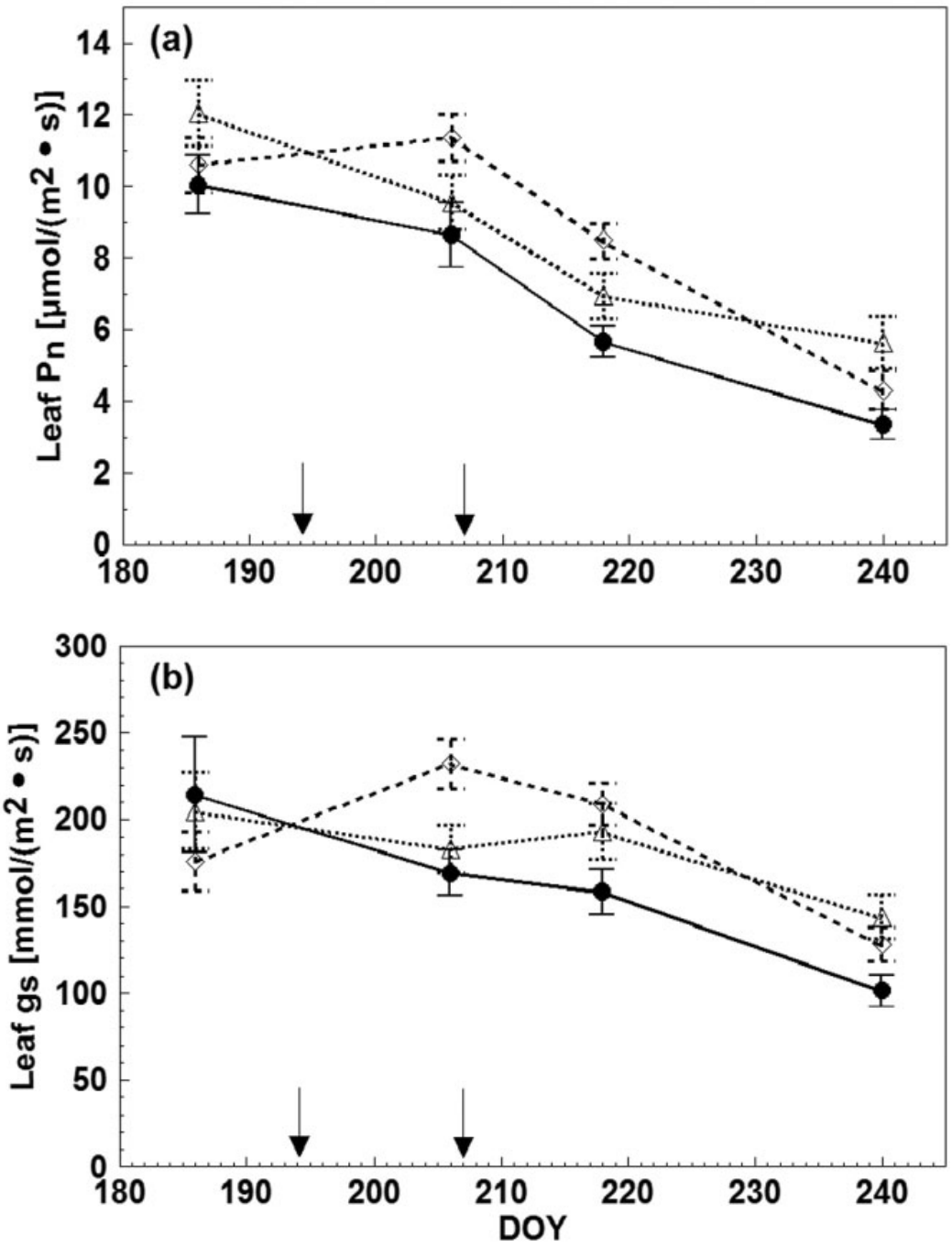

Figure 6. (a) Single-leaf net photosynthesis rate (Pn) and (b) stomatal conductance (gs) measured on DOYs 186, 206, 218 and 240 measured on Sangiovese grapevines commencing on day-of-the-year (DOY) 180: non-defoliated (C) (●), defoliated in preveraison (DEF-I) (◇) and postveraison (DEF-II) (△). Within each date, values were pooled over different leaf types and position. Vertical bars indicate SE (n = 24).

Stomatal conductance essentially reflected Pn trends, although on DOY 221 and 241, the two defoliation treatments did not differ. When the effect of leaf removal on single-leaf Pn was evaluated as a response relative to leaf position, hence age (Figure 7), significant compensation in basal leaves was recorded in DEF-I 23 days after leaf removal, while basal DEF-II leaves were less responsive. Not surprisingly, apical leaves showed marked compensation upon leaf removal although with a different dynamic: in DEF-I maximum Pn gain was scored 13 days after treatment and then this difference progressively decreased, whereas DEF-II showed significant Pn compensation in apical leaves only during the last set or readings. Lateral response was somewhat confounded by fairly large variability found in predefoliation readings, though it was apparent that DEF-II has a significantly higher rate than that of the C vines at DOY 240, whereas measurements taken 22 days earlier did not differ among treatments (Figure 7).

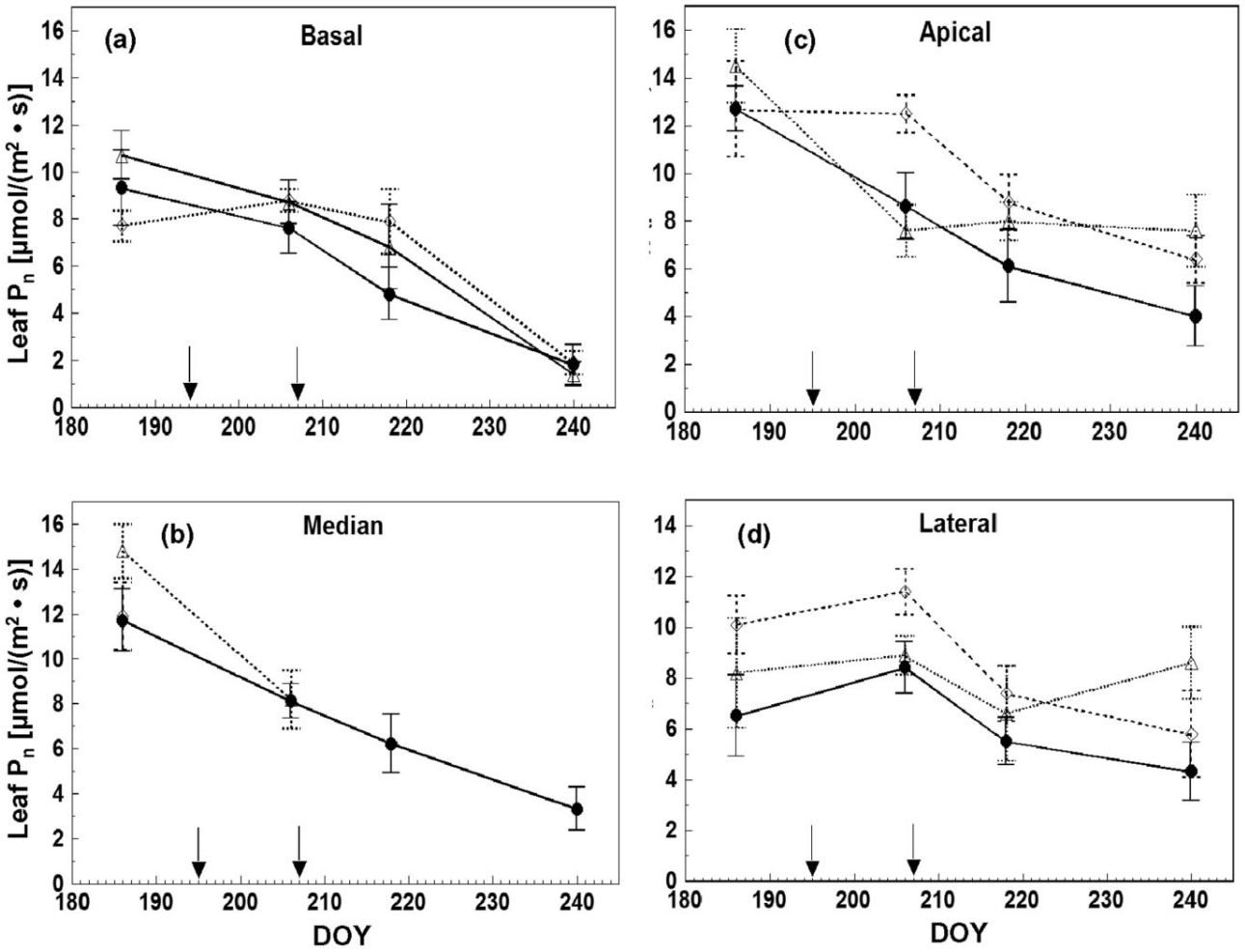

Figure 7. Single-leaf net photosynthesis rate (Pn) measured on day-of-the-year (DOY) 186, 206, 218 and 240 on primary, (a) basal, (b) median and (c) apical according to insertion along main stem, and (d) lateral leaves of Sangiovese grapevines commencing on DOY 180: non-defoliated (C) (●), defoliated in preveraison (DEF-I) (◇) and postveraison (DEF-II) (△). Vertical bars indicate SE (n = 6).

4.3.3. Vine performance and balance

The LA removed in both defoliation treatments was slightly less than 1 m2 and corresponded to 28.3 and 27.2% of final LA estimated on DEF-I and DEF-II, respectively (Table 1). Limited LA formation occurred after each defoliation as it can be inferred by the modest fraction of lateral LA that developed on defoliated vines as compared with that on C vines. When the removed LA was added to the final LA in the defoliated treatment, total LA was similar to that of the C vines (3.29 and 3.48 m² for DEF-I and DEF-II, respectively).

Yield per vine and its components did not differ among treatments. LA-to-yield ratio (cm²/g) calculated at harvest not unexpectedly showed higher source availability per

unit of crop for the C vines compared with that of the DEF-I and DEF-II treatments, although this difference disappeared when the source–sink balance was given as the actual amount of carbon available per g/berry FM. Moreover, foliage efficiency as g $CO_2/(m^2 \bullet s)$ calculated over the entire time span covered by the chamber readings (DOY 186–242) showed that DEF-I and DEF-II vines were 33 and the 36% more efficient, respectively, than the C vines (Table 1).

At first harvest (DOY 248), when only C vines were picked, must soluble solids accumulation was delayed in DEF-I and DEF-II by 1.3 and 2.4 °Brix, respectively, and TA was still significantly higher than that in C (Table 2). By contrast, must pH and anthocyanins and phenolic substances were similar among treatments. A week later (DOY 255), DEF-I and DEF-II showed full and partial recovery in soluble solids content, both reaching the minimum set threshold. The remaining parameters were almost constant, although some variation compared with the preharvest date may be related to the 30.6 mm of rain that fell between the first and second harvest (data not shown).

4.4. Discussion

Our whole-vine enclosure system, as with previous studies employing the same type of equipment (Intrieri et al. 1998, Petrie et al. 2003, 2009, Tarara et al. 2011), was effective in assessing the complex and dynamic changes brought about by leaf removal at different seasonal dates, changes that are usually cumbersome to follow when relying only on a single-leaf approach (Poni et al. 2009). Our objective (i) shows that the short-term decline in NCER/vine (Figure 2c) upon leaf removal was slightly less than the fraction of actually removed total LA (Table 1). This finding is not surprising as opening such a window in the canopy enables, especially at high solar angles, more light interception by the basal part of the canopy. It should also be taken into account that the photosynthetic contribution of the soon-to-be-removed leaves changes as per timing of leaf removal simply because their age and, hence, a change in their efficiency.

The strongest response returned by the whole-canopy readings was the capacity to compensate for photosynthesis at both leaf-removal dates in terms of amplitude and duration, a phenomenon that lasted until harvest. The data reported in Table 1 as g $CO_2/(m^2 \bullet s)$ show that the average whole-season photosynthetic foliage efficiency of defoliated canopies was at least 30% higher than that of C vines and, when expressed on a per canopy basis, the amount of assimilates available for ripening (mg C/g berry FM, last column of Table 1) was similar, thereby fitting with the fairly homogeneous ripeness pattern ultimately reached by all treatments. The indication provided by this last index diverges from what could have been inferred from just expressing the total leaf-to-fruit ratio, which, albeit almost universally used as an index of source–sink balance (Kliewer and Dokoozlian 2005), confirmed its limitation as being static and, hence, unsuitable for describing actual leaf function in our trial using potted vines.

The single-leaf readings further support Pn compensation, confirming it in terms of magnitude as compared with C rates and specifying that although the higher degree of compensation was provided the younger, main and lateral leaves in the apical shoot zone, basal leaves were also able to show significant Pn compensation in the DEF-I treatment. Thus, the combined whole-canopy and single-leaf approach of our study supports the finding that grapevine leaves are capable of notable Pn compensation when a significant

portion of LA is removed, a finding in agreement with others (Candolfi-Vasconcelos and Koblet 1991, Poni and Giachino 2000). Moreover, that whole-canopy and single-leaf readings yielded a similar outcome suggests, as recently reported by Tarara et al. (2011), that a good linear association between the two techniques can be achieved.

The defoliation DEF-I occurred on DOY 195 (13 July), i.e. still at preveraison when, according to Poni et al. (1994), working on the same cultivar, the leaves growing on the median part of the shoot (i.e. those actually removed) have the highest Pn rates. The data in Figure 7 for median leaves confirm that leaves pulled in DEF-I were active [mean Pn about 12 μmol/(m²•s) for data taken on DOY 186], though the Pn rates of apical leaves shown in the same figure were even slightly higher, thereby contradicting what Poni et al. (1994) reported. This mismatch, however, can be easily explained as those authors worked on untrimmed shoots because the shoot apical parts still included some young developing leaves at preveraison, whereas in the present study the shoots were trimmed 14 days before the first gas exchange readings. Thus, 'apical' leaves located underneath the trimming cut likely had enough time to reach full expansion and ripeness by the time measurements started. In addition, leaves classified as 'median' in the study by Poni et al. (1994) corresponded to the equivalent position leaves labelled 'apical' in the present study. Then too, if photosynthetic activity of each shoot zone was similar before leaf removal, it is perfectly understandable why NCER per unit LA calculated over 3 consecutive days after leaf stripping [3.78 μmol/(m²•s); Figure 5] was similar to pre-leaf removal rate [3.61 μmol/(m²•s), mean over DOY 190–192].

Postveraison, single-leaf Pn data again appeared to offer a somewhat different picture from that reported by Poni et al. (1994) and by Knoll and Redl (2012) for untrimmed shoots where the highest Pn in the apical shoot zone was assessed. Figure 7 shows that the measurements taken the day before DEF-II was defoliated (DOY 206) appear to show that the Pn rate was again similar across different position and leaf types [about 8 μmol/(m²•s)]. The same NCER per unit LA comparison previously shown for DEF-I confirms that the 3-day averaged NCER postveraison rate [4.19 μmol/(m²•s)] was even slightly higher than predefoliation rate [3.82 μmol/(m²•s)]. According to the NCER trend in Figure 5, DEF-I shows a decidedly improved compensation from DOY 215 on; this

response appears to match closely the improved basal leaf Pn recorded on DOY 218 (Figure 7). Note too that the total photosynthetic contribution of each shoot zone is also a function of leaf number and size within it. As the basal part of the canopy included the first six to seven basal leaves, and given that these are usually larger than upper located leaves, it is to be expected that the basal canopy sector plays a major role in overall canopy photosynthetic balance.

Table 1. Vegetative growth, yield components and vine balance indices determined on Sangiovese grapevines either nondefoliated (C) or defoliated preveraison (DEF-I) or postveraison (DEF-II)

	Removed LA (m^2)	**Total LA (m^2)**	**Primary LA (m^2)**	**Lateral LA (m^2)**	**Yield/vine (kg)**	**Bunches/ vine**	**Bunch mass (g)**	**Berry mass (g)**	**LA/FM (cm^2/g)**	**Carbon [g CO_2/ (m^2day)]**	**Carbon/Y (mg CO_2/ g berry FM)**
Control	0 a	3,4914 a	2,4655 a	1,0258 a	1,983	14,8	134,1	1,98	17,6 a	2,64 b	11,0
DEF-I	0,9335 b	2.3608 b	1,6643 b	0,6964 b	2,241	15,7	140,1	1,92	10,5 b	3,51 a	9,8
DEF-II	0,9483 b	2,5340 b	1,8766 b	0,6557 b	2,350	16,0	150,6	2,02	10,8 b	3,67 a	10,4
Significance	**	**	**	**	ns	ns	ns	ns	*	**	ns

* and ** denote a significant difference between treatments at $P < 0.05$ and 0.01 according to within column mean separation performed with SNK test. FM, berry fresh mass; LA, leaf area; ns, non significant.

When C vines were harvested upon reaching the minimum threshold of 18°Brix, the leaf-removal treatments showed a delayed sugar accumulation, which was more marked in DEF-II. Thus, a significant delay in soluble solids accumulation occurred despite the fact that seasonal source-to-sink ratio expressed as mg CO_2/g FM and calculated for the entire period the chambers were on was not significantly modified by the treatments (Table 1). This appears to indicate that a late season apical-to-bunch defoliation that induces even a short-term drop in NCER/vine (Figure 5, DEF-II behaviour) is effective at temporarily hindering berry sugar accumulation. This appears to find support in a recent paper by Palliotti et al. (2013), who showed in a 2-year field trial on Sangiovese, comparing mechanical postveraison apical-to-bunch zone defoliation and a non-defoliated control, that defoliated vines registered a 1.2 °Brix reduction at harvest, although final LA-to-fruit ratio was 11.3 cm^2/g, a value close to the one scored by DEF-II in the present study (Table 1).

Given a similar level of LA removed, crop level and season-averaged carbon-to-yield ratio in the two treatments (Table 1), higher sensitivity of DEF-II reflected by a greater delay in ripening is also quite puzzling and shifts the focus towards compensation and assimilate partitioning. It may be that berry ripening in DEF-I specifically benefits from significant Pn compensation shown by basal leaves on DOY 218, which is supported by evidence showing that certain leaves primarily feed the most closely located sinks (Flore and Lakso 1988). Quinlan and Weaver (1970) fed $^{14}CO_2$ at fruitset to a bunch adjacent leaf, and 24 h later autoradiographs showed that photosynthates were traced in the adjacent bunch with no movement towards other sinks.

Table 2. Grape composition of Sangiovese grapevines either nondefoliated (C) or defoliated preveraison (DEF-I) or postveraison (DEF-II) at two harvest dates

	Soluble solids (°Brix)	TA (g/L)	pH	Anthocyanins		Phenolic substances	
				(mg/g)	(mg/berry)	(mg/g)	(mg/berry)
Harvest 04/09							
Control	18.8 a	5.34 a	3.13	0.63	1.05	2.14	4.20
DEF-I	17.5 b	6.03 b	3.34	0.67	1.19	2.31	4.89
DEF-II	16.4 c	6.22 b	3.32	0.64	1.19	2.27	5.09
Significance	**	**	ns	ns	ns	ns	ns
Harvest 11/09							
Control	-	-	-	-	-	-	-
DEF-I	18.9	6.54	3.19	0.67	1.22	1.90	4.10
DEF-II	18.0	6.69	3.18	0.67	1.23	1.98	4.27
Significance	ns	ns	ns	ns	ns	ns	ns

* and ** denote a significant difference between treatments at $P < 0.05$ and 0.01 according to within column mean separation performed with SNK test. ns, non significant.

A week after first harvest, DEF-I had fully recovered to the sugar concentration of C, whereas DEF-II gained 1.6 °Brix compared with the 18 °Brix target of C. Although the chambers were dismantled on DOY 242 (i.e. before harvest), the robustness of seasonal whole-canopy, gas exchange data enable a comparison between estimated canopy NCER from DOY 249 to 254 and the amount of sugar accumulated by the berries over that period. Given the yield per vine and soluble solids data (Tables 1 and 2), 31.3 and 40.5 g of sugar accumulated, respectively, in DEF-I and DEF-II berries during the period elapsing between the two harvests. We can thus estimate that canopy NCER from DOY 249 to 254 approximately matches the last 5 days of actual readings (DOY 237–242), yielding an average daily assimilation of 12.5 and 17.1 g CO_2/vine for DEF-I and DEF-II, respectively. This estimate seems rather conservative because DOYs 237–242 were the hottest of the entire season, with maximum air T peaking around 40 °C, whereas DOYs 249–254 were clear and definitely cooler as maximum air T never exceeded 31 °C (Figure 2a) and, hence, more conducive to optimal NCER rates. If we then consider that total carbon gain per vine from DOY 249–254 was 75 and 102.6 g CO_2 in DEF-I and DEF II, respectively, and apply a cost conversion coefficient into biomass of 0.55 (Ollat and Gaudillere 2000), the result is an

accumulated dry matter of 41.3 and 56.4 g, respectively, which is in agreement with respective weekly sugar gain values of 31.3 and 40.5 g.

Few comparative data on the effect of late-season leaf removal for delaying ripening are available in the literature. Stoll et al. (2010) report that a significant delay in ripening in Riesling is reached when the leaf-to-fruit ratio was reduced from 14 to 8 cm^2/g. More recently, they have shown that mechanical defoliation at fruitset (BBCH 71 according to Lorenz et al. 1995) above the bunch area, which retains about 70% of the LA on control vines, had no effect on grape composition, vine yield and berry size (Stoll et al. 2013). Their explanation for the lack of effect is that the final LA-to-fruit ratio calculated for the leaf-removal treatment (12.3 cm^2/g vs 19.6 cm^2/g in C) was still non-limiting. Interestingly, neither the present study nor those of Stoll et al. (2010, 2013) report a decrease in berry size and/or yield because of leaf removal, which, given that the aim of our trial is a delay in ripening, is a positive feature as an increased solute concentration because of smaller berry size might offset the retarding effect in grape composition pursued with leaf removal.

Our results show that phenolic ripeness was unaffected by the leaf removal treatments, which registered no variation between first and second harvest. Kliewer and Dokoozlian (2005) report that maximum fruit skin coloration is reached when the LA-to-fruit ratio varies from 11 to 14 cm^2/g. There is a large body of literature (cited in Downey et al. 2006), however, showing that factors other than LA/Y have a higher impact on berry coloration, with berry size and bunch microclimate playing major roles. The first parameter was directly quantified in our study and showed inconsistent differences; the latter was not directly assessed. Leaf removal, however, was performed above the fruiting area and was unlikely to have caused abrupt modification in bunch microclimate. Indeed, the final amount of colour stored in berry skin was low. Air temperature was likely to have contributed to the lower concentration of anthocyanins as it rose over several days between veraison and harvest and finally peaked during the last week of chamber measurements (Figure 2b). It is well known that the optimal temperature for pigment-producing enzymes (17–26°C) is lower than the optimal range for sugar activity (18–33°C), and that berry overheating leads to poor coloration in red cultivars (Spayd et al. 2002,

Price et al. 2005). Moreover, Mori et al. (2007) have specifically shown that high T (≥ 35°C) can also enhance anthocyanin degradation postveraison.

4.5. Conclusions

Although conducted only over a single season, our trial using potted vines delivers data showing that there is a straightforward relationship between physiological adaptation to late-season leaf removal applied above the bunch area and final grape composition. Defoliation applied postveraison was more effective than that preveraison at temporarily limiting sugar accumulation, which was significantly lower at harvest than that in control vines. The gap in ripeness, however, was filled by the shift of harvest date in the defoliation treatments by just a week, a result that was also made possible by the amount and duration of photosynthetic compensation, which was clearly detected at both the single-leaf and whole-canopy level.

The data for final grape composition are promising. In effect, had the defoliated vines been picked at the first date, this would have led to delayed technological ripening, i.e. sugar concentration and TA, yet with the same phenolic ripeness. Thus, it appears there is room for a decoupling between the two ripeness types that would result in a wine style that is currently much in demand by the market: one that is less alcoholic to meet the trend for 'light drinking' while at the same time largely retaining its phenolic substances, which have a well-documented positive action on human heath (Casey 2012). More work is now needed in the field to verify over a longer term the consistent reproducibility of our findings under an array of different genotypes and environments.

4.6. References

Böttcher, C., Harvey, K., Forde, C.G., Boss, P.K. and Davies, C. (2011) Auxin treatment of pre-veraison grape (*Vitis vinifera* L.) berries both delays ripening and increases the synchronicity of sugar accumulation. Australian Journal of Grape and Wine Research 17, 1–8.

Caffarra, A. and Eccel, E. (2011) Projecting the impacts of climate change on the phenology of grapevine in a mountain area. Australian Journal of Grape and Wine Research 17, 52–61.

Candolfi-Vasconcelos, M. and Koblet, W. (1991) Influence of partial defoliation on gas-exchange parameters and chlorophyll content of field grown grapevines – mechanisms and limitations of the compensation capacity. Vitis 30, 129–141.

Casey, D. (2012) Elixir of immortality? Wine, resveratrol and sirtuins: an overview. Journal of Wine Research 23, 247–252.

Coombe, B.G. (1992) Research on development and ripening of the grape berry. American Journal of Enology and Viticulture 43, 101–110.

Coombe, B.G. and McCarthy, M.G. (2000) Dynamics of grape berry growth and physiology of ripening. Australian Journal of Grape and Wine Research 6, 131–135.

Downey, M.O., Dokoozlian, N.K. and Krstic, M.P. (2006) Cultural practices and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. American Journal of Enology and Viticulture 57, 257–268.

Duchêne, E. and Schneider, C. (2005) Grapevine and climatic changes: a glance in the situation in Alsace. Agronomy for Sustainable Development 25, 93–99.

Filippetti, I., Allegro, G., Mohaved, N., Pastore, C., Valentini, G. and Intrieri, C. (2011) Effects of late-season source limitations induced by trimming and anti-transpirants canopy sprays on grape composition during ripening in *Vitis vinifera* L. cv. Sangiovese. Proceedings 17th international symposium GiESCO, 29 August–2 September 2011; Alba, Italy. pp. 259–262.

Flore, J.A. and Lakso, A.N. (1988) Environmental and physiological regulation of photosynthesis in fruit crops. Horticultural Reviews 11, 111–157.

Ganichot, B. (2002) Évolution de la data des vendanges dans les Côtes du Rhone méridionales. Proceedings 6° Rencontres Rhodanniens (Institut Rhodanien, Orange France) pp. 38–41.

Iland, P.G. (1988) Leaf removal effects of fruit composition. In: Proceedings of the second international symposium for cool climate viticulture and oenology; 11–15 January 1988; Auckland, New Zealand. Eds. R.E. Smart, R. Thornto, S. Rodriguez and J. Young (New Zealand Society for Viticulture and Oenology: Auckland, New Zealand) pp. 137–138.

Intrieri, C., Poni, S., Rebucci, B. and Magnanini, E. (1998) Row orientation effects on whole-canopy gas exchange of potted and field grown grapevines. Vitis 37, 147–154.

Jackson, R.S. (2008) Wine science: principles and applications, Third ed (Academic Press: San Diego, CA, USA) p. 749.

Jones, G.V. (2012) Climate, grapes, and wine: structure and suitability in a changing climate. Acta Horticulturae 932, 19–28.

Jones, G.V., White, M.A., Cooper, O.R. and Storckmann, K. (2005) Climate change and global wine quality. Climatic Change 73, 319–343.

Kliewer, W.M. and Dokoozlian, N.K. (2005) Leaf area/crop weight ratios of grapevines: influence on fruit composition and wine quality. American Journal of Enology and Viticulture 56, 170–181.

Knoll, M. and Redl, H. (2012) Gas-exchange of field-grown *Vitis vinifera* L. cv. Zweigeit leaves in relation to leaf age and position along the stem. Journal International des Science de la Vigne et du Vin 46, 281–294.

Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J.M. and Zamora, F. (2011) Use of unripe grapes harvested during cluster thinning as a method for reducing alcohol content and pH of wine. Australian Journal of Grape and Wine Research 17, 230–238.

Kriedemann, P.E., Kliewer, W.M. and Harris, J.M. (1970) Leaf age and photosynthesis in *Vitis vinifera* L. Vitis 9, 97–104.

Lakso, A.N. and Kliewer, W.M. (1975) Physical properties of phosphoenolpyruvate carboxylase and malic enzyme in grape berries. American Journal of Enology and Viticulture 26, 75–78.

Long, S.P. and Hallgren, J.E. (1985) Measurement of CO2 assimilation by plants in the field and the laboratory. In: Techniques in bioproductivity and photosynthesis. Eds. J. Coombs, D.D. Hall, S.P. Long and J.M.O. Scurlock (Pergamon Press: Oxford, UK) p. 62.

Lorenz, D.H., Eichorn, K.W., Bleiholder, H., Klose, R., Meier, U. and Weber, E. (1995) Growth stages of the grapevine: phenological growth stages of the grapevine (*Vitis*

vinifera L. ssp vinifera) – Codes and descriptions according to the extended BBCH scale. Australian Journal of Grape and Wine Research 17, 230–238.

Marais, J., Hunter, J.J. and Haasbroek, P.D. (1999) Effect of canopy microclimate, season and region on Sauvignon blanc grape composition and wine quality. South African Journal of Enology and Viticulture 20, 19–30.

Mori, K., Goto-Yamamoto, N., Kitayama, M. and Hashizume, K. (2007) Loss of anthocyanins in red-wine grape under high temperature. Journal of Experimental Botany 58, 1935–1945.

Ollat, N. and Gaudillere, J.P. (2000) Carbon balance in developing grapevine berries. Acta Horticulturae 526, 345–350.

Palliotti, A., Poni, S., Berrios, J.G. and Bernizzoni, F. (2010) Vine performance and grape composition as affected by early-season source limitation induced with anti-transpirants in two red *Vitis vinifera* cultivars. Australian Journal of Grape and Wine Research 16, 426–433.

Palliotti, A., Panara, F., Silvestroni, O., Lanari, V., Sabbatini, P., Howell, G.S., Gatti, M. and Poni, S. (2013) Influence of mechanical post-veraison leaf removal apical to the cluster zone on delay of fruit ripening in Sangiovese (*Vitis vinifera* L.) grapevines. Australian Journal of Grape and Wine Research. doi: 10.1111/ajgw.12033

Petrie, P.R., Trought, M.C.T., Howell, G.S. and Buchan, G.D. (2003) The effects of leaf removal and canopy height on whole-vine gas-exchange and fruit development of *Vitis vinifera* L. Sauvignon blanc. Functional Plant Biology 30, 711–717.

Petrie, P.R., Trought, M.C.T., Howell, G.S., Buchan, G.D. and Palmer, I.W. (2009) Whole-canopy gas exchange and light interception of vertically-trained *Vitis vinifera* L. under direct and diffuse light. American Journal of Enology and Viticulture 60, 173–182.

Poni, S. and Giachino, E. (2000) Growth, photosynthesis and cropping of potted grapevines (*Vitis vinifera* L. cv. Cabernet Sauvignon) in relation to shoot trimming. Australian Journal of Grape and Wine Research 3, 216–226.

Poni, S., Intrieri, C. and Silvestroni, O. (1994) Interactions of leaf age, fruiting, and exogenous cytokinins in Sangiovese grapevines under non- irrigated conditions. I. Gas exchange. American Journal of Enology and Viticulture 45, 71–78.

Poni, S., Magnanini, E. and Rebucci, B. (1997) Measurements of whole-vine gas exchange using an automated chamber system. HortScience 32, 64–67.

Poni, S., Bernizzoni, F., Civardi, S., Gatti, M., Porro, D. and Camin, F. (2009) Performance and water-use efficiency (single-leaf vs. whole-canopy) of well watered and half stressed split-root Lambrusco grapevines. Agriculture, Ecosystem and Environment 129, 97–106.

Price, S.F., Breen, P.J., Valladao, M. and Watson, T. (2005) Cluster sun exposure and quercetin in Pinot noir grapes and wine. American Journal of Enology and Viticulture 46, 187–194.

Quinlan, J.D. and Weaver, J.R. (1970) Modification of pattern of photosynthate movement within and between shoots of *Vitis vinifera* L. Plant Physiology 46, 527–530.

Schultz, H.R. and Jones, G.V. (2010) Climate induced historic and future changes in viticulture. Journal of Wine Research 21, 137–145.

Seccia, A. and Maggi, G. (2011) Futuro roseo per i vitigni a bassa gradazione alcolica. L'informatore Agrario 13, 11–14.

Spayd, S.E., Tarara, J.M., Mee, D.L. and Ferguson, J.C. (2002) Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. American Journal of Enology and Viticulture 53, 171–182.

Stoll, M., Lafontaine, M. and Schultz, H.R. (2010) Possibilities to reduce the velocity of berry maturation through various leaf area to fruit ratio modifications in *Vitis vinifera* L. cv. Riesling. Progrès Agricole et Viticole 127, 68–71.

Stoll, M., Bischoff-Schaefer, M., Lafontaine, M., Tittmann, S. and Henschke, J. (2013) Impact of various leaf area modifications on berry maturation in *Vitis vinifera* L. cv. 'Riesling'. Acta Horticulturae 978, 293–299.

Symons, G.M., Davies, C., Shakurov, Y., Dry, I.B., Reid, J.B. and Thomas, M.R. (2006) Grapes and steroids. Brassinosteroids are involved in grape berry ripening. Plant Physiology 140, 150–158.

Tarara, J.M., Perez Peña, J.E.P., Keller, M., Schreiner, R.P. and Smithyman, R.P. (2011) Net carbon exchange in grapevine canopies responds rapidly to timing and extent of regulated deficit irrigation. Functional Plant Biology 38, 386–400.

Tomasi, D., Pascarella, G., Gaiotti, F. and Sivilotti, P. (2006) Ambiente, conduzione e qualità aromatica delle uve. L'Informatore Agrario 14, 12–14.

5. Differential responses of sugar, organic acids and anthocyanins to source-sink modulation in Cabernet Sauvignon and Sangiovese grapevines

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Abstract

Grape berry composition mainly consists of primary and secondary metabolites. Both are sensitive to environment and viticultural management. As a consequence, climate change can affect berry composition and modify wine quality and typicity. Leaf removal techniques can impact berry composition by modulating the source-to-sink balance and, in turn, may mitigate some undesired effects due to climate change. The present study investigated the balance between technological maturity parameters such as sugars and organic acids, and phenolic maturity parameters anthocyanins in response to source-sink modulation. Sugar, organic acid, and anthocyanin profiles were compared under two contrasting carbon supply levels in two grape cultivars. Grape berries of cv. Cabernet Sauvignon and Sangiovese were collected at 9 and 14 developmental stages. In addition, whole-canopy net carbon exchange rate was monitored for Sangiovese vines and a mathematic model was used to calculate the balance between carbon fixation and berry sugar accumulation. Carbon limitation affected neither berry size, nor the concentration of organic acids at harvest. However, it significantly reduced the accumulation of sugars and total anthocyanins in both cultivars. Most interestingly, carbon limitation decreased total anthocyanin concentration by 84.3 % as compared to the non source-limited control, whereas it decreased sugar concentration only by 27.1 %. This suggests that carbon limitation led to a strong imbalance between sugars and anthocyanins. Moreover, carbon limitation affected anthocyanin profiles in a cultivar dependent manner. Mathematical

analysis of carbon-balance indicated that berries used a higher proportion of fixed carbon for sugar accumulation under carbon limitation (76.9%) than under carbon sufficiency (48%). Thus, under carbon limitation, the grape berry can manage the metabolic fate of carbon in such a way that sugar accumulation is maintained at the expense of secondary metabolites.

Keywords: *Vitis vinifera* L, wine alcohol content, wine color, climate change, leaf-to-fruit ratio, berry composition.

5.1. Introduction

Grapevine is an important perennial crop cultivated in many countries (7519 mha in 2013) (OIV, 2014). Its fruits are used predominantly for wine making, yet also for juice, raisins and fresh consumption. Grape berry composition, which is important for the grape growers and the wine industry, is mainly determined by sugars, organic acids, and various secondary metabolites (e.g. anthocyanins) (Conde et al., 2007). The accumulation of these components along berry development and ripening depends on the genotype and on the environment (Jackson and Lombard, 1993).

Climate change already affects the physiology of the grapevine (Schultz, 2000), causing increased sugar concentration and, consequently, higher alcohol content in wines (Duchêne and Schneider, 2005; Bock et al., 2013), reduced organic acids and anthocyanins (Barnuud et al., 2013, 2014), and modified aroma profiles (Keller, 2010a). In the long term, the sustainability of wine production in several viticultural regions may be threatened by climate change (Schultz and Jones, 2010; Hannah et al., 2013). To face such challenges, the mechanisms controlling the accumulation of quality-related metabolites in grapes must be better understood. This will allow promoting innovative viticultural practices resulting in easier adaptation of wine production to climate change (van Leeuwen et al., 2013).

Among the different viticultural practices affecting berry composition (Keller, 2010a; Dai et al., 2011; Kuhn et al., 2014), source-sink modulation by summer pruning (i.e. leaf removal or shoot and cluster thinning) is an important tool that may control the relationship between yield and quality, and adjust the complex chemical composition of grape berry (Kliewer and Dokoozlian, 2005). For example, the berry sugar concentration is often positively correlated with leaf area-to-yield ratio when the ratio is below a threshold value of about 1 m^2/Kg of fruit mass (Kliewer and Dokoozlian, 2005; Duchêne et al., 2012). Above this value, the sugar concentration usually reaches a plateau and becomes less responsive to source-sink modulation (Kliewer and Dokoozlian, 2005). The responses of organic acids to source-sink modulation have been less thoroughly studied, and contradictory reports showed that a lower leaf area-to-yield ratio caused either an increase (Wolpert et al., 1983; Ollat and Gaudillere, 1998; Wu et al., 2013), decline (Bravdo et al.,

1985), or lack of response (Reynolds et al., 1994; Parker et al., 2015) of organic acids compared with a high leaf to yield ratio.

In addition to primary metabolites, secondary metabolites (e.g. anthocyanins) also play an essential role in shaping wine quality and typicity. Anthocyanins are responsible for grape color, which is an important determinant of wine color. Grape anthocyanins derive from five anthocyanidins: cyanidin (Cy), delphinidin (Dp), peonidin (Pn), petunidin (Pt) and malvidin (Mv). They have different patterns of hydroxylation (di- or tri-hydroxylated forms), methylation, and can be further modified by acylation (Mazza, 1995). The fine-tuning of anthocyanin composition has important impacts on the color hue and color stability of the resultant wines (Mazza, 1995). Source-sink modulation impacts berry coloration (Weaver, 1963; Kliewer and Weaver, 1971; Petrie et al., 2000a), and recently its effects on anthocyanin content and composition (Guidoni et al., 2008; Pastore et al., 2011, 2013; Filippetti et al., 2015) drew attention of many research groups. For example, Wu et al. (2013) showed that retaining two leaves only in a girdled shoot with one cluster completely inhibited berry coloration. Moreover, Guidoni et al. (2008) reported that total anthocyanins were reduced by source limitation, with di-hydroxylated anthocyanins more sensitive than tri-hydroxylated ones in cv. Nebbiolo berries. However, other authors recently showed that a post-veraison source limitation resulting from either shoot trimming (Filippetti et al., 2015), removal of leaves above the clusters (Palliotti et al., 2013b; Poni et al., 2013) or late-season application of anti-transpirants (Palliotti et al., 2013a) significantly reduced the speed of sugar accumulation but did not affect the concentration of berry anthocyanins at harvest. As the source-sink modulation techniques also bring about concomitant modifications in the fruit zone microclimate (i.e. light and temperature regimes), the results must be interpreted with caution. It is well established that especially temperature significantly affects anthocyanin accumulation (Spayd et al., 2002; Pereira et al., 2006; Mori et al., 2007). Therefore, experiments that are more precisely controlled and avoid confounding between the effects of source-sink modulation and microclimate are needed to quantify the actual response of anthocyanins to carbon availability.

The accumulation of carbon in primary and secondary metabolites is interconnected and results from a complicated metabolic network. For instance, sugar levels positively correlate with total anthocyanin levels (Vitrac et al., 2000; Dai et al., 2014), yet negatively correlate with organic acids (Keller, 2010b). Interestingly, the accumulation of sugars and anthocyanins can be uncoupled by environmental conditions such as high temperature (Sadras and Moran, 2012). In contrast, the effect of source-sink modulation on the sugar-anthocyanin uncoupling seems more complicated (Sadras and Moran, 2012). The anthocyanin : sugar ratio has been reported to be increased (Guidoni et al., 2002), decreased (Sadras et al., 2007), or unchanged (Petrie and Clingeleffer, 2006) by increasing source-sink ratio. The mechanisms underlying this diversity of responses warrant further investigation. If sugars and anthocyanins do have different sensitivities to source-sink modulation, such de-synchronization may help to define a window of source-sink ratios, within which sugars are reduced while anthocyanins are unaffected. This would provide valuable clues to mitigate the negative influences of climate change (Keller, 2010a).

Therefore, the present study aims to quantify the relative sensitivities of different berry compounds (sugars, organic acids, and anthocyanins) to changes in source-sink modulation under controlled or semi-controlled conditions. Monitoring the carbon fixation rate of the whole-canopy and dynamic profiling of metabolites allowed us to conduct a quantitative analysis of carbon demand and supply, and to obtain detailed information on the source-sink balance. In addition, a detailed HPLC analysis allowed us to compare the effects of source-sink modulation on the developmental changes in anthocyanin profiles in two distinct cultivars, Cabernet Sauvignon and Sangiovese.

5.2. Material and Methods

Two experiments were conducted with cv. Cabernet Sauvignon in Bordeaux (latitude 44° 46' 46'' N, longitude 00° 34' 01'' W), France, and cv. Sangiovese in Piacenza (latitude 45°02'52''N, longitude 9°42'2''E), Italy.

5.2.1. Plant material and sampling

5.2.1.1. Exp 1. Cabernet Sauvignon

Fruiting-cuttings made of one shoot bearing one grape cluster of cv. Cabernet Sauvignon were prepared as described in Mullins and Rajasekaran (1981) and grown in a naturally lighted and semi-controlled greenhouse with chemical disease control applied every two weeks. Environmental conditions (air temperature, radiation at canopy level, and relative humidity) were recorded hourly throughout the experiment (Supplementary Figure S1).

Thirty homogeneous fruiting-cuttings were subjected to two source-to-sink ratios at one week before veraison; a group of 15 plants had 12 leaves per cluster per vine (12L) while the remaining vines had three leaves per cluster per vine (3L). At 63 DAF (days after flowering), leaves underneath the basal cluster were removed in both treatments to standardize the microclimate effects; therefore, above the cluster, 3 and 12 leaves were maintained, yielding a total of 7 and 16 nodes per shoot for the 3L and 12L treatments, respectively. The remaining leaves and all secondary shoots were removed over the measurement period. The plants were randomly assigned to three blocks and each block composed of 5 plants of each treatment.

Berries were sampled 9 times at one-week interval from one week after treatment (70 DAF) to 126 DAF. In order to ensure the capture of maturity in 3L treated vines, the last sampling date corresponded to an over-ripe stage. At each sampling date, two berries were sampled from the top and the middle of a single cluster, and the resulting 10 berries from 5 clusters (vines) of a given treatment within a plot were pooled to form a biological

replicate. Three biological replicates were obtained for each treatment at each sampling date. At harvest, all berries were sampled, counted, and weighed.

5.2.1.2. Exp 2. Sangiovese

The experiment was conducted on 4-year-old cane-pruned cv. Sangiovese grapevines grafted on M3 rootstock and grown outdoors in 40 L pots. The pots were filled with a mixture of sand, loam and clay (65, 20 and 15% by volume, respectively) and kept well watered throughout the trial season. Each vine had a 1m long fruiting cane with 8-9 dormant buds. Shoot thinning was applied to retain one main shoot per node and, on each shoot, the basal cluster only was maintained. Vines were arranged along a single, vertically shoot-positioned, 35oNE-SW oriented row and hedgerow-trained. Eight uniform vines were assigned in a completely randomized design to the following two treatments one week before veraison: 3 leaves per cluster (shoot) or 12 leaves per cluster (shoot). As in the Cabernet Sauvignon experiment, at 40 DAF, leaves beneath the basal cluster were removed in both treatments to standardize the microclimate effects; therefore, above the cluster, 3 and 12 leaves were maintained, for 3L and 12L treatment, respectively. Shoots were trimmed to 8 and 16 nodes per shoot, for 3L and 12L treatment, respectively. The remaining leaves and all secondary shoots were removed throughout the measuring period.

Berries were sampled 14 times at one-week interval from one week before treatment to 8 weeks after treatment, and thereafter at 4-day intervals for better capturing maturity. At each sampling date, three berries from a cluster were sampled and the 24 or 27 berries from 8 or 9 clusters (shoot) of a given vine under a treatment were pooled to form a biological replicate. Four biological replicates were obtained for each treatment at each sampling date. At harvest, all berries of a vine were sampled, counted, and weighed.

5.2.2. Berry pretreatment

Sampled berries from both experiments were immediately put into a pre-weighed tube and dropped into liquid nitrogen. The tubes were reweighed after deep freeze to calculate berry fresh weight and then stored in -80 °C for later biochemical analysis.

Berries stored in -80 °C were slightly thawed and separated quickly into skin, pulp, and seed in the laboratory. The skin and pulp were immediately ground into fine powder in liquid nitrogen using a ball grinder MM200 (Retsch, Haan, Germany).

5.2.3. Sugars and organic acids

An aliquot of 500 mg fine powder of pulp was extracted sequentially with ethanol (80% and 50%), dried in Speed-Vac, and re-dissolved in 2.5 mL de-ionized water. Glucose and fructose content were measured enzymatically with an automated micro-plate reader (Elx800UV, Biotek Instruments Inc., VT, USA) according to the method of Gomez et al. (2007). Tartaric acid content was assessed by using the colorimetric method based on ammonium vanadate reactions (Pereira et al., 2006). Malic acid was determined using an enzyme-coupled spectrophotometric method that measures the change in absorbance at 340 nm from the reduction of NAD+ to NADH (Pereira et al., 2006).

5.2.4. Analysis of anthocyanins

An aliquot of 500 mg of berry skin powder was freeze-dried for 72 h and the dried powder (~50 mg) were extracted in 1.0 mL methanol containing 0.1% HCL (v/v). Extracts were filtered through a 0.45 µm polypropylene syringe filter (Pall Gelman Corp., Ann Harbor, USA) for HPLC analysis. Each individual anthocyanin was analyzed as described in Hilbert et al. (2003) and Acevedo De la Cruz et al. (2012) with HPLC. Quantification was carried out by peak area integration at 520nm, and Malvidin-3-glucoside (Extrasynthèse, Lyon, France) standard was used for quantify the anthocyanin concentration.

5.2.5. Leaf area measurement

For Cabernet Sauvignon experiment, leaf area was estimated using the relationship between specific leaf area (m2 fresh area gDW-1) and total leaf dry weight as described in Castelan-Estrada et al. (2002). Leaf areas of the removed leaves for 3L treatment were determined at the initiation of treatment and whole plant leaf areas were determined for both treatments at the end of the experiment.

For Sangiovese experiment, leaf area was measured on the leaves that were removed the day of treatment and after harvest of all berries. Leaf area was determined by measuring the surface of each lamina with a leaf area meter (LI-3000A, LI-COR Biosciences, Lincoln, NE, USA).

5.2.6. Chlorophyll concentration

Six leaves per plant for 12L vines and 3 leaves per plant for 3L vines were measured using the portable Chlorophyll Meter SPAD 502 (Minolta Corp., Ramsey, NJ). On each leaf, five SPAD readings were taken at each leaf lobe and then averaged.

5.2.7. Single-leaf gas exchange

Net photosynthesis (Pn), evapotranspiration (E) and stomatal conductance (gs) rates of 6 leaves per plant were measured only in the Sangiovese experiment at 95 DAF using a CIRAS-2 portable photosynthesis system (PP Systems, Amesbury, MA, USA). On each vine, two shoots were chosen in basal and apical positions along the cane and, on each shoot, three mature leaves located in the basal, median, and apical positions of the main stem were measured in rapid sequence. Readings were performed in the morning hours (10h00-12h00) under constant saturating light (≈1500 μmol m^{-2} s^{-1}) imposed with an additional external lamp mounted on top of the leaf chamber. Measurements were taken at ambient relative humidity and the flow fed to the broad-leaf chamber (4.5 cm^2 window size) was 300 mL min^{-1}. To ensure stability of the inlet reference CO_2 concentration [CO_2], a CO_2 minicartridge was used to provide automatic control of inlet [CO_2] at 380 mmol L^{-1}.

5.2.8. Whole-canopy gas exchange

Whole-canopy net CO2 exchange rate (NCER) measurements were performed only in the Sangiovese experiment using the multi-chamber system reported in Poni et al. (2014) featuring alternating current, centrifugal blowers (Vorticent C25/2M Vortice, Milan, Italy) delivering a maximum air flow of 950m3 h–1; flexible plastic polyethylene chambers allowing 88% light transmission, 6% diffuse light enrichment and no alteration of the light spectrum. System also features a CIRAS-EGM4 single-channel absolute CO2 infrared gas

analyser (PP Systems, Amesbury, MA, USA) set at a 0–1000 parts per million measurement range and a CR1000 data logger wired to an AM16/ 32B Multiplexer (Campbell Scientific, Shepshed, UK). To facilitate air mixing and ensure higher stability in inlet CO2 concentration, air was forced through a buffer tank (500 L) before being directed to the chambers. Switching of air sampling from one chamber to another was achieved at programmed time intervals (90 s) using a set of solenoid valves (SIRAI, Padova, Italy); the air-flow rate to each chamber was controlled by a butterfly valve (Ghibson, Monteveglio, Italy) and measured with a Testo 510 digital manometer (Farnell, Lainate, Italy) using the flow restriction method (Osborne, 1977). The flow rate fed to the chambers was set at 7.1 L/s and kept until the leaf removal treatment, when the flow rate was changed to 5 L/s. Whole-canopy NCER per vine (μmol CO_2/s) and per leaf area (LA) unit (μmol CO_2/m^2s) was calculated from flow rates and CO_2 differentials after.

The chambers were set up on each vine and continuously operated 24 h per day from one week before treatment (2 July) until 95 days after flowering (1 September). Ambient (inlet) air temperature and the air temperature at each chamber's outlet were measured by shielded 1–0.2 mm diameter PFA–Teflon insulated type-T thermocouples (Omega Engineering, Stamford, CT, USA), and direct and diffuse radiation were measured with a BF2 sunshine sensor (Delta-T Devices, Ltd, Cambridge, England) placed horizontally on top of a support stake next to the chambers enclosing the canopies. Ambient (inlet) relative humidity and the relative humidity at each chamber's outlet were measured by a HIH-4000 humidity sensor (Honeywell, Freeport, IL, USA) mounted upstream of the EGM4.

5.2.9. Data analysis

All data analysis were conducted with R software (R Development Core Team, 2010). Student t-test was used to verify the differences between the two source-sink ratios at each developmental stage.

Carbon allocation analysis was conducted as it follows. First, the carbon accumulated in berries throughout development was calculated as a function of hexose concentration and berry fresh weight with a carbon transformation coefficient of 0.4 g

carbon g-1 hexose. Second, the total carbon accumulated (C) in berries per vine was fitted to the following sigmoid curve (Sadras et al., 2008):

$$C = \frac{C_{max}}{1 + e^{\left[\frac{t_0 - t}{b}\right]}}$$

where t is the number of days after flowering, Cmax is the maximal quantity of carbon (g), t0 is the number days after flowering when carbon quantity is half the maximum, and b represents the carbon accumulation duration from 0.25 Cmax to 0.75 Cmax. Third, the carbon accumulation rate (g Carbon/ day) was calculated by using the first order derivation of the sigmoid curve. Finally, the relationship between the berry accumulated carbon and the photosynthesized carbon (obtained from whole-canopy gas exchange measurement) was quantified by their ratio to estimate the supply vs. demand carbon balance.

5.3. Results

5.3.1. Leaf-to-fruit ratio

As expected, leaf removal effectively reduced the total leaf area per vine in 3L treatment in Cabernet Sauvignon and Sangiovese (Tables 1 and 2). It also resulted in a significantly lower leaf-to-fruit ratio (LA/F) in 3L than 12L treatments in both cultivars. 3L vines all had a leaf area to yield ratio lower than 1.0 m^2/Kg. On the other hand, both 12 L Cabernet Sauvignon vines and Sangiovese vines had a LA/F of 3.98 m^2/Kg and 1.15 m^2/kg, respectively (Tables 1 and 2).

Table 1. Effect of source-sink modulation on leaf area (LA), leaf area-to-yield ratio, and leaf chlorophyll content of Cabernet Sauvignon grapevines. Data are means of nine plants.

Treatment ǂ	Pre-trimming LA/vine (cm^2)	Removed LA/vine (cm^2)	Final LA/vine (cm^2)	LA / yield (m^2/Kg)	SPAD
3L	1123	858	265	0.67	51.0
12L	999	0	999	3.98	44.1
Sig.(t test)+	ns	**	**	**	**

ǂ3L: plants with three leaves per cluster; 12L: plants with twelve leaves per cluster.
+ *,**, and ns indicate statistical significance at *P*=0.05, 0.001, and not significant, respectively.

Table 2. Effect of source-sink modulation on leaf area (LA), whole net carbon exchange rate (NCER) per vine and per unit of leaf area, and leaf-to-yield ratio of Sangiovese grapevines. Data are means of four plants. NCER/vine and NCER/LA were averaged for all the post-treatment measurements.

Treatmentǂ	Pre-trimming LA (m^2)	Removed LA (m^2)	Final LA (m^2)	NCER/vine ($\mu mols^{-1}$)		NCER/LA ($\mu molm^{-2}s^{-1}$)		gCO2/vine (cumulated over trial period)	LA/yield (m^2/Kg)
				Pre	Post	Pre	Post		
3L	1.86	1.55	0.31	8.19	1.94	4.39	6.30	142	0.33
12L	1.70	0.68	1.02	8.04	5.64	4.67	5.56	321	1.15
Sig. (t test)+	ns	**	**	ns	**	ns	ns	**	**

ǂ3L: plants with three leaves per cluster; 12L: plants with twelve leaves per cluster.
+ *,**, and ns indicate statistical significance at *P*=0.05, 0.001, and not significant, respectively.

5.3.2. Berry weight

In Cabernet Sauvignon, the 3L treatment limited the increase in berry size that normally occurs between 80-90 DAF (days after flowering), but extended the growth duration to 110 DAF, when berries under 12L conditions already reached their maximal fresh weights (Figure 1A). The longer growth duration compensated the decreased growth rate and resulted in a very similar berry weight under both growth conditions at harvest. Conversely, 3L treatment did not affect the developmental profile of berry size in Sangiovese berries (Figure 1B). At harvest, Sangiovese berries were bigger than those of Cabernet Sauvignon and berries of both cultivars doubled their size from veraison to maturity. In addition, berry dehydration occurred in Cabernet Sauvignon berries as indicated by the decrease in berry fresh weight from 112 DAF to 126 DAF (Figure 1A).

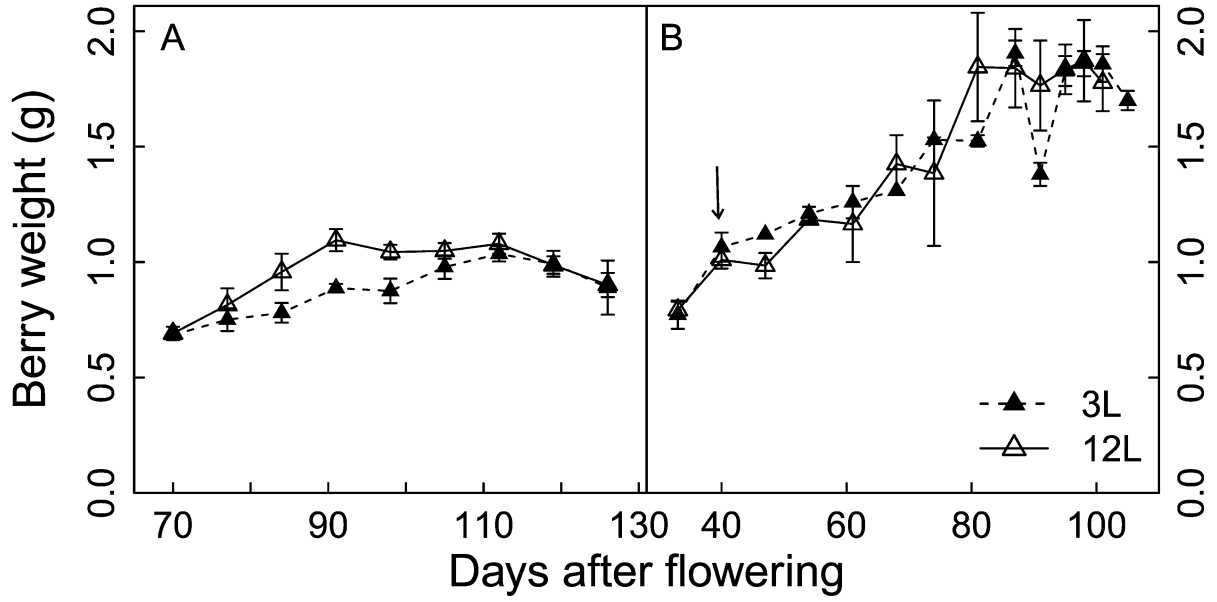


Figure 1. Effect of source-sink modulation on seasonal berry weight of Cabernet Sauvignon (A) and Sangiovese (B) vines having either three leaves (3L) or twelve leaves per cluster (12L). The solid arrow indicates date of source-sink modulation. Vertical bars indicate standard error (SE) (n=3 for Cabernet Sauvignon, and n=4 for Sangiovese).

5.3.3. Sugar concentration

Hexose (glucose + fructose) concentrations of Cabernet Sauvignon and Sangiovese berries were significantly reduced by source limitation (Figure 2). The negative effects of source limitation were observed one week after treatment for Cabernet Sauvignon and two weeks after treatments for Sangiovese. At harvest, 3L treatment caused a 17.5% reduction of hexose concentration in Cabernet Sauvignon and a 36.7% reduction in Sangiovese compared to 12L treated berries.

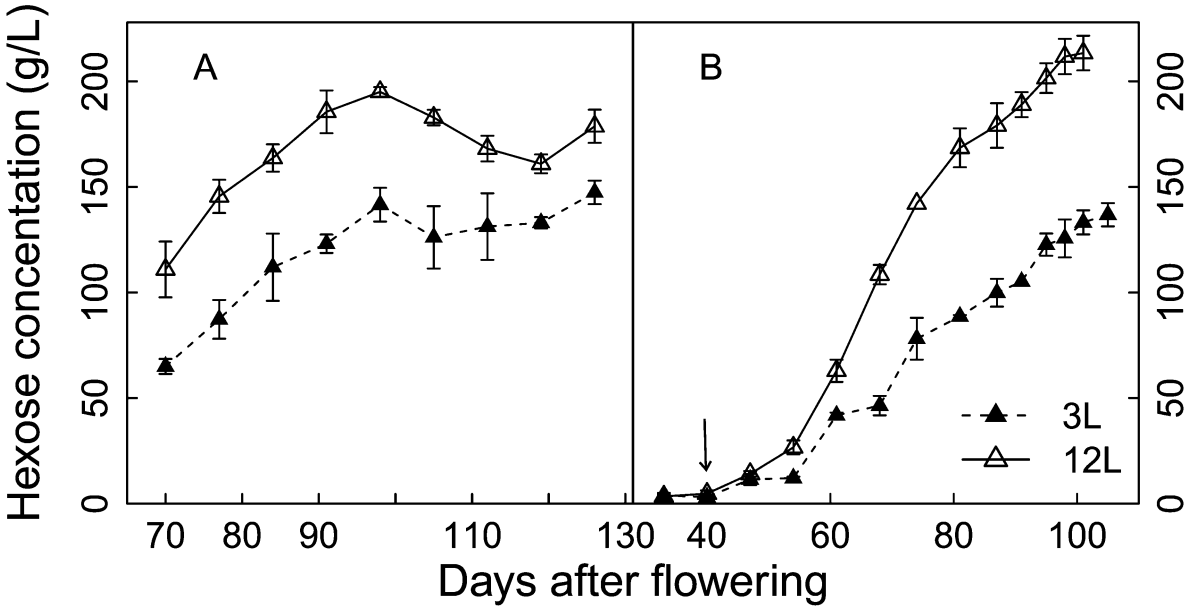


Figure 2. Effect of source-sink modulation on seasonal hexose concentrations of Cabernet Sauvignon (A) and Sangiovese (B) berries sampled from vines having either three leaves (3L) or twelve leaves per cluster (12L). The solid arrow indicates date of source-sink modulation. Vertical bars indicate standard error (SE) (n=3 for Cabernet Sauvignon, and n=4 for Sangiovese).

5.3.4. Organic acids

The developmental profiles of malic and tartaric acids were slightly affected by the source-sink modulation in Cabernet Sauvignon berries (Figure 3A and 3C). From veraison to near harvest, the concentrations of malic and tartaric acids were higher in the 3L than in the 12L treatment, while no significant differences were found at harvest. On the other hand, the developmental profiles of organic acids in Sangiovese berries were not significantly affected by source-sink modulation (Figure 3B and 3D). In addition, the flat

trend of tartaric acids in Cabernet Sauvignon berries is due to the fact that sampling started at a later stage (Figure 3C). At harvest, the concentrations of malic acid were 1.78 and 1.5 g/L and those of tartaric acids were 6.61 and 7.13 g/L for Cabernet Sauvignon and Sangiovese, respectively.

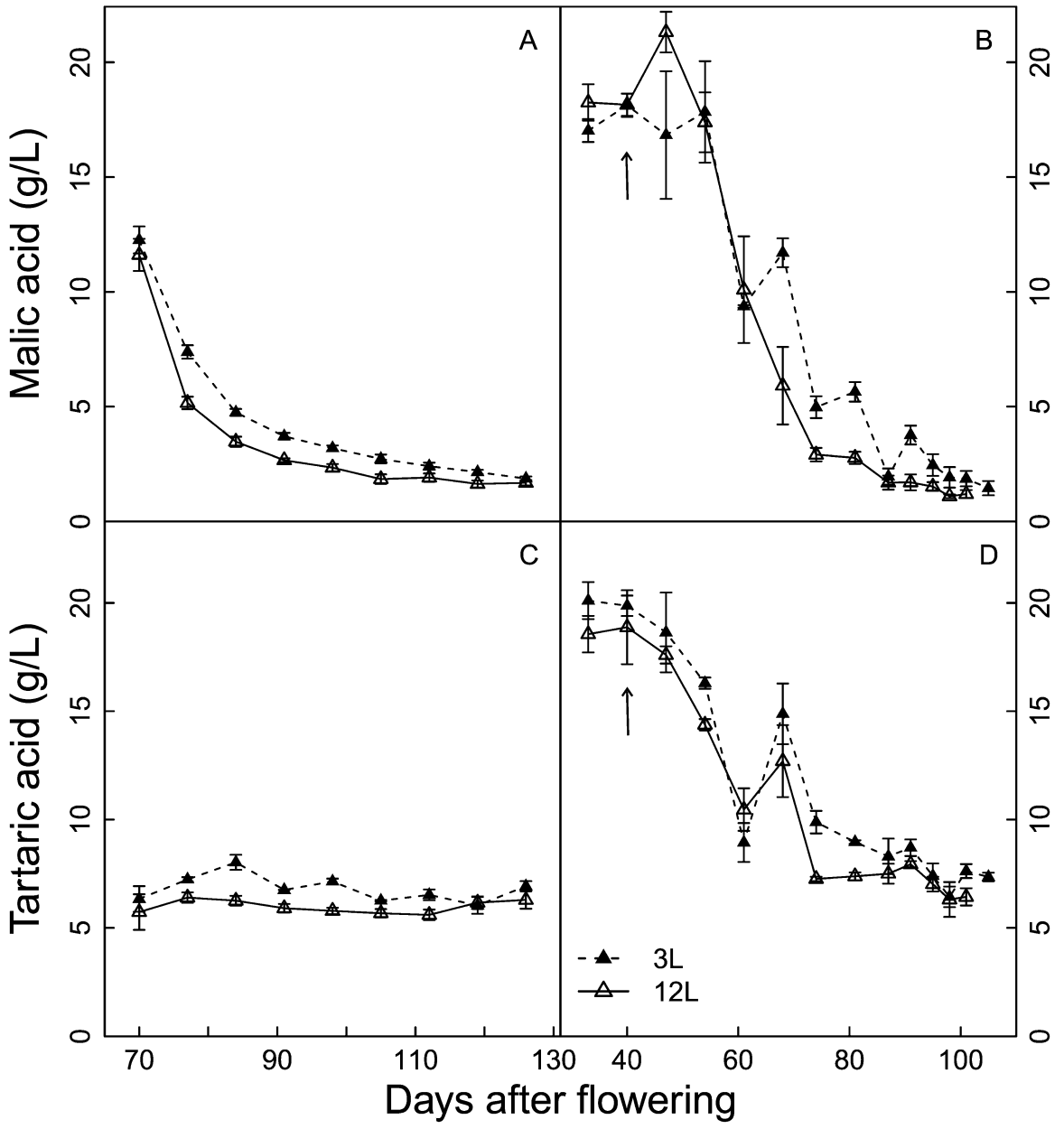


Figure 3. Effect of source-sink modulation on seasonal malic acid (A, B) and tartaric acid (C, D) concentration of Cabernet Sauvignon (A) and Sangiovese (B) berries from vines having either three leaves (3L) or twelve leaves per cluster (12L). The solid arrows indicate date of source-sink modulation. Vertical bars indicate standard error (SE) (n=3 for Cabernet Sauvignon, and n=4 for Sangiovese).

5.3.5. Anthocyanin composition and concentration

Cabernet Sauvignon and Sangiovese berries showed different anthocyanin profiles under adequate source supply (Figure 4), with malvidin-derivatives dominant in the former and cyanidine-3-glucoside dominant in the latter. Moreover, all the acylated forms of anthocyanins were absent in Sangiovese (Figure 4A). These differences in composition were further affected by source limitation (Figure 4B and 4C). At harvest, the proportion of malvidin-derivatives was increased to 93.7% in 3L-treated berries in comparison with 79.7% in 12L-treated berries of Cabernet Sauvignon (Figure 4B). Sangiovese was less affected by source limitation, with 55.6% of cynidin-3-glucoside in 3L-treated berries and 42.4% in 12L-treated berries (Figure 4C).

The developmental profiles of anthocyanin composition and concentration were significantly affected by source limitation in both cultivars (Figures 5 and 6). In Cabernet Sauvignon, the amount of total anthocyanins was systematically higher in 12L treated berries than in 3L treated berries throughout berry development (Figure 5A). 12L treated berries increased their anthocyanins sharply from 70 to 77 DAF, remained at a plateau until 98 DAF, and thereafter decreased progressively. 3L berries exhibited a similar developmental profile but with a much lower plateau than 12L berries. In Sangiovese, 12L berries started to accumulate anthocyanins from 68 DAF and reached a maximum at harvest. By contrast, 3L treatment almost completely depressed the accumulation of total anthocyanins, with only a slight increase between 95 to 105 DAF (Figure 5B). At harvest, 3L treatment caused a 74.8% reduction in the concentration of total anthocyanins in Cabernet Sauvignon (1.32 mg/g FW in 3L versus 5.27 mg/g FW in 12L) and a 94.5% reduction in Sangiovese (0.22 mg/gFW in 3L versus 3.32 mg/gFW in 12L).

Tri-hydroxylated and di-hydroxylated anthocyanins showed different developmental profiles and distinct responses to source limitation (Figure 5C-5H). Cabernet Sauvignon berries had higher tri-hydroxylated anthocyanins than di-hydroxylated ones in both treatments (Figure 5C, 5E, and 5G), while the reverse was observed in Sangiovese (Figure 5D, 5F, and 5H). In Cabernet Sauvignon, 3L treatment decreased more di-hydroxylated (Figure 5C) than tri-hydroxylated anthocyanins (5E),

resulting in a lower ratio of di- to tri-hydroxylated anthocyanins (Figure 5G). By contrast, 3L treatment equally decreased both the di- and tri-hydroxylated anthocyanins in Sangiovese, leaving the ratio of di- to tri-hydroxylated anthocyanins unaffected (Figure 5H).

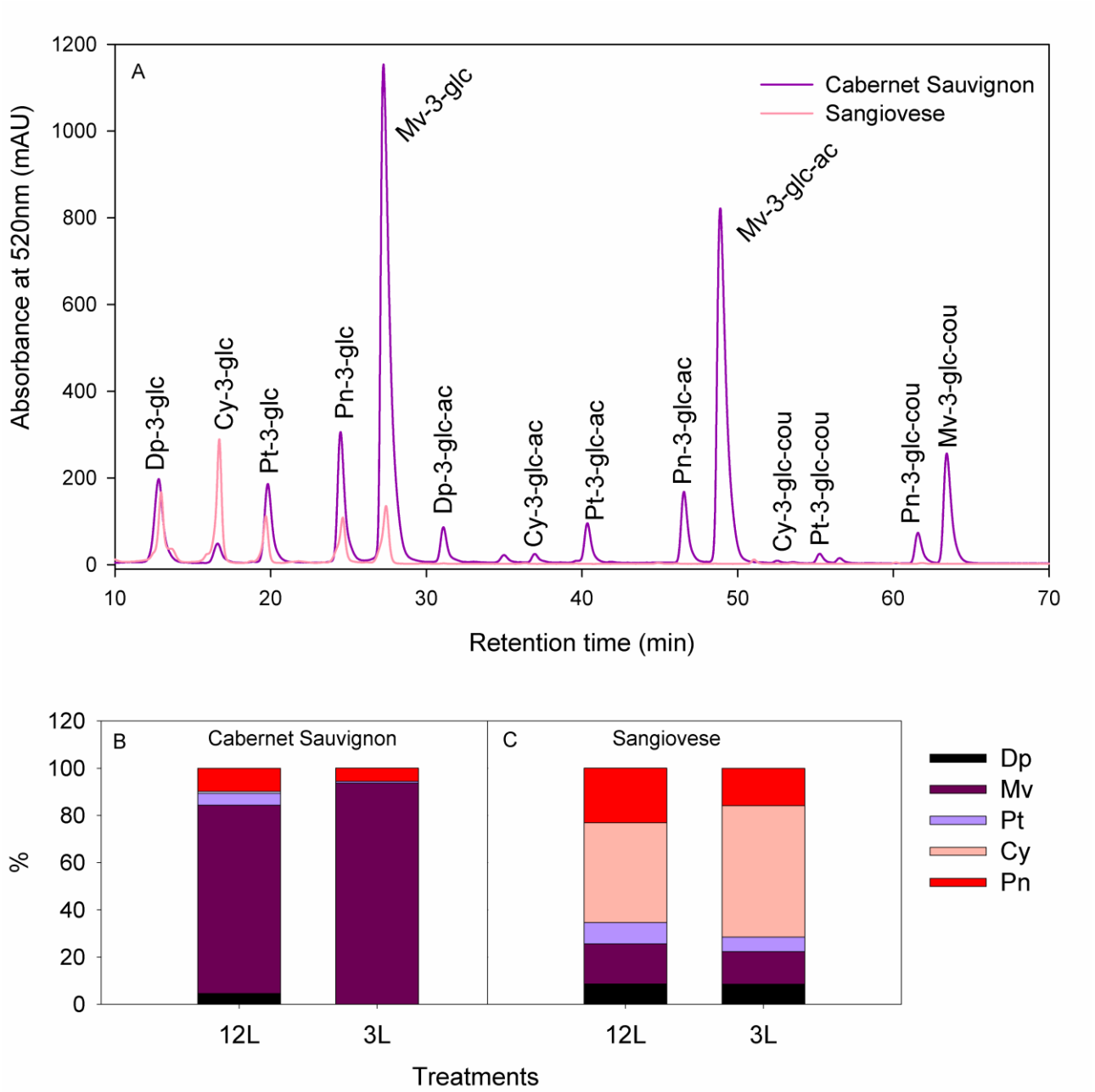


Figure 4. Typical HPLC chromatograms of anthocyanins in berry skins of Cabernet Sauvignon and Sangiovese grape berries grown under non limited carbon supply (A); and effects of source-sink modulation on anthocyanin composition of Cabernet Sauvignon (B) and Sangiovese (C) berry skins at harvest from vines with either three leaves (3L) or twelve leaves per cluster (12L). Abbreviations: Dp = delphinidin, Mv = malvidin, Pt = petunidin, Cy = cyanidine, Pn = peonidin, glc = glucoside, ac = acetyl, cou = coumarate.

Tri-hydroxylated and di-hydroxylated anthocyanins showed different developmental profiles and distinct responses to source limitation (Figure 5C-5H). Cabernet Sauvignon berries had higher tri-hydroxylated anthocyanins than di-hydroxylated ones in both treatments (Figure 5C, 5E, and 5G), while the reverse was observed in Sangiovese (Figure 5D, 5F, and 5H). In Cabernet Sauvignon, 3L treatment decreased more di-hydroxylated (Figure 5C) than tri-hydroxylated anthocyanins (5E), resulting in a lower ratio of di- to tri-hydroxylated anthocyanins (Figure 5G). By contrast, 3L treatment equally decreased both the di- and tri-hydroxylated anthocyanins in Sangiovese, leaving the ratio of di- to tri-hydroxylated anthocyanins unaffected (Figure 5H).

The effect of source-sink modulation on anthocyanin acylation was further investigated in Cabernet Sauvignon berries (Figure 6). 3L treatment decreased more strongly the nonacylated anthocyanins than the acylated ones in comparison with 12L treated berries (Figure 6A and 6B). This unbalanced modification caused a significant increase in the proportion of acylated anthocyanins in 3L treated berries than 12L treated berries (Figure 6D). The proportion of acylated anthocyanins reached 58.4% in 3L and was 46.1% in 12L at harvest (Figure 6D).

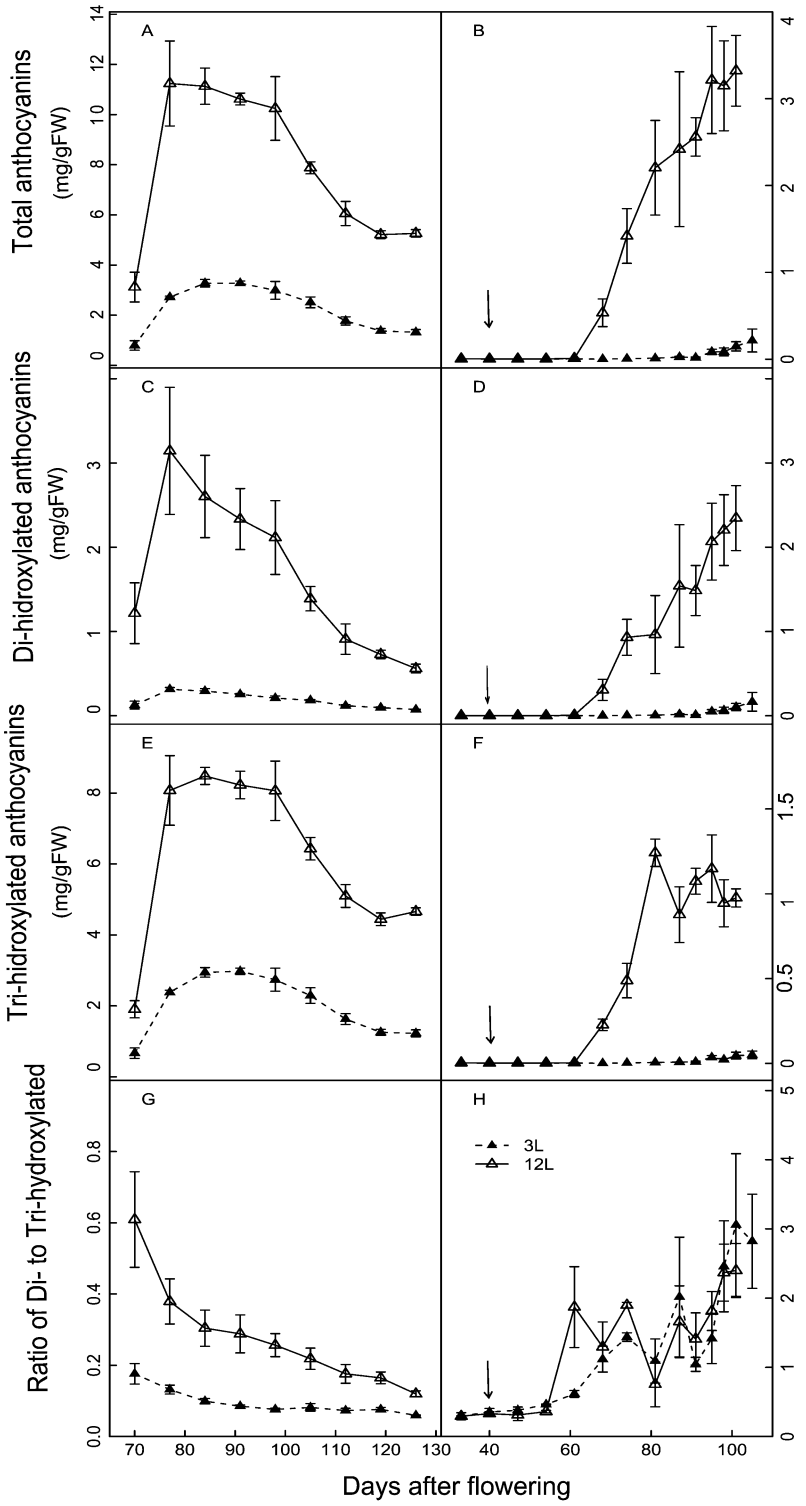


Figure 5. Effect of source-sink modulation on the accumulation of total anthocyanins (A, B), di-hydroxylated anthocyanins (C,D), tri-hydroxylated anthocyanins (E,F), and the ratio of Di- to Tri-hydroxylated anthocyanins (G, H) in the skin of Cabernet Sauvignon (left panel) and Sangiovese berries (right panel). The two carbon supply levels were obtained by treating vines with either three leaves (3L) or twelve leaves per cluster (12L). The solid arrows indicate date of source-sink modulation. Vertical bars indicate standard error (SE) (n=3 for Cabernet Sauvignon, and n=4 for Sangiovese).

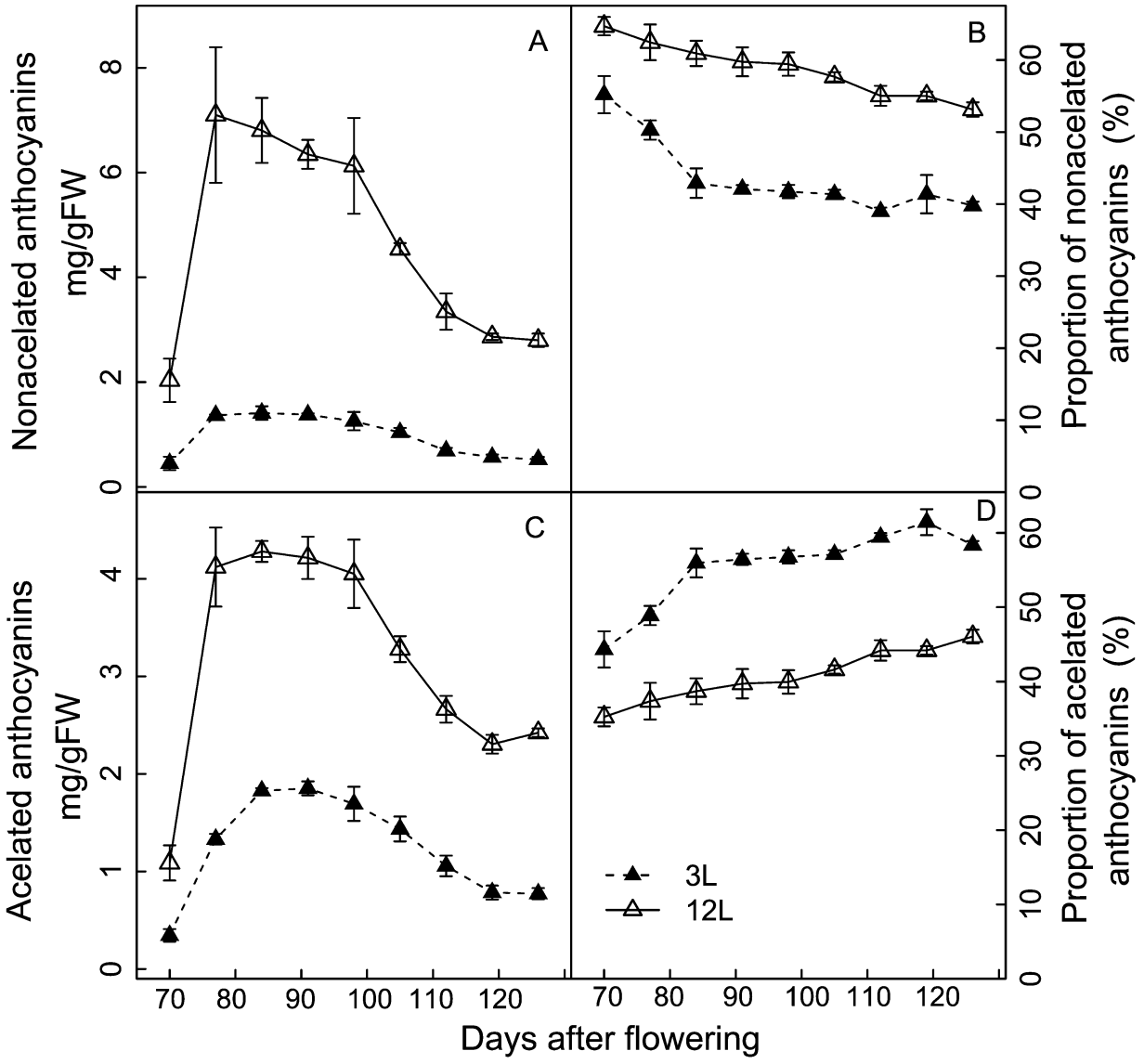


Figure 6. Effect of source-sink modulation on the quantity (A, C) and proportion (B,D) of nonacylated anthocyanins (A,B) and acylated anthocyanins (C,D) in the skin of Cabernet Sauvignon berries from vines with either three leaves (3L) or twelve leaves per cluster (12L). Vertical bars indicate standard error (SE) (n=3).

5.3.6. Leaf carbon fixation and berry carbon utilization

Whole-canopy net CO_2 exchange rates (NCER) were measured in Sangiovese and are shown in Figure 7. The NCER per unit leaf area (μmol m^{-2} s^{-1}) was very similar (≈4.5 μmol m^{-2} s^{-1}) for the two groups of vines before treatment (Figure 7A and Table 2). After treatment, it became slightly higher in 3L (in average 6.30 μmol m^{-2} s^{-1}) than in 12L treatment (in average 5.56 μmol m^{-2} s^{-1}), without reaching a significant difference though (Table 2). However, when those marginal differences in each day were cumulated, the carbon fixed by a unit of leaf area over the experimental period was clearly higher in 3L than 12L treatment (Figure 7C). Single leaf photosynthesis rate at harvest, measured under optimal conditions at saturating light, was significantly higher in 3L treated leaves (15.1 μmol m^{-2} s^{-1}) than in 12L treatment (13.8 μmol m^{-2} s^{-1}). Moreover, for both Cabernet Sauvignon (Table 1) and Sangiovese (Table 3), leaves from 3L plants had higher chlorophyll content than that of 12L plants (Table 3).

Table 3. Effect of source-sink modulation on transpiration (E), stomatal conductance (gs), net photosynthesis (Pn) and chlorophyll content (SPAD) measured on six leaves per vine in Sangiovese. Data are means of four plants**.**

Treatment[‡]	E (mmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	P_n (μmol m^{-2} s^{-1})	SPAD
3L	8.59	0.368	15.1	40.9
12L	8.86	0.345	13.8	38.0
Sig.(t test) [+]	ns	ns	**	*

[‡]3L: plants with three leaves per cluster; 12L: plants with twelve leaves per cluster.
[+] *,**, and ns indicate statistical significance at *P*=0.05, 0.001, and not significant, respectively.

The NCER per vine was calculated as the product of NCER per leaf area and total leaf area per vine (Figure 7B). Before treatment, leaves of both groups had very similar NCER/vine (8.12 umol s^{-1}). During the treatment, 40% and 83.3% leaf area were removed in comparison with pre-treatment for 12L and 3L vines, respectively (Table 2). These reductions in total leaf area per vine resulted in an abrupt proportional decrease of NCER/vine of 38.8% for 3L vines and 82.4% for 12L vines, averaged during the first three days after treatment (Figure 7B). Thereafter, vines reacted to their treatments, and the 3L plants showed a 66.1% reduction in NCER/vine in parallel with a 69.6% reduction in leaf

area per vine, as compared to 12L plants (Figure 7B and Table 2). When the NCER per vine was cumulated over the post-treatment period, the cumulated carbon per vine at harvest in 3L treatment was only reduced by 55.8% compared to 12L (Figure 7D).

To investigate the carbon supply-demand balance, carbon accumulation rate in all the berries of a vine was also calculated and compared with the carbon fixed by photosynthesis (Figure 8). As expected, 3L berries accumulated much lower carbon (43.4 g) than those of 12L treatment (81.9 g) at harvest (Figure 8A). In parallel, the carbon accumulation rate in berries (g carbon per day) was also decreased in 3L berries compared to 12L berries (Figure 8B). Considering the carbon accumulation rate as carbon utilization and the NCER per vine as carbon supply, the proportion of the former to the latter was calculated (Figure 8C). Total accumulated carbon per day in 3L berries accounted for ~76.9% of that fixed by photosynthesis during the rapid sugar accumulation period (namely from 54 to 81 DAF), while it accounted for only ~48% in 12L berries over the same period. Interestingly, this proportion jumped to very high levels, even more than 100%, in several specific days in both treatments. Further analysis revealed that those days corresponded to cloudy days (Supplementary Figure S1) when NCERs per vine were very low (Figure 7B).

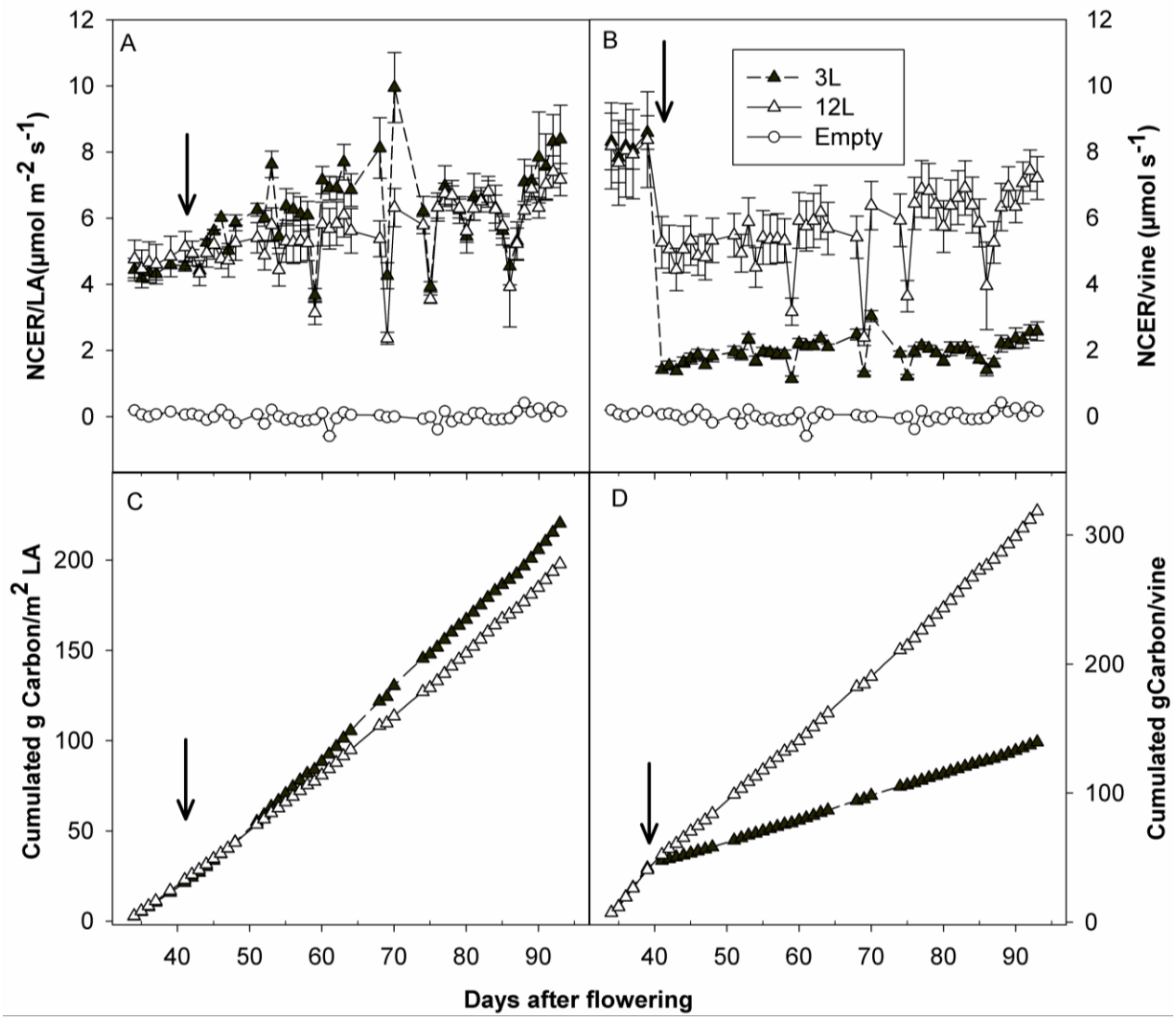


Figure 7. Effect of source-sink modulation on net carbon exchange rate (NCER) per unit of leaf area (A) and per vine (B), as well as cumulated carbon fixation per unit of leaf area (C) and per vine (D), recorded daily with a whole-canopy gas exchange system throughout the experimental period on Sangiovese vines either with three leaves (3L) or twelve leaves per cluster (12L). Values from empty chambers were also indicated (A, B) as a reference. The solid arrows indicate date of source-sink modulation. Vertical bars indicate standard error (SE) (n=4).

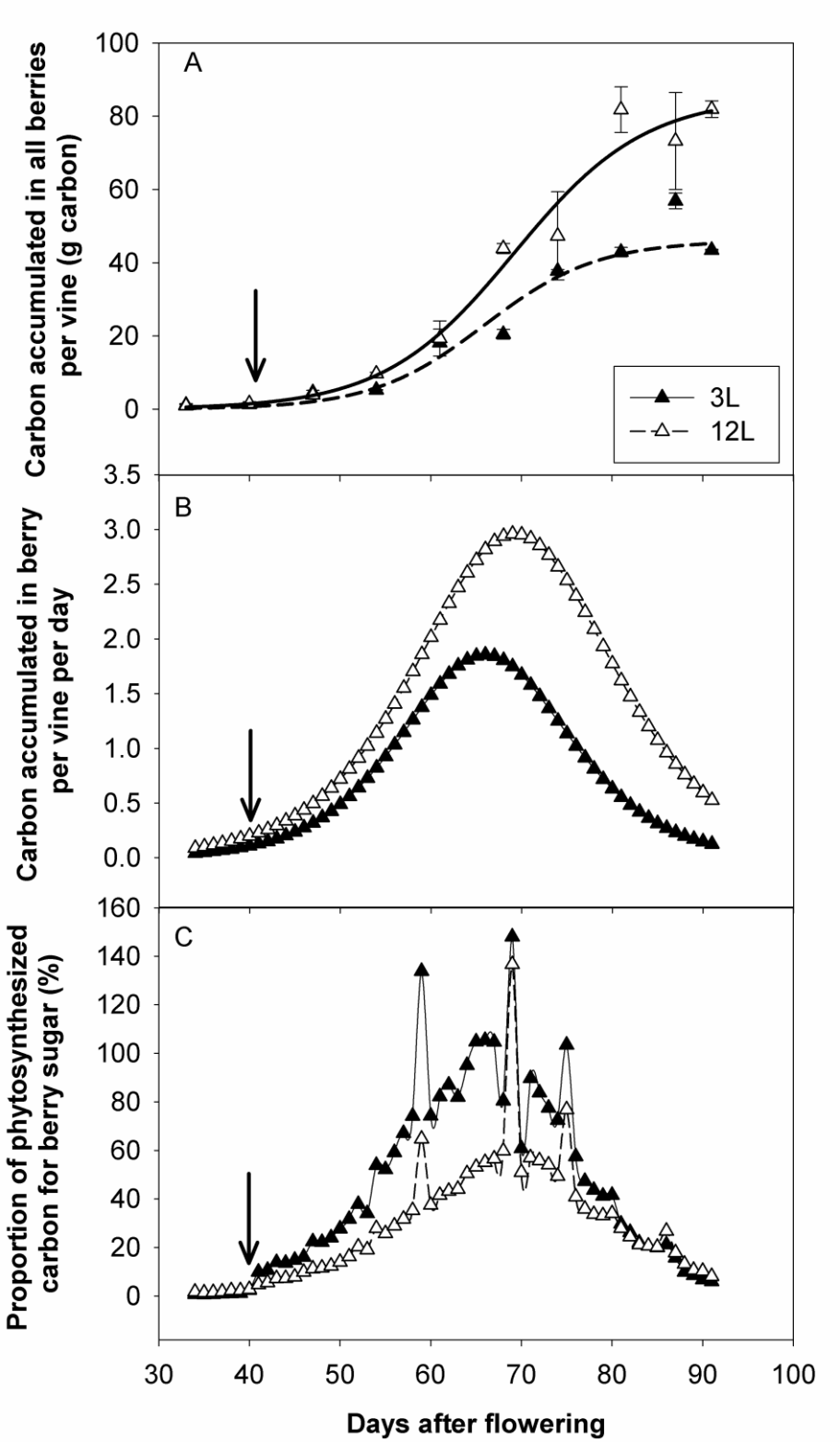


Figure 8. Effect of source-sink modulation on carbon allocation in Sangiovese grown with three leaves (3L) or twelve leaves (12L) per cluster. Carbon accumulated in all berries per vine(A), carbon accumulation rate in all berries per vine per day (B), and proportion of photosynthesized carbon used for berry sugar accumulation during the developmental period (C).The solid arrows indicate date of source-sink modulation. (will be updated soon by including the 1 in 12L).

5.4. Discussion

Source limitation induced by severe leaf removal in Cabernet Sauvignon and Sangiovese caused a significant reduction in the accumulation of sugars in berries. This result confirms many previous studies with different other *Vitis vinifera* L. cultivars, that reported a decrease in sugar accumulation following carbon limitation induced by late leaf removal (manual or mechanical), shoot trimming at fruit set and veraison (Poni and Giachino, 2000; Heuvel et al., 2005; Stoll et al., 2010; Poni et al., 2013; Palliotti et al., 2013b; Filippetti et al., 2015; Parker et al., 2015). However, there are also studies conversly showing that sugar accumulation is unaffected (Percival et al., 1994; Chorti et al., 2010; Sabbatini and Stanley Howell, 2010; Pastore et al., 2013) or even slightly increased (Bledsoe et al., 1988; Percival et al., 1994; Guidoni et al., 2002; Poni et al., 2006a, 2008; Palliotti et al., 2011; Pastore et al., 2011; Bubola and Persuric, 2012; Gatti et al., 2012; Palliotti et al., 2012; Pastore et al., 2013) after a diminishing LA/Fruit ratio. These contradictory observations are most likely the result of the differences in the timing and severity of source to sink modulations. In fact, Kliewer and Dokoozlian (2005) have shown that a LA/Fruit above 0.8 m^2/Kg is critical for full ripening of the grapes. Studies reporting no effect of source-sink modulations on sugar accumulation often did not go below this threshold value. In the present study, the LA/Fruit was lower than 0.8 m^2/Kg in source limited vines for both cultivars, and accounting of the fact that a significant effect was observed. It is therefore of the utmost importance to consider the magnitude of LA/Fruit when compare trials on the effect of source-sink modulations on berry sugars.

In contrast to the decrease in sugar concentration under source limitation, no significant differences were found in organic acids content at harvest in the two cultivars studied in this work. In a recent detailed developmental analysis, Parker et al. (2015) also observed lost of synchronization between sugar and organic acid in response to lowering LA/Fruit ratios, with sugar accumulation reduced but organic acids largely unaffected. Some other studies (Bledsoe et al., 1988; Poni et al., 2006a, 2009; Tardaguila et al., 2010; Pastore et al., 2013) reported that a decrease in source-sink ratio by leaf removal lead to decreased total acidity and malic acid, whereas tartaric acids was unaffected or even increased compared to vines without leaf removal. The authors of these studies frequently

pointed out a likely confounding effect of leaf removal and modified microclimate, making their result not really comparable with those found in the present study. Our results and those from Parker et al. (2015) both confirm that the organic acids are less responsive to carbon limitation than sugars.

In addition to the relative sensitivity of sugars and organic acids, we also studied the response of important secondary metabolites such as anthocyanins. Source limitation caused 75% and 93.5% reductions in anthocyanin concentrations in Cabernet Sauvignon and Sangiovese berries at harvest, paralleled by only 17.5% and 36.7% reductions in sugars, as compared to the non source limited berries. After recalculation from the dataset of recent reports, we found that source limitation caused a 99.2% reduction in anthocyanins with a 38.5% reduction in sugars in cv. Jingyan (*Vitis vinifera*) (Wu et al., 2013), and a 17.5% or 19.1% reduction in anthocyanins with a 8.4% or 6.8% reduction in sugars in cv. Sangiovese (Pastore et al., 2011, 2013). These results clearly indicate that the accumulation of sugars and anthocyanins are uncoupled under source limitation, and that carbon is preferentially allocated for sugar accumulation rather than anthocyanins. Several theories have been developed in literature to describe the relationship between primary and secondary metabolites in plants, and the two most relevant ones are the carbon-nutrient balance (CNB) hypothesis and the growth-differentiation balance (GDB) hypothesis (reviewed in Koricheva et al., 1998). CNB predicts that concentrations of Cabon-based secondary metabolites will decrease in cases where carbon fixation is reduced more than growth as a result of decreased available carbon pool for allocation to secondary metabolites. GDB provides a similar prediction, and a meta-analysis showed that both hypothesizes are valid for describing the dependence of total C-based secondary metabolites, particularly phenylpropanoid-derived compounds (including anthocyanins), on carbon availability in the leaves of woody plant (Koricheva et al., 1998). Arnold et al. (2004) confirmed, with a series of elegant experiments, that the phenolic content and coloration of poplar (Populus nigra x P. deltoides) sink leaves is reduced by disrupted carbon flow from source to sink, namely reduced carbon availability. In cell suspensions of cv. Gamay Freaux that constructively produce anthocyanins, Guardiola et al. (1995) proved with a mathematical modeling approach that primary (sugars) and secondary

(anthocyanins) metabolisms compete for carbon substrate when substrate is scarce. Our results provide a piece of evidence to the validity of CNB and GDB hypothesizes in a productive sink (berries) and pave the way for modeling the sugars and anthocyanins accumulations in grape berry under various source-sink ratios. Interestingly, (Sadras and Moran, 2012) also reported the uncoupling between sugars and anthocyanins accumulation under high temperatures conditions. However, the biological mechanisms underlying this uncoupling effect should be different from those under carbon limitation, because temperature has direct effect on anthocyanin biosynthesis and degradation by modulating gene expression and enzyme activities of related enzymes (Mori et al., 2007). Few studies have been conducted to understand the inhibitory effect of source limitation on anthocyanin accumulation at protein and/or transcription levels. Two recent genome-wide transcriptome analyses showed that carbon limitation reduced the transcript abundance of UDP glucose:flavonoid-3-O-glucosyltransferase (UFGT) and GST4, which are known as important regulators of anthocyanin accumulation and transport (Pastore et al., 2011, 2013). A proteomic analysis showed that the abundances of chalcone synthase and dihydroflavonol reductase, which are both involved in anthocyanin pathway, were strongly reduced by source limitation (Wu et al., 2013). Our observed reduction in total anthocyanin in both cultivars under source limitation should also result from modifications in the key regulators of anthocyanin pathways, although further transcriptomic and proteomic experiments are needed to confirm these speculations.

Since it is known that different molecules of anthocyanins have different color hues and stabilities (He et al., 2010), we also studied the alternation of anthocyanin composition in response to carbon limitation. The concentration and composition of anthocyanins are different between Cabernet Sauvignon and Sangiovese berries under normal (carbon sufficient) condition; Cabernet Sauvignon berries had higher concentration of malvidin (tri-hydroxylated) derivatives and acylated anthocyanins, whereas Sangiovese berries were richer in cyanidin-3-glucoside (di-hyroxylated), and no acylated anthocyanins were found. These results are in agreement with a previous report (Mattivi et al., 2006). Carbon limitation increased the proportion of cyanidin-3-glucoside in Sangiovese. In the same cultivar, other authors (Filippetti et al., 2007; Pastore et al., 2011, 2013) also found that a

decrease in source-sink ratio increased the proportion of cyanidin-3-glucoside. On the contrary, the proportion of the predominantly accumulated anthocyanin peonidin-3-glucoside (di-hydroxylated) in cv. Nebbiolo was decreased by low source-sink ratio. In Cabernet Sauvignon, we found the proportion of di-hydroxylated anthocyanins (cyanidin and peonidin derivatives) was reduced by carbon limitation. These results indicate that the modification in anthocyanin composition in response to source limitation is cultivar dependent. It is known that the ratio between di- and tri-hydroxylated anthocyanins is under the control of the relative activity of F3'H and F3'5'H (Castellarin et al., 2006). Transcriptome analysis showed that carbon limitation increased the transcript abundance of F3'Hb, which is responsible for the biosynthesis of di-hydroxylated, and explained the observed modification in anthocyanin composition (Pastore et al., 2013). In Cabernet Sauvignon, we also observed that source limitation significantly increased the proportion of acylated anthocyanins in compared to non source limitation condition. The molecular regulation of anthocyanin acylation is largely unknown, although acylation can improve anthocyanin stability (He et al., 2010). Further efforts are warranted to investigate why the activities or expression of F3'H and F3'5'H respond to source limitation differentially between cultivars and why acylated anthocyanins are preferably accumulated under source limitation.

Source and sink can communicate interactively and exert mutual influences on each other. When the source-to-sink ratio is reduced, the leaves (source) on the grapevine can increase leaf efficiency toward a compensation of their photosynthetic rate to meet the demand of berries (sink) (Candolfi-Vasconcelos and Koblet, 1990; Petrie et al., 2003; Kliewer and Dokoozlian, 2005). We observed that the source limited vines increased their NCER per unit of leaf area compared to source sufficient vines. This was further confirmed by a higher content of chlorophyll (7% more in Sangiovese vines and 13.5% more in Cabernet Sauvignon vines), and a higher photosynthesis capacity measured on single leaf under optimal conditions. Similar effects of source limitation on leaf chlorophyll content have been observed by other authors (Candolfi-Vasconcelos and Koblet, 1990; Petrie et al., 2000b). Such photosynthetic compensation can explain why the source limited vines lost 69.6% of their leaf area although carbon fixation was reduced

only 55.8% over berry ripening. However, it is clear that the compensation is partial, and this may be due to the fact that only the main leaves from the primary shoot were retained with all new growth being removed during our experiment. Mature leaves are less responsive to source-sink modulation (Candolfi-Vasconcelos et al., 1994b).

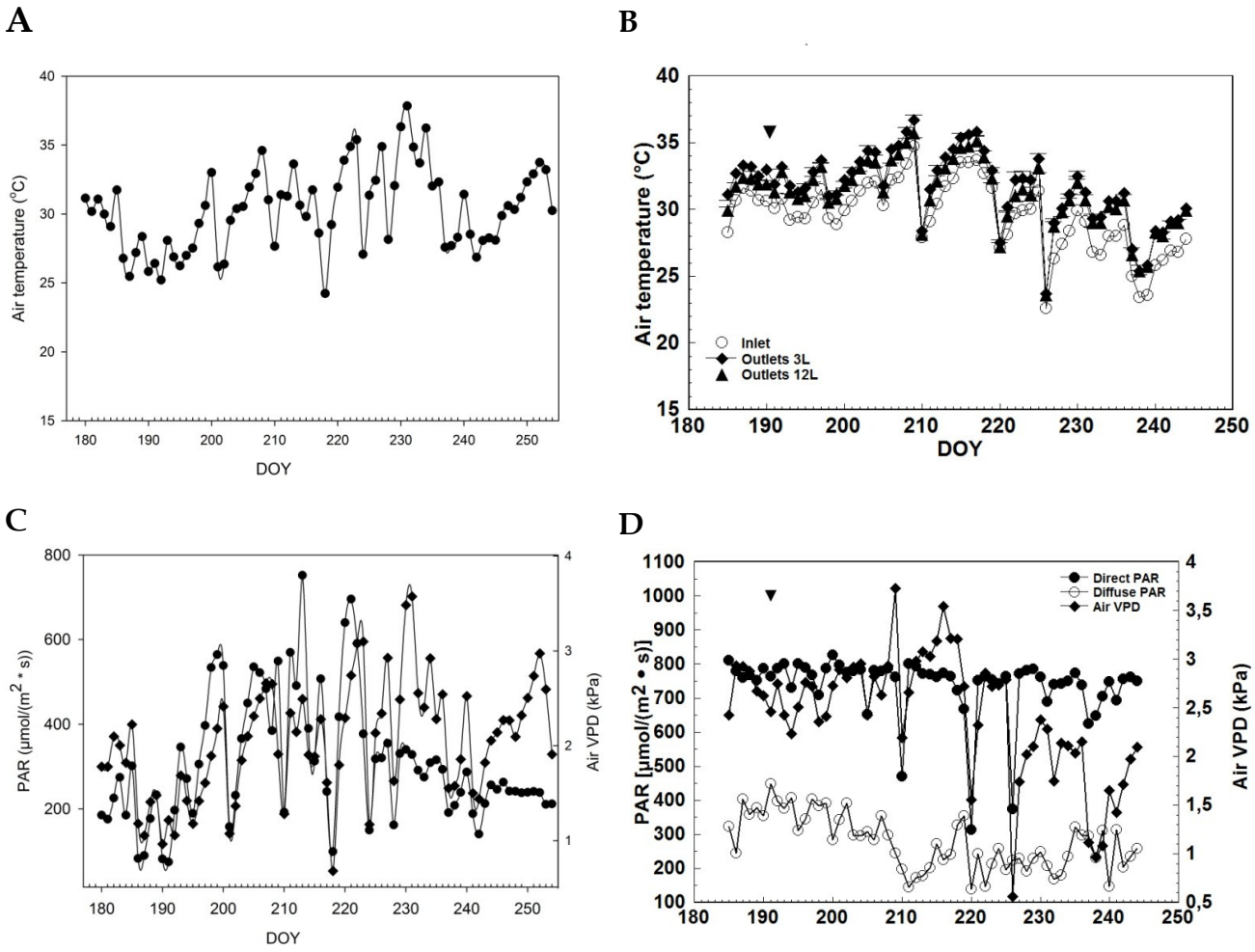
Comparing carbon fixation by leaves and carbon utilization by berries can provide a valuable estimation of the carbon balance between demand and supply. This information is essential to understand the physiology of vines and may help to develop mechanistic models (Cola et al., 2014; Poni et al., 2006b). However, this comparison is often missing in the source-sink modulation experiments due to the lack of suitable facilities to measure it. The whole-canopy gas exchange approach (Poni et al., 2014) makes it possible to monitor the seasonal NCER and to quantify the carbon fixed following the source-sink modulation. In addition, we followed the dynamics of sugar accumulation of berries and calculated the quantity of carbon used in the berries via a mathematic sugar accumulation function (Sadras et al., 2008). This provided a good estimate of carbon utilization in the most important sink (berries) (Gutierrez et al., 1985; Coombe, 1989). The mathematical analysis of carbon-balance indicated that berry carbon utilization accounted for a higher proportion of fixed carbon for sugar accumulation under carbon limitation (73.4%) than under carbon adequacy (40.7%) during the sugar accumulation stages (54 to 81 DAF). This indicates that carbon allocation is not proportional to the carbon offer but with priorities to berries under source limitation, providing direct evidence to support the most applied assumption in grapevine carbon allocation models (Gutierrez et al., 1985; Bindi et al., 1996; Poni et al., 2006a; Pallas et al., 2008). The biological mechanisms behind this phenomenon have been poorly investigated. Pastore et al. (2011) showed that the transcript abundance of pyruvate decarboxylase isozyme 2 involved in glycolysis was reduced by a low source-sink ratio. However, we found that the carbon limitation increased enzyme activities involved in primary carbohydrate metabolisms (Dai et al. unpublished data). These increases in metabolic enzymes may confer higher sink strength and therefore allow berries attracting a higher proportion of carbon under source limitation. It is worth to note that our calculation do not consider the carbon utilization for maintenance and reserves, nor the potential contribution of reserve remobilization for carbon offer (Gutierrez et al.,

1985), but rather provides a quantitative indicator of carbon allocation. Therefore, the proportions close to 100% observed between 64 to 67 DAF in 3L vines are hardly realistic in real vines; instead they strongly indicate that carbon reserves are remobilized for berry sugar accumulation and/or vine maintenance. The same explanations go to the points where a proportion more than 100% was observed when it was cloudy and the vine photosynthesis rate was extremely low. Although we did not quantify the reserve remobilization, Weaver (1963) reported that reserves (both soluble sugars and starch) in shoots were significantly reduced by source limitation in cv. Carignane and Zinfandel vines. Kliewer and Antcliff (1970) estimated that as much as 40% of the total sugars in berries may come from storage tissues of the vine. Using 14C-labeling, (Candolfi-Vasconcelos et al., 1994a) showed that carbon reserves from the woody storage tissues can be actively reallocated into berries under source limitations. An experimental coupling 14C-labeling, whole-canopy net carbon exchange rate measurement, and carbon content assessment in various tissues (leaf, shoot, wood, fruit, and root) would provide more valuable dataset to quantify the carbon balance and allocation.

5.5. Conclusions

Source limitation induced by leaf removal one week before veraison significantly reduced the concentration of sugars and anthocyanins but did not alter the concentration of organic acids in Cabernet Sauvignon and Sangiovese. Moreover, the magnitude of reduction was much higher in anthocyanins than sugars in response to source limitation, attesting a decoupling between sugars and anthocyanins in both cultivars. Although the patterns of responses of sugars, organic acids, and total anthocyanins to source limitation are rather consistent between cultivars, the modification of anthocyanin compositions is cultivar dependent. Therefore grape berry can manage the metabolic fate of carbon in such a way that sugar accumulation is maximally maintained at the expense of secondary metabolites (e.g. anthocyanins) under source limitation.

5.6. Supplementary data



Supplementary Figure 1. Seasonal trends of (A) air temperature and (B) air vapour pressure deficit (VPD) and photosynthetically active radiation (PAR) measured in a semi-control greenhouse where Cabernet Sauvignon fruit cuttings were grown; (C) inlet and outlet chamber air temperature, (D) air vapour pressure deficit (VPD), direct and diffuse photosynthetically active radiation (PAR) measured in the whole-canopy gas exchange system where Sangiovese grapevines were grown. In (C) and (D), the solid triangle represent date of treatment.

Supplementary Figure 2. The two different leaf removal treatments: three leaves per cluster and twelve leaves per cluster. All the plants are in an custom-built flow-through multichamber system.

A **B**

Supplementary Figure 3. (A) Grape cluster from a plant with twelve leaves per cluster at harvest; (B) grape cluster from a plant with three leaves per cluster at harvest.

5.7. References

Acevedo De la Cruz, A., Hilbert, G., Rivière, C., Mengin, V., Ollat, N., Bordenave, L., Decroocq, S., Delaunay, J. C., Delrot, S., Mérillon, J. M., et al. (2012). Anthocyanin identification and composition of wild Vitis spp. accessions by using LC-MS and LC-NMR. Anal. Chim. Acta 732, 145–152. doi:10.1016/j.aca.2011.11.060.

Arnold, T., Appel, H., Patel, V., Stocum, E., Kavalier, A., and Schultz, J. (2004). Carbohydrate translocation determines the phenolic content of Populus foliage: A test of the sink-source model of plant defense. New Phytol. 164, 157–164. doi:10.1111/j.1469-8137.2004.01157.x.

Barnuud, N. N., Zerihun, A., Gibberd, M., and Bates, B. (2014). Berry composition and climate: Responses and empirical models. Int. J. Biometeorol. 58, 1207–1223. doi:10.1007/s00484-013-0715-2.

Barnuud, N. N., Zerihun, A., Mpelasoka, F., Gibberd, M., and Bates, B. (2013). Responses of grape berry anthocyanin and titratable acidity to the projected climate change across the Western Australian wine regions. Int. J. Biometeorol. 58, 1279–1293. doi:10.1007/s00484-013-0724-1.

Bindi, M., Fibbi, L., Gozzini, B., Orlandini, S., and Miglietta, F. (1996). Modelling the impact of future climate scenarios on yield and yield variability on grapevine. Clim. Res. 7, 213–224.

Bledsoe, A. M., Kliewer, W. M., and Marois, J. J. (1988). Effects of iming and severity of leaf removal on yield and fruit composition of sauvignon blanc grapevines. Am. J. Enol. Vitic. 39, 49–54.

Bock, A., Sparks, T. H., Estrella, N., and Menzel, A. (2013). Climate-Induced changes in grapevine yield and must sugar content in Franconia (Germany) between 1805 and 2010. PLoS One 8, 1–10. doi:10.1371/journal.pone.0069015.

Bravdo, B., Hepner, Y., Loinger, C., Cohen, S., and Tabacman, H. (1985). Effect of crop level and crop load on growth, yield, must and wine composition, and quality of Cabernet Sauvignon. Am. J. Enol. Vitic. 36, 125–131.

Bubola, M., and Persuric, D. (2012). Yield components, vegetative growth and fruit composition of "Istrian Malvasia" (*Vitis vinifera* L.) as affected by the timing of partial defoliation. Agric. Conspec. Sci. 77, 21–26.

Candolfi-Vasconcelos, M. C., Candolfi, M., and Kohlet, W. (1994a). Retranslocation of carbon reserves from the woody storage tissues into the fruit as a response to

defoliation stress during the ripening period in *Vitis vinifera* L. Planta 192. doi:10.1007/BF00203595.

Candolfi-Vasconcelos, M. C., and Koblet, W. (1990). Yield, fruit quality, bud fertility and starch reserves of the wood as a function of leaf removal in *Vitis vinifera*-Evidence of compensation and stress recovering. Vitis 29, 199–221.

Candolfi-Vasconcelos, M. C., Koblet, W., Howell, G. S., and Zweifel, W. (1994b). Influence of defoliation, rootstock, training system, and leaf position on gas exchange of Pinot noir grapevines. Am. J. Enol. Vitic. 45, 173–180.

Castelan-Estrada, M., Vivin, P., and Gaudillière, J. P. (2002). Allometric relationships to estimate seasonal above-ground vegetative and reproductive biomass of *Vitis vinifera* L. Ann. Bot. 89, 401–408. doi:10.1093/aob/mcf059.

Castellarin, S. D., Di Gaspero, G., Marconi, R., Nonis, A., Peterlunger, E., Paillard, S., Adam-Blondon, A.-F., and Testolin, R. (2006). Colour variation in red grapevines (*Vitis vinifera* L.): genomic organisation, expression of flavonoid 3′-hydroxylase, flavonoid 3',5'-hydroxylase genes and related metabolite profiling of red cyanidin-/blue delphinidin-based anthocyanins in berry skin. BMC Genomics 7, 12. doi:10.1186/1471-2164-7-12.

Chorti, E., Guidoni, S., and Ferrandino, A. (2010). Effect of different cluster sunlight exposure levels on ripening and anthocyanin accumulation in Nebbiolo grapes. Am. J. Enol. Vitic. 61, 23–30.

Cola, G., Mariani, L., Salinari, F., Civardi, S., Bernizzoni, F., Gatti, M., and Poni, S. (2014). Description and testing of a weather-based model for predicting phenology, canopy development and source-sink balance in *Vitis vinifera* L. cv. Barbera. Agric. For. Meteorol. 184, 117–136. doi:10.1016/j.agrformet.2013.09.008.

Conde, C., Silva, P., Fontes, N., Dias, A. C. P., Tavares, R. M., Sousa, M. J., Agasse, A., Delrot, S., and Gerós, H. (2007). Biochemical changes throughout grape berry development and fruit and wine quality. Food 1, 1–22.

Coombe, B. G. (1989). The grape berry as a sink. Acta Hortic. 29, 149–158.

Dai, Z. W., Meddar, M., Renaud, C., Merlin, I., Hilbert, G., Delrot, S., and Gomès, E. (2014). Long-term in vitro culture of grape berries and its application to assess the effects of sugar supply on anthocyanin accumulation. J. Exp. Bot. 65, 4665–4677. doi:10.1093/jxb/ert489.

Dai, Z. W., Ollat, N., Gomes, E., Decroocq, S., Tandonnet, J. P., Bordenave, L., Pieri, P., Hilbert, G., Kappel, C., van Leeuwen, C., et al. (2011). Ecophysiological, genetic and molecular causes of variation in grape berry weight and composition. Am. J. Enol. Vitic. 62, 413–427.

Duchêne, E., Dumas, V., Jaegli, N., and Merdinoglu, D. (2012). Deciphering the ability of different grapevine genotypes to accumulate sugar in berries. Aust. J. Grape Wine Res. 18, 319–328. doi:10.1111/j.1755-0238.2012.00194.x.

Duchêne, E., and Schneider, C. (2005). Grapevine and climatic changes: a glance at the situation in Alsace. Agron. Sustain. Dev. 25, 93–99. doi:10.1051/agro:2004057.

Filippetti, I., Movahed, N., Allegro, G., Valentini, G., Pastore, C., Colucci, E., and Intrieri, C. (2015). Effect of post-veraison source limitation on the accumulation of sugar, anthocyanins and seed tannins in *Vitis vinifera* cv. Sangiovese berries. Aust. J. Grape Wine Res. 21, 90–100. doi:10.1111/ajgw.12115.

Filippetti, I., Ramazzotti, S., Centinari, M., Bucchetti, B., and Intrieri, C. (2007). Effects of cluster thinning on grape composition: Preliminary experiences on "Sangiovese" grapevines. in Acta Horticulturae, 227–234.

Gatti, M., Bernizzoni, F., Civardi, S., and Poni, S. (2012). Effects of cluster thinning and preflowering leaf removal on growth and grape composition in cv. Sangiovese. Am. J. Enol. Vitic. 63, 325–332. doi:10.5344/ajev.2012.11118.

Gomez, L., Bancel, D., Rubio, E., and Vercambre, G. (2007). The microplate reader : an efficient tool for the separate enzymatic analysis of sugars in plant tissues – validation of a micro-method. J. Sci. Food Agric. 87, 1893–1905. doi:10.1002/jsfa.

Guardiola, J., Iborra, J. L., and Canovas, M. (1995). A model that links growth and secondary metabolite production in plant cell suspension cultures. Biotechnol. Bioeng. 46, 291–297.

Guidoni, S., Allara, P., and Schubert, A. (2002). Effect of cluster thinning on berry skin anthocyanin composition of *Vitis vinifera* cv. Nebbiolo. Am. J. Enol. Vitic. 53, 224–226.

Guidoni, S., Ferrandino, A., and Novello, V. (2008). Effects of seasonal and agronomical practices on skin anthocyanin profile of Nebbiolo grapes. Am. J. Enol. Vitic. 59, 22–29.

Gutierrez, A. P., Williams, D. W., and Kido, H. (1985). A model of grape growth and development: the mathematical structure and biological considerations. Crop Sci. 25, 721–728.

Hannah, L., Roehrdanz, P. R., Ikegami, M., Shepard, a. V., Shaw, M. R., Tabor, G., Zhi, L., Marquet, P. a., and Hijmans, R. J. (2013). Climate change, wine, and conservation. Proc. Natl. Acad. Sci. 110, 6907–6912. doi:10.1073/pnas.1210127110.

He, F., Mu, L., Yan, G.-L., Liang, N.-N., Pan, Q.-H., Wang, J., Reeves, M. J., and Duan, C.-Q. (2010). Biosynthesis of anthocyanins and their regulation in colored grapes. Molecules 15, 9057–91. doi:10.3390/molecules15129057.

Heuvel, J. E. Vanden, Robidoux, J. L., Autio, W. R., and Neto, C. C. (2005). Timing of partial defoliation affects carbohydrate concentration of vegetative tissues and concentration of phenolics in berries of potted De Chaunac (Vitis sp .) grapevines. Can. J. Plant Sci.

Hilbert, G., Soyer, J. P., Molot, C., Giraudon, J., Milin, S., and Gaudillere, J. P. (2003). Effects of nitrogen supply on must quality and anthocyanin accumulation in berries of cv . Merlot. Vitis 42, 69–76.

Jackson, D. I., and Lombard, P. B. (1993). Environmental and management practices affecting grape composition and wine quality - A Review. Am. J. Enol. Vitic. 44, 409–430.

Keller, M. (2010a). Managing grapevines to optimise fruit development in a challenging environment: a climate change primer for viticulturists. Aust. J. Grape Wine Res. 16, 56–69. doi:10.1111/j.1755-0238.2009.00077.x.

Keller, M. (2010b). The Science of Grapevines: Anatomy and Physiology. , ed. Elsevier Burlington, MA: Academic Press doi:10.1016/B978-0-12-374881-2.00012-X.

Kliewer, W. M., and Antcliff, A. J. (1970). Influence of defoliation, leaf darkening, and cluster shading on the growth and composition of Sultana grapes. Am. J. Enol. Vitic. 21, 26–36.

Kliewer, W. M., and Dokoozlian, N. K. (2005). Leaf area / crop weight ratios of grapevines : Influence on fruit composition and wine quality. Am. J. Enol. Vitic. 56, 170–181.

Kliewer, W. M., and Weaver, R. J. (1971). Effect of crop level and leaf area on growth, composition, and coloration of "Tokay" grapes. Am. J. Enol. Vitic. 22, 172–177.

Koricheva, J., Larsson, S., Haukioja, E., and Keinanen, M. (1998). Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. OIKOS 83, 212–226.

Kuhn, N., Guan, L., Dai, Z. W., Wu, B.-H., Lauvergeat, V., Gomès, E., Li, S.-H., Godoy, F., Arce-Johnson, P., and Delrot, S. (2014). Berry ripening: recently heard through the grapevine. J. Exp. Bot. 65, 1–17. doi:10.1093/jxb/ert395.

Van Leeuwen, C., Schultz, H. R., Garcia de Cortazar-Atauri, I., Duchêne, E., Ollat, N., Pieri, P., Bois, B., Goutouly, J.-P., Quénol, H., Touzard, J.-M., et al. (2013). Why climate change will not dramatically decrease viticultural suitability in main wine-producing areas by 2050. Proc. Natl. Acad. Sci. U. S. A. 110, 3051–3052. doi:10.1073/pnas.1307927110.

Mattivi, F., Guzzon, R., Vrhovsek, U., Stefanini, M., and Velasco, R. (2006). Metabolite profiling of grape: flavonols and anthocyanins. J. Agric. Food Chem. 54, 7692–7702.

Mazza, G. (1995). Anthocyanins in grapes and grape products. Crit. Rev. Food Sci. Nutr. 35, 341–371. doi:10.1097/NT.0b013e31823db374.

Mori, K., Goto-Yamamoto, N., Kitayama, M., and Hashizume, K. (2007). Loss of anthocyanins in red-wine grape under high temperature. J. Exp. Bot. 58, 1935–45. doi:10.1093/jxb/erm055.

Mullins, M. G., and Rajasekaran, K. (1981). Fruiting cuttings: Revised method for producing test plants of grapevine cultivars. Am. J. Enol. Vitic. 32, 35–40.

OIV (2014). State of the Vitiviniculture World Market. in, 14.

Ollat, N., and Gaudillere, J. P. (1998). The effect of limiting leaf area during stage I of berry growth on development and composition of berries of *Vitis vinifera* L. cv. Cabernet Sauvignon. Am. J. Enol. Vitic. 49, 251–258.

Osborne, W. C. (1977). "Fans," in International series of monographs in heating, ventilation and refrigeration (Pergamon Press).

Pallas, B., Louarn, G., Christophe, A., Lebon, E., and Lecoeur, J. (2008). Influence of intra-shoot trophic competition on shoot development in two grapevine cultivars (*Vitis vinifera*). Physiol. Plant. 134, 49–63. doi:10.1111/j.1399-3054.2008.01100.x.

Palliotti, A., Gardi, T., Berrios, J. G., Civardi, S., and Poni, S. (2012). Early source limitation as a tool for yield control and wine quality improvement in a high-yielding red *Vitis vinifera* L. cultivar. Sci. Hortic. (Amsterdam). 145, 10–16. doi:10.1016/j.scienta.2012.07.019.

Palliotti, A., Gatti, M., and Poni, S. (2011). Early leaf removal to improve vineyard efficiency: Gas exchange, source-to-sink balance, and reserve storage responses. Am. J. Enol. Vitic. 62, 219–228. doi:10.5344/ajev.2011.10094.

Palliotti, A., Panara, F., Famiani, F., Sabbatini, P., Howell, G. S., Silvestroni, O., and Poni, S. (2013a). Postveraison application of antitranspirant Di-1-p-Menthene to control sugar accumulation in sangiovese grapevines. Am. J. Enol. Vitic. 64, 378–385. doi:10.5344/ajev.2013.13015.

Palliotti, A., Panara, F., Silvestroni, O., Lanari, V., Sabbatini, P., Howell, G. S., Gatti, M., and Poni, S. (2013b). Influence of mechanical postveraison leaf removal apical to the cluster zone on delay of fruit ripening in Sangiovese (*Vitis vinifera* L.) grapevines. Aust. J. Grape Wine Res. 19, 369–377. doi:10.1111/ajgw.12033.

Parker, A. K., Hofmann, R. W., Van Leeuwen, C., McLachlan, A. R. G., and Trought, M. C. T. (2015). Manipulating the leaf area to fruit mass ratio alters the synchrony of total soluble solids accumulation and titratable. Aust. J. Grape Wine Res. doi:10.1111/ajgw.12132.

Pastore, C., Zenoni, S., Fasoli, M., Pezzotti, M., Tornielli, G. B., and Filippetti, I. (2013). Selective defoliation affects plant growth, fruit transcriptional ripening program and flavonoid metabolism in grapevine. BMC Plant Biol. 13, 30. doi:10.1186/1471-2229-13-30.

Pastore, C., Zenoni, S., Tornielli, G. B., Allegro, G., Dal Santo, S., Valentini, G., Intrieri, C., Pezzotti, M., and Filippetti, I. (2011). Increasing the source/sink ratio in *Vitis vinifera* (cv Sangiovese) induces extensive transcriptome reprogramming and modifies berry ripening. BMC Genomics 12, 631. doi:10.1186/1471-2164-12-631.

Percival, D. C., Fisher, K. H., and Sullivan, J. a. (1994). Use of fruit zone leaf removal with *Vitis vinifera* L. cv. Riesling grapevines. II.Effect on fruit composition, yield, and occurrence of bunch rot (Botrytis cinerea Pers.:Fr.). Am. J. Enol. Vitic. 45, 133–140.

Pereira, G. E., Gaudillere, J. P., Pieri, P., Hilbert, G., Maucourt, M., Deborde, C., Moing, A., and Rolin, D. (2006). Microclimate Influence on Mineral and Metabolic Profiles of Grape Berries. J. Agric. Food Chem. 54, 6765–6775.

Petrie, P. R., and Clingeleffer, P. R. (2006). Crop thinning (hand versus mechanical), grape maturity and anthocyanin concentration : outcomes from irrigated Cabernet Sauvignon (*Vitis vinifera* L .) in a warm climate. Aust. J. Grape Wine Res. 12, 21–29.

Petrie, P. R., Trought, M. C. T., and Howell, G. S. (2000a). Fruit composition and ripening of Pinot Noir (*Vitis vinifera* L .) in relation to leaf area. Aust. J. Grape Wine Res. 6, 46–51.

Petrie, P. R., Trought, M. C. T., and Howell, G. S. (2000b). Influence of leaf ageing, leaf area and crop load on photosynthesis, stomatal conductance and senescence of grapevine (*Vitis vinifera* L. cv. Pinot noir) leaves. Vitis 39, 31–36.

Petrie, P. R., Trought, M. C. T., Howell, G. S., and Buchan, G. D. (2003). The effect of leaf removal and canopy height on whole-vine gas exchange and fruit development of *Vitis vinifera* L. Sauvignon Blanc. Funct. plant Biol. 30, 711–717.

Poni, S., Bernizzoni, F., and Civardi, S. (2008). The effect of early leaf removal on whole-canopy gas exchange and vine performance of *Vitis Vinifera* L . " Sangiovese ." Vitis 47, 1–6.

Poni, S., Bernizzoni, F., Civardi, S., and Libelli, N. (2009). Effects of pre-bloom leaf removal on growth of berry tissues and must composition in two red *Vitis vinifera* L. cultivars. Aust. J. Grape Wine Res. 15, 185–193. doi:10.1111/j.1755-0238.2008.00044.x.

Poni, S., Casalini, L., Bernizzoni, F., Civardi, S., and Intrieri, C. (2006a). Effects of early defoliation on shoot photosynthesis, yield components, and grape composition. Am. J. Enol. Vitic. 57, 397–407.

Poni, S., Gatti, M., Bernizzoni, F., Civardi, S., Bobeica, N., Magnanini, E., and Palliotti, A. (2013). Late leaf removal aimed at delaying ripening in cv. Sangiovese: physiological assessment and vine performance. Aust. J. Grape Wine Res. 19, 378–387. doi:10.1111/ajgw.12040.

Poni, S., and Giachino, E. (2000). Growth, photosynthesis and cropping of potted grapevines (*Vitis vinifera* L. cv. Cabernet Sauvignon). Aust. J. Grape Wine Res. 6, 216–226.

Poni, S., Merli, M. C., Magnanini, E., Galbignani, M., Bernizzoni, F., Vercesi, A., and Gatti, M. (2014). An improved multichamber gas exchange system for determining whole-canopy water-use efficiency in grapevine. Am. J. Enol. Vitic. 65, 268–276. doi:10.5344/ajev.2014.13117.

Poni, S., Palliotti, A., and Bernizzoni, F. (2006b). Calibration and evaluation of a STELLA software- based daily CO 2 balance model in *Vitis vinifera* L . J. Am. Soc. Hortic. Sci. 131, 273–283.

R Development Core Team (2010). R Development Core Team. Vienna, Austria: R Foundation for Statistical Computing.

Reynolds, A. G., Price, S. F., Wardle, D. A., and Watson, B. T. (1994). Fruit environment and crop level effects on Pinot noir. I.Vine performance and fruit composition in British Columbia. Am. J. Enol. Vitic. 45, 452–459.

Sabbatini, P., and Stanley Howell, G. (2010). Effects of early defoliation on yield, fruit composition, and harvest season cluster rot complex of grapevines. HortScience 45, 1804–1808.

Sadras, V. O., Collins, M., and Soar, C. J. (2008). Modelling variety-dependent dynamics of soluble solids and water in berries of *Vitis vinifera*. Aust. J. Grape Wine Res. 14, 250–259. doi:10.1111/j.1755-0238.2008.00025.x.

Sadras, V. O., and Moran, M. a. (2012). Elevated temperature decouples anthocyanins and sugars in berries of Shiraz and Cabernet Franc. Aust. J. Grape Wine Res. 18, 115–122. doi:10.1111/j.1755-0238.2012.00180.x.

Sadras, V. O., Stevens, R. M., Pech, J. M., Taylor, E. J., Nicholas, P. R., and McCarthy, M. G. (2007). Quantifying phenotypic plasticity of berry traits using an allometric-type approach: A case study on anthocyanins and sugars in berries of Cabernet Sauvignon. Aust. J. Grape Wine Res. 13, 72–80. doi:10.1111/j.1755-0238.2007.tb00237.x.

Schultz, H. R. (2000). Climate change and viticulture: A European perspective on climatology, carbon dioxide and UV-B effects. Aust. J. Grape Wine Res. 6, 2–12.

Schultz, H. R., and Jones, G. V (2010). Climate induced historic and future changes in viticulture. J. Wine Res. 21, 137–145.

Spayd, S. E., Tarara, J. M., Mee, D. L., and Ferguson, J. C. (2002). Separation of Sunlight and Temperature Effects on the Composition of *Vitis vinifera* cv. Merlot Berries. Am. J. Enol. Vitic. 53, 171–182.

Stoll, M., Scheidweiler, M., Lafontaine, M., and Schultz, H. R. (2010). Possibilities to reduce the velocity of berry maturation through various leaf area to fruit ratio modifications in *Vitis Vinifera* L. Riesling. Progrès Agric. Vitic. 127, 68–71.

Tardaguila, J., Toda, F. M. De, Poni, S., and Diago, M. P. (2010). Impact of early leaf removal on yield and fruit and wine composition of *Vitis vinifera* L. Graciano and Carignan. Am. J. Enol. Vitic. 61, 372–381.

Vitrac, X., Larronde, F., Krisa, S., Decendit, A., Deffieux, G., and Mérillon, J. M. (2000). Sugar sensing and Ca2+-calmodulin requirement in *Vitis vinifera* cells producing anthocyanins. Phytochemistry 53, 659–665. doi:10.1016/S0031-9422(99)00620-2.

Weaver, R. J. (1963). Effect of leaf to fruit ratio on fruit quality and shoot development in Carignane and Zinfandel wine grapes. Am. J. Enol. Vitic. 14, 1–12.

Wolpert, J. A., Howell, G. S., and Mansfield, T. K. (1983). Sampling Vidal blanc grapes. I. Effect of training system, pruning severity, shoot exposure, shoot origin, and cluster thinning on cluster weight and fruit quality. Am. J. Enol. Vitic. 34, 72–76. Available at: http://www.ajevonline.org/cgi/content/abstract/34/2/72.

Wu, B.-H., Niu, N., Li, J.-H., and Li, S.-H. (2013). Leaf:fruit ratio affects the proteomic profile of grape berry skins. J. Am. Soc. Hortic. Sci. 138, 416–427.

6. General conclusions

During the three years of PhD, different leaf-to-fruit ratios were studied to clarify action mechanisms vs the ripening process. In this research was studied the effect of severity, timing and position of leaf removal on two cultivars, Sangiovese and Cabernet Sauvignon.

Pre-véraison and post-véraison treatments on Sangiovese berries were both successful at delaying ripening. Specifically the delay in sugar accumulation of one week, for the berries with low carbon availability in comparison with no source limitation. However the postponement in sugar accumulation did not interfere with the anthocyanin accumulation, neither with total acidity and organic acids concentration at harvest in Sangiovese grapes. In both treatment, pre-véraison and post-véraison leaf removal, the leaves had a full compensation of photosynthesis which have been observed previously by other authors (Candolfi-Vasconcelos and Koblet, 1990; Petrie et al., 2003; Kliewer and Dokoozlian, 2005; Poni et al., 2008). This response of vine compensation after source-to-sink modulation was seen also when measurement was done on single leaves.

From the results obtained in the first year, we can affirm that the timing of leaf removal is very important, because of the difference in sugar content of pre- and post-véraison, whereas the latter had a lower sugar concentration. We can specify that if leaf area to fruit modulations are done later in the season, a higher delay in ripening is possible.

Moreover, the position in which leaves were removed, apical to the cluster zone, perfectly fits the target for berry ripening retardation. In this case, the cluster is protected from high light and temperature, which is detrimental for anthocyanin accumulation. For this reason, there are no differences in the anthocyanin concentration at harvest in all plants, but only low sugar concentration in treated vines, which is due mainly to low carbon availability.

After véraison defoliation in fruit cuttings of Cabernet Sauvignon, grapes berries at harvest had lower concentration of sugar compared to control plants. This response, even

with a different variety, of source limited plants is the confirmation of the first work, specifically the two experiments had a leaf removal apical to cluster zone at véraison. The only difference was the severity of leaf removal, which in the second case was more severe (63% of leaves removed on Sangiovese, and 76% of leaves removed on Cabernet Sauvignon). This difference had a great impact on anthocyanin accumulation, that decreased considerably with no possibility of compensation. As for the first experiment, no changes in organic acids and acidity was found.

In the light of the last two research, another study was necessary to answer the questions of vine photosynthesis after severe leaf removal, and the repeatability of the treatment on real vines and another variety.

The results of the third experiment was comparable with the previous ones, and the delay in technical ripening (sugar concentration) was evident also for Sangiovese grape berries. However, the low color content found in berries of Sangiovese can be explained by the difference responses of different cultivars; in fact Cabernet Sauvignon anthocyanin profile is very different from Sangiovese, with the latest having more di-hidroxylated anthocyanins, which are more unstable compared to tri-hydroxylated anthocyanins. Another important fact is that the severe source limitation in Sangiovese vines had only a slight compensation of whole-vine photosynthesis, probably because of the timing of leaf removal, the age of remaining leaves, and the absence of secondary leaves.

In the two similar researches on severity of source to sink modulations on Cabernet Sauvignon and Sangiovese, the decoupling between sugars and anthocyanins is demonstrated when calculation of carbon fixation by leaves and carbon utilization by berries is done. According to the last study, the carbon reserves are remobilized for berry sugar accumulation and/or vine maintenance, not for anthocyanin accumulation.

In conclusion, limitation of carbon availability in grapevines can delay ripening, however it is strongly recommended to consider the severity and the timing of source limitation. This canopy management technique can be a valuable tool to delay berry sugar accumulation and reduce alcohol content in wines, because of its easy mechanization and low cost.

7. References

Andersen, C. P. 2003. Source-sink balance and carbon allocation below ground in plants exposed to ozone. New Phytologist 157:213–228.

Battaglini, A., G. Barbeau, M. Bindi, and F.-W. Badeck. 2008. European winegrowers' perceptions of climate change impact and options for adaptation. Regional Environmental Change 9:61–73.

Bergqvist, J., N. Dokoozlian, and N. Ebisuda. 2001. Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the central San Joaquin Valley of California. American Journal of Enology and Viticulture 52:3–9.

Böttcher, C., K. Harvey, C. G. Forde, P. K. Boss, and C. Davies. 2011. Auxin treatment of pre-veraison grape (*Vitis vinifera* L.) berries both delays ripening and increases the synchronicity of sugar accumulation. Australian Journal of Grape and Wine Research 17:1–8.

Camps, J. O., and M. C. Ramos. 2012. Grape harvest and yield responses to inter-annual changes in temperature and precipitation in an area of north-east Spain with a Mediterranean climate. International journal of biometeorology 56:853–64.

Candolfi-Vasconcelos, M. C., and W. Koblet. 1990. Yield, fruit quality, bud fertility and starch reserves of the wood as a function of leaf removal in *Vitis vinifera*-Evidence of compensation and stress recovering. Vitis 29:199–221.

Caspari, H. W., A. Lang, and P. Alspach. 1998. Effects of girdling and leaf removal on fruit set and vegetative growth in grape. American Journal of Enology and Viticulture 49:359–366.

Chorti, E., S. Guidoni, and A. Ferrandino. 2010. Effect of different cluster sunlight exposure levels on ripening and anthocyanin accumulation in Nebbiolo grapes. American Journal of Enology and Viticulture 61:23–30.

Conde, C., P. Silva, N. Fontes, A. C. P. Dias, R. M. Tavares, M. J. Sousa, A. Agasse, S. Delrot, and H. Gerós. 2007. Biochemical changes throughout grape berry development and fruit and wine quality. Food 1:1–22.

Coombe, B. G. 1987. Influence of temperature on composition and quality of grapes. Acta Horticulturae nternational Society for Horticultural Science 206:23–36.

Coombe, B. G. 1992. Research on Development and Ripening of the Grape Berry. Am. J. Enol. Vitic. 43:101–110.

Cormier, F., R. Couture, C. B. Do, T. Q. Pham, and V. H. Tong. 1997. Properties of anthocyanins from grape cell culture. Journal of Food Science 62:246–248.

Diago, M. P., B. Ayestarán, Z. Guadalupe, Á. Garrido, and J. Tardaguila. 2012. Phenolic composition of Tempranillo wines following early defoliation of the vines. Journal of the science of food and agriculture 92:925–34.

Diago, M. P., M. Vilanova, and J. Tardaguila. 2010. Effects of timing of manual and mechanical early defoliation on the aroma of *Vitis vinifera* L . Tempranillo wine. American Journal of Enology and Viticulture 3:382–391.

Dokoozlian, N. 2000. Grape berry growth and development. Pages 30–37 Raisin Production Manual.

Downey, M. O., N. K. Dokoozlian, and M. P. Krstic. 2006. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. American Journal of Enology and Viticulture 57:257–268.

Duchêne, E., and C. Schneider. 2005. Grapevine and climatic changes: a glance at the situation in Alsace.

English, J. T., C. S. Thomas, J. J. Marois, and W.D. Gubler. 1989. Microclimates of grapevine canopies associated with leaf removal and control of Botrytis bunch rot. The American Phytopathological Society 79:395–401.

Farrar, J. F., and D. L. Jones. 2000. The control of carbon acquisition by roots. New Phytologist 147:43–53.

Ferguson, J. C., J. M. Tarara, L. J. Mills, G. G. Grove, and M. Keller. 2011. Dynamic thermal time model of cold hardiness for dormant grapevine buds. Annals of botany 107:389–96.

Fraga, H., a C. Malheiro, J. Moutinho-Pereira, and J. a Santos. 2012. Future scenarios for viticultural zoning in Europe: ensemble projections and uncertainties. International journal of biometeorology.

Fraga, H., A. C. Malheiro, J. Moutinho-Pereira, and J. A. Santos. 2013. An overview of climate change impacts on European viticulture. Food and Energy Security 1:94–110.

Fregoni, M. 1998. Viticoltura di qualità. L'informatore agrario, Verona.

Friend, A. P., and M. C. T. Trought. 2007. Delayed winter spur-pruning in New Zealand can alter yield components of Merlot grapevines. Australian Journal of Grape and Wine Research 13:157–164.

Gaal, M., M. Moriondo, and M. Bindi. 2012. Modelling the impact of climate change on the hungarian wine regions using random forest. Applied ecology and environmental research 10:121–140.

Galbreath, J. 2012. Climate change response: Evidence from the Margaret River wine region of Australia. Business Strategy and the Environment 10.

Greer, D. H., and C. Weston. 2010. Heat stress affects flowering, berry growth, sugar accumulation and photosynthesis of *Vitis vinifera* cv. Semillon grapevines grown in a controlled environment.

Guidoni, S., A. Ferrandino, and V. Novello. 2008. Effects of seasonal and agronomical practices on skin anthocyanin profile of Nebbiolo grapes. American Journal of Enology and Viticulture 59:22–29.

Hannah, L., P. R. Roehrdanz, M. Ikegami, a. V. Shepard, M. R. Shaw, G. Tabor, L. Zhi, P. a. Marquet, and R. J. Hijmans. 2013. Climate change, wine, and conservation. Proceedings of the National Academy of Sciences 110:6907–6912.

Haselgrove, L., R. Botting, V. Heeswijck, P. HOJ, P. Dry, C. Ford, and P. Iland. 2000. Canopy microclimate and berry composition: The effect of bunch exposure on the phenolic composition of *Vitis vinifera* L. cv. Shiraz grape berries. Australian Journal Of Grape And Wine Research 6:141–149.

He, F., L. Mu, G.-L. Yan, N.-N. Liang, Q.-H. Pan, J. Wang, M. J. Reeves, and C.-Q. Duan. 2010. Biosynthesis of anthocyanins and their regulation in colored grapes. Molecules 15:9057–91.

Howell, G. S. 2001. Sustainable grape productivity and the growth-yield relationship: A review. American Journal of Enology and Viticulture 52:165–174.

Hunter, J. J., O. T. De Villiers, and J. E. Watts. 1991. The effect of partial defoliation on quality characteristics of *Vitis vinifera* L. cv. Cabernet Sauvignon grapes. II. Skin color, skin sugar, and wine quality. American Journal of Enology an Viticulture 42:13–18.

Hunter, J. J., and J. H. Visser. 1990. The effect of partial defoliation on growth characteristics of *Vitis vinifera* L. cv. Cabernet Sauvignon II. Reproductive growth. South African Journal of Enology and Viticulture 11:26–32.

Iland, P., P. Dry, T. Proffitt, and S. Tyerman. 2011. The grapevine: From the science to the practice of growing vines for wine. Page 320. Hardback. Patrick Iland Wine Promotions.

Intrieri, C., I. Filippetti, G. Allegro, M. Centinari, and S. Poni. 2008. Early defoliation (hand vs mechanical) for improved crop control and grape composition in Sangiovese (*Vitis vinifera* L.). Australian Journal of Grape and Wine Research 14:25–32.

IPCC. 2014. IPCC Climate Change 2014: Fifth Assessment Report. Intergovernmental Panel on Climate Change:138.

Jackson, D. I., and P. B. Lombard. 1993. Environmental and management practices affecting grape composition and wine quality - A Review. American Journal of Enology and Viticulture 44:409–430.

Jeong, S. T., N. Goto-Yamamoto, S. Kobayashi, and M. Esaka. 2004. Effects of plant hormones and shading on the accumulation of anthocyanins and the expression of anthocyanin biosynthetic genes in grape berry skins. Plant Science 167:247–252.

Jogaiah, S., D. P. Oulkar, A. N. Vijapure, S. R. Maske, A. K. Sharma, and R. G. Somkuwar. 2013. Influence of canopy management practices on fruit composition of wine grape cultivars grown in semi-arid tropical region of India. African Journal pf Agricultural Research 8:3462–3472.

Jones, G. V., and F. Alves. 2012. Impact of climate change on wine production: a global overview and regional assessment in the Douro Valley of Portugal. International Journal of Global Warming 4:383–406.

Jones, G. V., M. A. White, O. R. Cooper, and K. Storchmann. 2005. Climate Change and Global Wine Quality. Climatic Change 73:319–343.

Joscelyne, V. L., M. O. Downey, M. Mazza, and S. E. P. Bastian. 2007. Partial shading of Cabernet Sauvignon and Shiraz vines altered wine color and mouthfeel attributes, but increased exposure had little impact. Journal of agricultural and food chemistry 55:10888–10896.

Keller, M. 2010. Managing grapevines to optimise fruit development in a challenging environment: a climate change primer for viticulturists. Australian Journal of Grape and Wine Research 16:56–69.

Kemp, B. S., R. Harrison, and G. L. Creasy. 2011. Effect of mechanical leaf removal and its timing on flavan-3-ol composition and concentrations in *Vitis vinifera* L. cv. Pinot Noir wine. Australian Journal of Grape and Wine Research 17:270–279.

Kennedy, J. 2002. Understanding grape berry development. Practical winery & vineyard:2–5.

Kennedy, J. A., C. C. Saucier, and Y. Glories. 2006. Grape and wine phenolics: History and perspective. American Journal of Enology and Viticulture 57:239–249.

King, P. D., D. J. McClellan, and R. E. Smart. 2012. Effect of severity of leaf and crop removal on grape and wine composition of Merlot vines in Hawke's Bay vineyards. American Journal of Enology and Viticulture 63:500–507.

Kliewer, W. M. 1966. Sugars and organic acids of *Vitis vinifera*. Plant physiology 41:923–31.

Kliewer, W. M. 1968. Changes in the concentration of free amino acids in grape berries during maturation. American Journal of Enology an Viticulture 19:166–174.

Kliewer, W. M. 1973. Berry composition of *Vitis vinifera* cultivars as influenced by photo- and nycto-temperatures during maturation. Amer Soc Hort Sci J.

Kliewer, W. M. 1977. Influence of temperature, solar radiation and nitrogen on coloration and composition of Emperor grapes. American Journal of Enology and Viticulture 28:96–103.

Kliewer, W. M., and N. K. Dokoozlian. 2005. Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality. American Journal of Enology and Viticulture 56:170–181.

Kliewer, W. M., L. A. Lider, and H. B. Schultz. 1967. Influence of artificial shading of vineyards on the concentration of sugar and organic acid in grapes. Am. J. Enol. Vitic 18:78–86.

Kliewer, W. M., and C. S. Ough. 1970. The effect of leaf area and crop level on the concentration of amino acids and total nitrogen in Thompson seedless grapes. Vitis 9:196–206.

Kliewer, W. M., and R. J. Weaver. 1971. Effect of crop level and leaf area on growth, composition, and coloration of "Tokay" grapes. American Journal of Enology and Viticulture 22:172–177.

Koch, K. E. 1996. Carbohydrated-modulated gene expression in plants. Annual Review of Plant Physiology and Plant Molecular Biology 47:509–540.

Kotseridis, Y., A. Georgiadou, P. Tikos, S. Kallithraka, and S. Koundouras. 2012. Effects of severity of post-flowering leaf removal on berry growth and composition of three red *Vitis vinifera* L. cultivars grown under semiarid conditions. Journal of agricultural and food chemistry 60:6000–6010.

Lakso, A. N., and W. M. Kliewer. 1975. The Influence of Temperature on Malic Acid Metabolism in Grape Berries I-Enzyme Responses. Plant Physiology 56:370–372.

Lebon, G., G. Wojnarowiez, B. Holzapfel, F. Fontaine, N. Vaillant-Gaveau, and C. Clément. 2008. Sugars and flowering in the grapevine (*Vitis vinifera* L.). Journal of experimental botany 59:2565–78.

Lee, J., and R. P. Schreiner. 2010. Free amino acid profiles from "Pinot noir" grapes are influenced by vine N-status and sample preparation method. Food Chemistry 119:484–489.

Lee, J., and P. A. Skinkis. 2013. Oregon "Pinot noir" grape anthocyanin enhancement by early leaf removal. Food Chemistry 139:893–901.

Lohitnavy, N., S. Bastian, and C. Collins. 2010. Berry sensory attributes correlate with compositional changes under different viticultural management of Semillon (*Vitis vinifera* L.). Food Quality and Preference 21:711–719.

Mattivi, F., R. Guzzon, U. Vrhovsek, M. Stefanini, and R. Velasco. 2006. Metabolite profiling of grape: flavonols and anthocyanins. Journal of Agricultural and Food Chemistry 54:7692–7702.

Mazza, G., L. Fukumoto, P. Delaquis, B. Girard, and B. Ewert. 1999. Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. Journal of Agricultural and Food Chemistry 47:4009–4017.

Mira de Orduña, R. 2010. Climate change associated effects on grape and wine quality and production. Food Research International 43:1844–1855.

Mori, K., N. Goto-Yamamoto, M. Kitayama, and K. Hashizume. 2007. Loss of anthocyanins in red-wine grape under high temperature. Journal of experimental botany 58:1935–45.

Morrison, J. C., and A. C. Noble. 1990. The effects of leaf and cluster shading on the composition of Cabernet Sauvignon grapes and on fruit and wine sensory properties. American Journal of Enology and Viticulture 41:193–200.

Mullins, M. G., A. Bouquet, and L. E. Williams. 1992. Biology of the grapevine. Cambridge University Press.

OIV. 2014. The wine market : evolution and trends May 2014. Pages 1–14.

Ollat, N., P. Diakou-Verdin, J. P. Carde, F. Barrieu, J.-P. Gaudillere, and A. Moing. 2002. Grape berry development: A review. Journal International des Sciences de la Vigne et du Vin (France) 36.

Ollat, N., and J. P. Gaudillere. 1998. The effect of limiting leaf area during stage I of berry growth on development and composition of berries of *Vitis vinifera* L. cv. Cabernet Sauvignon. American Journal of Enology and Viticulture 49:251–258.

Ough, C. S., and R. M. Stashak. 1974. Further studies on proline concentration in grapes and wines. American Journal of Enology and Viticulture 25:7–12.

Palliotti, A., M. Gatti, and S. Poni. 2011. Early leaf removal to improve vineyard efficiency: Gas exchange, source-to-sink balance, and reserve storage responses. American Journal of Enology and Viticulture 62:219–228.

Palliotti, A., F. Panara, F. Famiani, P. Sabbatini, G. S. Howell, O. Silvestroni, and S. Poni. 2013. Postveraison application of antitranspirant Di-1-p-Menthene to control sugar accumulation in sangiovese grapevines. American Journal of Enology and Viticulture 64:378–385.

Palliotti, A., S. Tombesi, O. Silvestroni, V. Lanari, M. Gatti, and S. Poni. 2014. Changes in vineyard establishment and canopy management urged by earlier climate-related grape ripening: A review. Scientia Horticulturae 178:43–54.

Petrie, P. R., M. C. T. Trought, and G. S. Howell. 2000a. Influence of leaf ageing, leaf area and crop load on photosynthesis, stomatal conductance and senescence of grapevine (*Vitis vinifera* L. cv. Pinot noir) leaves. Vitis 39:31–36.

Petrie, P. R., M. C. T. Trought, and G. S. Howell. 2000b. Fruit composition and ripening of Pinot Noir (*Vitis vinifera* L .) in relation to leaf area. Australian Journal Of Grape And Wine Research 6:46–51.

Petrie, P. R., M. C. T. Trought, G. S. Howell, and G. D. Buchan. 2003. The effect of leaf removal and canopy height on whole-vine gas exchange and fruit development of *Vitis vinifera* L. Sauvignon Blanc. Functional plant biology 30:711–717.

Pirie, A., and M. G. Mullins. 1977. Interrelationships of sugars, anthocyanins, total phenols and dry weight in the skin of grape berries during ripening. American Journal of Enology an Viticulture 28:204–209.

Poni, S., F. Bernizzoni, and S. Civardi. 2008. The effect of early leaf removal on whole-canopy gas exchange and vine performance of *Vitis Vinifera* L . " Sangiovese ." Vitis 47:1–6.

Poni, S., F. Bernizzoni, S. Civardi, and N. Libelli. 2009. Effects of pre-bloom leaf removal on growth of berry tissues and must composition in two red *Vitis vinifera* L. cultivars. Australian Journal of Grape and Wine Research 15:185–193.

Poni, S., L. Casalini, F. Bernizzoni, S. Civardi, and C. Intrieri. 2006. Effects of early defoliation on shoot photosynthesis, yield components, and grape composition. American Journal of Enology and Viticulture 57:397–407.

Price, S. F., P. J. Breen, M. Valladao, and B. T. Watson. 1995. Cluster sun exposure and quercetin in Pinot noir grapes and wine. American Journal of Enology and Viticulture 46:187–194.

Di Profio, F., A. G. Reynolds, and A. Kasimos. 2011. Canopy management and enzyme impacts on Merlot, Cabernet franc, and Cabernet Sauvignon. I. Yield and berry composition. American Journal of Enology and Viticulture 62:139–151.

Risco, D., D. Pérez, A. Yeves, J. R. R. Castel, and D. S. S. Intrigliolo. 2014. Early defoliation in a temperate warm and semi-arid Tempranillo vineyard: Vine performance and grape composition. Australian Journal of Grape and Wine Research 20:111–122.

Rolland, F., E. Baena-Gonzalez, and J. Sheen. 2006. Sugar sensing and signaling in plants: conserved and novel mechanisms. Annual review of plant biology 57:675–709.

Ruml, M., A. Vuković, M. Vujadinović, V. Djurdjević, Z. Ranković-Vasić, Z. Atanacković, B. Sivčev, N. Marković, S. Matijašević, and N. Petrović. 2012. On the use of regional climate models: Implications of climate change for viticulture in Serbia. Agricultural and Forest Meteorology 158:53–62.

Sadras, V. O., A. Montoro, M. A. Moran, and P. J. Aphalo. 2012. Elevated temperature altered the reaction norms of stomatal conductance in field-grown grapevine. Agricultural and Forest Meteorology 165:35–42.

Sadras, V. O., and M. a. Moran. 2012. Elevated temperature decouples anthocyanins and sugars in berries of Shiraz and Cabernet Franc. Australian Journal of Grape and Wine Research 18:115–122.

Sadras, V. O., P. R. Petrie, and M. A. Moran. 2013. Effects of elevated temperature in grapevine. II juice pH, titratable acidity and wine sensory attributes. Australian Journal of Grape and Wine Research 19:107–115.

Santos, J., A. Malheiro, J. Pinto, and G. Jones. 2012. Macroclimate and viticultural zoning in Europe: observed trends and atmospheric forcing. Climate Research 51:89–103.

Schultz, H. R. 2000. Climate change and viticulture: A European perspective on climatology, carbon dioxide and UV-B effects. Australian Journal Of Grape And Wine Research 6:2–12.

Schultz, H. R., and G. V Jones. 2010. Climate induced historic and future changes in viticulture. Journal of Wine Research 21:137–145.

Sheen, J., L. Zhou, and J. C. Jang. 1999. Sugars as signaling molecules. Current Opinion in Plant Biology 2:410–418.

Skinkis, P. A., and A. J. Vance. 2013. Understanding vine balance: An important concept in vineyard management. Extension work, Oregon state University:1–10.

Smart, R. E., J. K. Dick, I. M. Gravett, and B. M. Fisher. 1990. Canopy management to improve grape yield and wine quality - principles and practices. South African journal of Enology and Viticulture 11:3–18.

Smart, R. E., J. B. Robinson, G. R. Due, and C. J. Brien. 1985. Canopy microclimate modification for the cultivar Shiraz II. Effects on must and wine composition. Vitis 24:119–128.

Smart, R., and M. Robinson. 1991. Sunlight into wine: a handbook for winegrape canopy management. Winetitles.

Soar, C. J., V. O. Sadras, and P. R. Petrie. 2008. Climate drivers of red wine quality in four contrasting Australian wine regions. Australian Journal of Grape and Wine Research 14:78–90.

Spayd, S. E., J. M. Tarara, D. L. Mee, and J. C. Ferguson. 2002. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. American Journal of Enology and Viticulture 53:171–182.

Staff, S. L., D. C. Percival, J. A. Sullivan, and K. H. Fisher. 1997. Fruit zone leaf removal influences vegetative, yield, disease, fruit composition, and wine sensory attributes of *Vitis vinifera* L. "Optima" and "Cabernet franc." Canadian Journal of Plant Science:149–153.

Sternad Lemut, M., K. Trost, P. Sivilotti, and U. Vrhovsek. 2011. Pinot Noir grape colour related phenolics as affected by leaf removal treatments in the Vipava Valley. Journal of Food Composition and Analysis 24:777–784.

Stines, A. P., J. Grubb, H. Gockowiak, P. A. Henschke, P. B. Høj, and R. Heeswijck. 2000. Proline and arginine accumulation in developing berries of *Vitis vinifera* L. in Australian vineyards: Influence of vine cultivar, berry maturity and tissue type. Australian Journal of Grape and Wine Research 6:150–158.

Stines, A. P., D. J. Naylor, P. B. Høj, and R. van Heeswijck. 1999. Proline accumulation in developing grapevine fruit occurs independently of changes in the levels of delta1-pyrroline-5-carboxylate synthetase mRNA or protein. Plant physiology 120:923.

Stoll, M., M. Scheidweiler, M. Lafontaine, and H. R. Schultz. 2010. Possibilities to reduce the velocity of berry maturation through various leaf area to fruit ratio modifications in *Vitis Vinifera* L. Riesling. Progrès agricole et viticole 127:68–71.

Sweetman, C., V. O. Sadras, R. D. Hancock, K. L. Soole, and C. M. Ford. 2014. Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit. Journal of experimental botany 65:5975–88.

Tarara, J. M., J. Lee, S. E. Spayd, and C. F. Scagel. 2008. Berry temperature and solar radiation alter acylation, proportion, and concentration of anthocyanin in Merlot grapes. American Journal of Enology and Viticulture 59:235–247.

Tardaguila, J., J. a. Blanco, S. Poni, and M. P. Diago. 2012. Mechanical yield regulation in winegrapes: comparison of early defoliation and crop thinning. Australian Journal of Grape and Wine Research 18:344–352.

Tardaguila, J., F. M. De Toda, S. Poni, and M. P. Diago. 2010. Impact of early leaf removal on yield and fruit and wine composition of *Vitis vinifera* L. Graciano and Carignan. American Journal of Enology and Viticulture 61:372–381.

Vasconcelos, M. C., and S. Castagnoli. 2000. Leaf canopy structure and vine performance. American Journal of Enology and Viticulture Viticulture 51:390–396.

Vilanova, M., M. P. Diago, Z. Genisheva, J. M. Oliveira, and J. Tardaguila. 2012. Early leaf removal impact on volatile composition of Tempranillo wines. Journal of the science of food and agriculture 92:935–42.

Yamane, T., T. J. Seok, N. Goto-Yamamoto, Y. Koshita, and S. Kobayashi. 2006. Effects of temperature on anthocyanin biosynthesis in grape berry skins. American Journal of Enology and Viticulture 57:54–59.

Zoecklein, B. W., T. K. Wolf, N. W. Duncan, J. M. Judge, and M. K. Cook. 1992. Effects of fruit zone leaf removal on yield, fruit composition, and fruit rot incidence of Chardonnay and white Riesling (*Vitis vinifera* L.) grapes. American Journal of Enology and Viticulture 43:139–148.