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Leaf respiration in grapevine (*Vitis vinifera* 'Chasselas') in relation to environmental and plant factors

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Summary

The leaf respiration (R_D) of grapevine (*Vitis vinifera* 'Chasselas') was measured under field conditions during the growing season in leaves of different physiological ages in relation to the temperature and plant water status. R_D increased with the temperature and was particularly high in young growing leaves on primary and lateral shoots. The R_D response to the temperature evolved over the season according to the type and age of the leaves and their phenology. Leaf aging (senescence) induced a decrease in R_D at the end of the season. At constant temperatures (20 °C), the highest R_D rates were measured during the rapid plant growth phase (the Q_{10} values were also the highest), and they progressively decreased to reach their lowest rates at the end of the growing season. The lowest R_D values were measured on leaves that were inserted opposite the clusters of primary shoots at any period during the season. Water stress led to a reduction in R_D, especially when the leaf temperature was above 20 °C. The nocturnal R_D evolution showed that the R_D rates were greatest at nightfall when the nocturnal temperatures were still high and leaf carbohydrate availability was at its highest; the rates gradually decreased to reach the lowest R_D values just before dawn.

K e y w o r d s : leaf dark respiration; leaf temperature; leaf age; phenology; grapevine.

Introduction

Respiration represents an essential process in plant survival and growth, and it contributes greatly to the global carbon balance in plants (Lakso *et al*. 2001). Leaf respiration (R_D) provides energy (ATP), reducing equivalents (NAD(P)H) and carbon-skeleton intermediates necessary for biosynthetic reactions (Amthor 2000). As a catabolic process, R_D involves the use of respiratory substrates, which are mostly sugars that formed during photosynthesis, but which also include organic and amino acids, among others. Respiratory rates vary according to plant age and differ from one organ to another, depending on the carbon substrate availability, developmental stage and environmental conditions. In general, the highest respiration rates are measured in the leaves (Amthor 1989), except those that are sometimes found in fruits during ripening (Blanke and Lenz 1989).

McCree (1970) demonstrated that global respiration in plant tissues was a combination of so-called growth respiration for synthesizing new biomass and maintenance respiration for renewing or maintaining the biomass in a healthy state. These two components of respiration evolve on a daily basis and throughout the growth period, and they depend in part on the amount of sugar substrates that are acquired during photosynthesis (Penning de Vries 1974, Azcon-Bieto *et al*. 1983, Stahl and McCree 1988, Amthor 2000). A large part of the energy spent on maintenance respiration is thought to be associated with protein turnover and the maintenance of ion gradients across membranes (Lambers *et al*. 1998). Thus, leaves that present a high nitrogen investment in Rubisco and other photosynthetic enzymes have a correspondingly high maintenance respiration (Van der Werf *et al*. 1992). High R_D rates may be equally related to the significant energy costs of loading photosynthates in the phloem, which represents an active process.

The amounts of CO_2 daily losses that occur through R_D are largely dependent on environmental conditions (Wright *et al*. 2006), and especially on temperature (Atkin *et al*. 2000a). R_D is able to adapt to temperature changes during the day and over the season (Körner and Larcher 1988, Atkin *et al*. 2000b, Wright *et al*. 2006), depending on the plants and growing conditions. Moreover, plant factors such as the leaf age, leaf nitrogen content, plant water status and phenology, among others, have a strong influence on R_D rates and on the capacity of leaves to acclimatize to changing environmental conditions. In general, the higher the metabolic activity of a tissue or given organ (and also its rate of growth) is, the greater its respiratory activity (Keller 2010). R_D rates and leaf photosynthesis are often coupled (Loveys *et al*. 2003, Whitehead *et al*. 2004, Wertin and Teskey 2008, Slot *et al*. 2013), and leaf nitrogen content is positively correlated with R_D (Atkin *et al*. 2008, Reich *et al*. 2008).

Studies on grapevines (Schultz 1991, 2003, Vanden Heuvel *et al*. 2004) have shown that the light conditions to which grapevine leaves are exposed during the season also play a role in R_D rates, with shade leaves having low-

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er R_D rates than sun leaves, regardless of the temperature. Moreover, estimated values of day respiration (Rd) were about 0.3 of night respiration (Rn) for adult non-senescent leaves (Schultz 2003) according to Brooks and Farquhar (1985), although the ratio (Rd/Rn) seems to vary during ontogenetic development. Results from field-grown 'Riesling' grapevines (Weyand and Schultz 2006) showed that respiratory losses by leaves depend greatly on night temperature and time during the season but varied between 3 and 7 % of the amount of carbon gained during the day. The age-related decline in R_D has also been reported by others (Palliotti and Cartechini 2005).

Based on a CO_2 balance model (Poni *et al*. 2006) in *Vitis vinifera*, the daily total respiration was estimated about 25 % of the total daily photosynthesis with the highest contribution from leaves (~ 45 %) in comparison with clusters (~ 20 %) and stems (~ 35 %). By including root respiration in the total organs respiration, up to 30-60 % of carbon obtained by photosynthesis is used in whole vine respiration according to Escalona *et al*., (2012) and Medrano *et al*. (2015), assuming the largest respiratory losses come from the root system. Nonetheless, leaves are generally responsible for most of the whole plant above-ground respiration in grapevines (Palliotti *et al*. 2004).

The objective of the present study was to measure leaf R_D rates in relation to temperature and other plant factors, such as the leaf age, grapevine phenology and plant water status. Nocturnal and seasonal R_D observations were made under field conditions at different phenological stages in the grapevine canopy.

Material and Methods

Plant material and experimental sites: The experiments were conducted from 1997 to 1998 on 15-year-old field-grown 'Chasselas' grapevines (clone 14/33-4 on 3309C rootstock) at the Agroscope, a research centre in Pully, Switzerland (46°52'N; 6°72'E), and in 2010 on 17-year-old field-grown 'Chasselas' (clone 14/33-4 on 3309C rootstock) at the Agroscope, in the experimental vineyard in Leytron, Switzerland (46°11'N; 7°12'E). The vines were trained with the Guyot system (vertical shoot-positioned) at 1.8 m x 1.0 m spacing. The experimental plots were both south-facing on 15 % slopes. The soil in Pully is deep and fertile, with a high water-holding capacity (> 200 mm) and a 2-meter soil depth. The average annual precipitation over 30 years (1980-2010) was approximately 1100 mm. The experimental site in Leytron lies on very stony (peyrosol > 60 % large elements, stones, blocks, and gravel) and deep soil (> 2.5 m vine root depth), with an estimated water-holding capacity of 150 mm. The average annual rainfall over 30 years (1980-2010) was 570 mm.

Determination of physiological leaf age: The ages of the leaves were expressed in plastochrons. The leaf plastochron index (LPI) was used to define the position of the leaf on the shoot according to the concept defined by Erickson and Michelini (1957). The details of the LPI calculation have been outlined previously (Schultz 1993). The vine leaves were classified into 6 age or plastochron classes. The determination of the age classes was based on the individual leaf surface development and maximum photosynthetic rate, or A_{max} (Fig. 1). The first age class consisting of 4 plastochrons (LPI 3-5) corresponds to very young growing leaves in rapid development. The age category from 6-10 represents a developmental stage in which 90-95 % of the photosynthetic potential has been attained and the leaf area expansion is complete. Leaves with a LPI > 10 were pooled into one class because the A_{max} was relatively constant along single shoots. Not included in this class were leaves within 1-2 leaves in close proximity to the fruit (leaves near the cluster), which were placed in a separate category because their photosynthetic activity is highest on the shoot (Zufferey *et al.* 2000). The leaves on the secondary shoots were grouped into apical and basal lateral leaves only because the plastochron concept was not applicable as a result of irregular growth. At the end of vegetative development, the LPI simply represents the position of the leaf on the shoot.

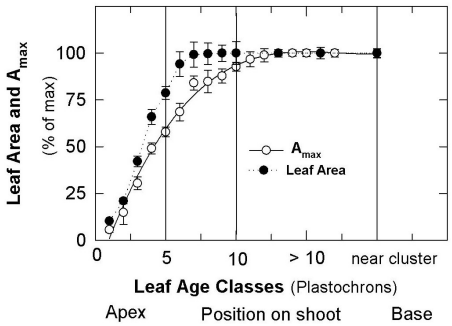

Fig. 1: A schematic presentation of leaf age groups, as expressed in plastochrons, on primary shoots. The age groups were selected on the basis of the leaf area development and maximum photosynthetic activity (A_{max}). 'Chasselas', Pully (CH).

Measurements of leaf dark respiration: The apparent leaf dark respiration (R_D) at the ambient nighttime CO_2 concentration (~ 400 ppm) was measured by using an open gas exchange system (ADC-System, LCA-4, Hoddesdon, England) equipped with a Parkinson leaf chamber. The leaf temperatures were measured with a thermocouple-sensor fixed inside the leaf chamber that was equipped with a ventilator to prevent the excessive heating of the chamber above the ambient temperature. R_D measurements were conducted on well-watered plants at the experimental vineyard in Pully during the different phenological stages (defined by Eichhorn and Lorenz 1977), and on water-stressed plants at the experimental vineyard in Leytron. R_D measurements were made throughout the night (complete darkness, PPFD = 0 $\mu molm^{-2} \cdot s^{-1}$) and sometimes during the late afternoon to obtain R_D data over a wide range of temperatures. The equilibration time to steady state after darkening (with black cloth) was less than 10 min and depended on the leaf age, ambient temperature and CO_2 concentration (Schultz 1991). A leaf area of 6.2 cm^2 was enclosed in the chamber and the whole leaf was completely darkened with a black cloth. All gas exchange parameters were calculated by using the equations of Von Caemmerer and Farquhar (1981).

Modeling the temperature and age response: A simple exponential relation between the R_D and temperature, which has been shown to apply to grapevine plants (SCHULTZ 1991) and other plant species (BUTLER and LANDSBERG 1981), was used in this study as follows: $R_D = a\, e^{(K \times Tleaf)}$, equation (1), where a = the base coefficient (=R_D if T_{leaf} = 0), K = the temperature coefficient of R_D and T_{leaf} = the leaf temperature. The Q_{10}-value (the factor by which the rate of respiration increases for each 10 °C increase in temperature) was estimated from least square regression of natural-log transformed R_D on leaf temperature: $\ln (R_D) = a + b \times T_{leaf}$, equation (2). In equation (2), a (the intercept) and b (the slope) are specific constants, used to estimate Q_{10} as $Q_{10} = e^{10b}$. The relationship R_D as a function of the leaf age (LPI) was described by using the following formula according to SCHULTZ (2003): $R_D = a_1 e^{(a2 \times LPI)} + a_3$, equation (3), where a_1, a_2 and a_3 are parameters describing the shape and the y-axis offset of the curve.

Measurements of plant water status: The pre-dawn leaf water potential (Ψ_{PD}) was measured by using a pressure chamber (SCHOLANDER *et al*. 1965) between 4 a.m. and 5 a.m. in complete darkness, on undamaged and non-senescent leaves of mature height. At the experimental vineyard in Leytron, the effect of the plant water status on the R_D was tested by using irrigated and non-irrigated vines during the 2010 season. Two different irrigation treatments were established. In the first treatment, 9 L m^{-2} (16 L per vine) was drip-fed weekly from bloom to veraison. During the second treatment, no irrigation was applied during the whole growing season. The experiment was performed

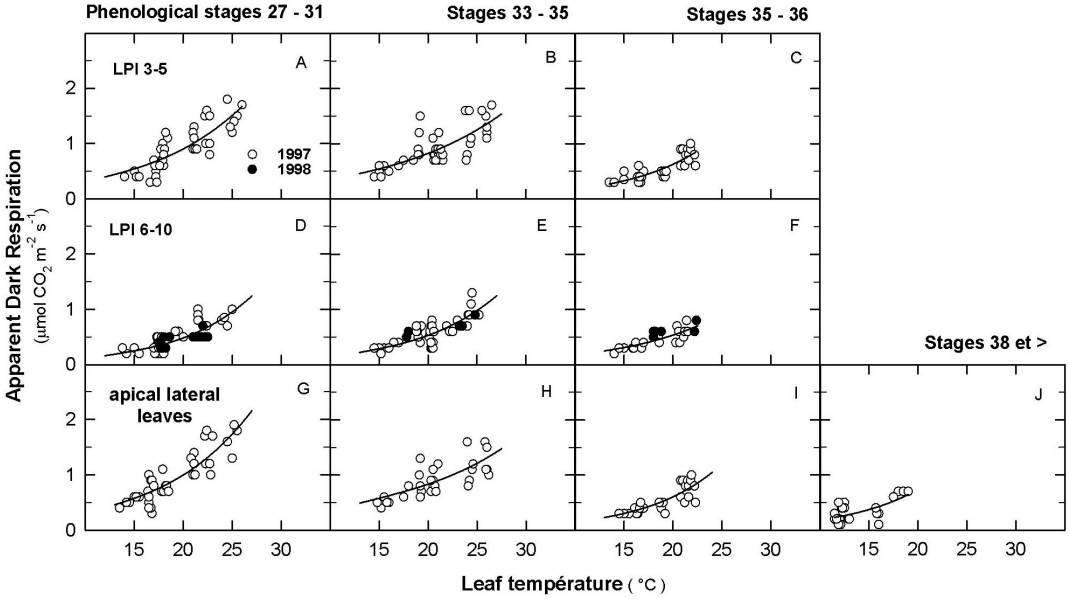

Fig. 2: Influence of the leaf temperature on the apparent dark respiration of young leaves from primary and secondary shoots (LPI 3-5, 6-10 and apical lateral leaves) at different phenological stages on the Eichhorn and Lorenz scale over the growing season. Data (symbols) are presented. Model curves were calculated according to equation (1). 'Chasselas', Pully (CH).

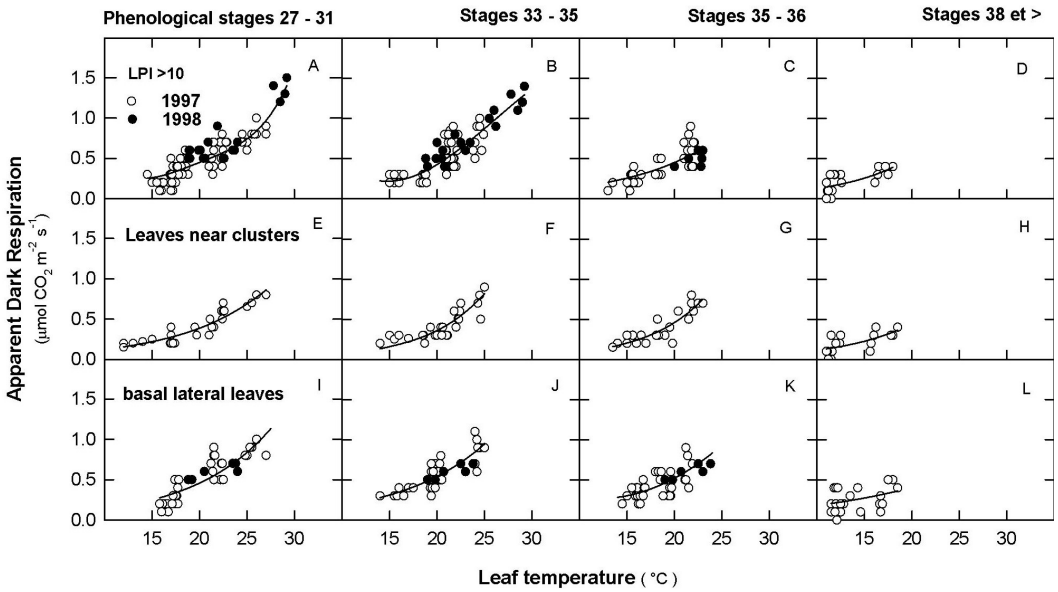

Fig. 3: Influence of the leaf temperature on the apparent dark respiration of adult leaves from primary and secondary shoots (LPI > 10, leaves near clusters, basal lateral leaves) at different phenological stages on the Eichhorn and Lorenz scale over the growing season. Data (symbols) are presented. Model curves were calculated according to equation (1). 'Chasselas', Pully (CH).

on 40 plants per treatment and set out in four randomized blocks of 10 vines each with buffer rows to the left and right.

Results

Effects of the temperature, leaf age and phenology: The R_D increased with the temperature, independent of the leaf type and age and the given period during the season (Figs 2 and 3). Young growing leaves showed higher R_D rates than adult leaves over the whole growing period (Fig. 2). In fact, the R_D values in very young leaves (LPI 3-5) on primary shoots were two times greater than those of mature leaves (LPI > 10, leaves opposite clusters) during the pre-veraison phenological stages (stages 27-35). In addition, the apical leaves on the lateral shoots showed higher R_D values in comparison with the basal leaves during the rapid plant growth phase (stages 27-35). Following veraison (stages > 35), a reduction in the R_D was observed, regardless of the leaf type and age. At the end of the growth period (stages 38 and >), the R_D dropped to very low values (> -0.5 µmol CO_2 $m^{-2} \cdot s^{-1}$) in the adult leaves on primary and lateral shoots. In general, the exponential function gave a good estimate of the R_D response to the leaf temperature. The Q_{10} values, which were estimated by using adjustments of equation 1, tend to decrease during the growing season from the end of June (stages 27-31), reaching 2.1 and 2.0 in July respectively (stages 33-35) then down to 1.7 in August (stage 35, results not presented in this study). During the ripening stage (stages 36-37), a Q_{10} value of approximately 1.5 was observed.

Position of the leaf on the shoot: The position of the leaf on the shoot (and consequently its age) played an important role in the R_D rates. When temperatures were above 15 °C, an increase in the R_D was measured primarily in apical leaves on primary shoots (LPI < 6) (Fig. 4 B-D). The respiratory intensity of leaves that were inserted at the bases of shoots (LPI > 35) changed little with the leaf temperature and remained low. From veraison on, these basal leaves begin to age, and their photosynthetic and respiratory activities gradually shut down. Both the temperature and leaf position on the shoot (which are representatives of age) are the strongest influences on the R_D as long as plant growth takes place. At the end of the growing season, low R_D rates were recorded from senescent leaves, regardless of temperature.

Effects of the plant water status: A rise in the water stress, which was represented by increasingly negative values of Ψ_{PD}, was accompanied by a decrease in the R_D in adult leaves on primary shoots (Fig. 5). The highest R_D values were measured in vines without water restriction, regardless of the temperature. The effect of water stress on the R_D was even more marked under high temperature conditions (Fig. 5 B). A significant drop in the R_D was observed with an increase in water stress when leaf temperatures were between 20 and 25 °C.

Nocturnal and seasonal evolution of R_D: The nocturnal evolution of the R_D in the leaves of different ages on primary and lateral shoots is presented in Fig. 6. The R_D was highest during the first part of the night (before midnight) when the leaf temperature and sugar content (results not presented) were greatest, and they decreased sharply at the end of the night. The highest R_D levels were recorded in the young leaves (LPI 3-5) of primary and lateral shoots (Fig. 6 A, D). Leaves inserted in opposite clusters showed the lowest R_D values (Fig. 6 C).

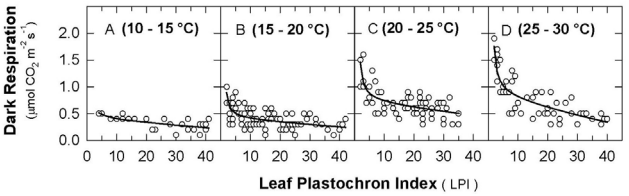

Fig. 4: Influence of the leaf position on the shoot (LPI) in the apparent dark respiration of leaves on primary shoots at various leaf temperature intervals and phenological stages from 31-36. Data are presented. Model curves were calculated according to equation (3). 'Chasselas', Pully (CH).

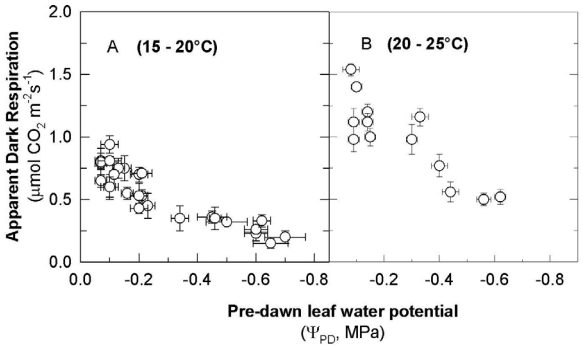

Fig. 5: Relationship between pre-dawn leaf water potential measurements (ψ_{PD}) and the apparent dark respiration of mature and non-senescent leaves on the primary shoots (LPI > 10) at various leaf temperature intervals during phenological stages 31-36. 'Chasselas', Leytron (CH).

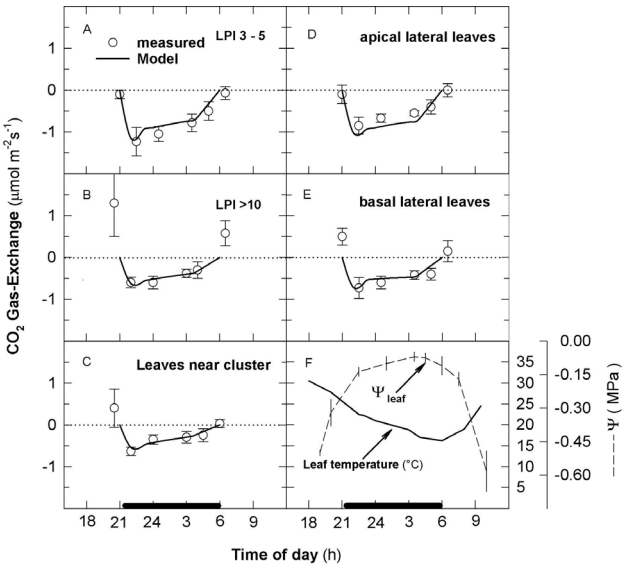

Fig. 6: Nocturnal evolution of the net CO_2 leaf gas exchange on primary (LPI 3-5, >10, leaves near cluster) and secondary shoots (apical and basal lateral leaves) of differing ages (July 21-22, 1997, phenological stages 31-33, A-E). Data (symbols) are presented. Model lines were calculated according to equation (1). Fig. F presents the leaf temperature (°C) and the leaf water potential (Ψ_{leaf}) evolution during the night. 'Chasselas', Pully (CH).

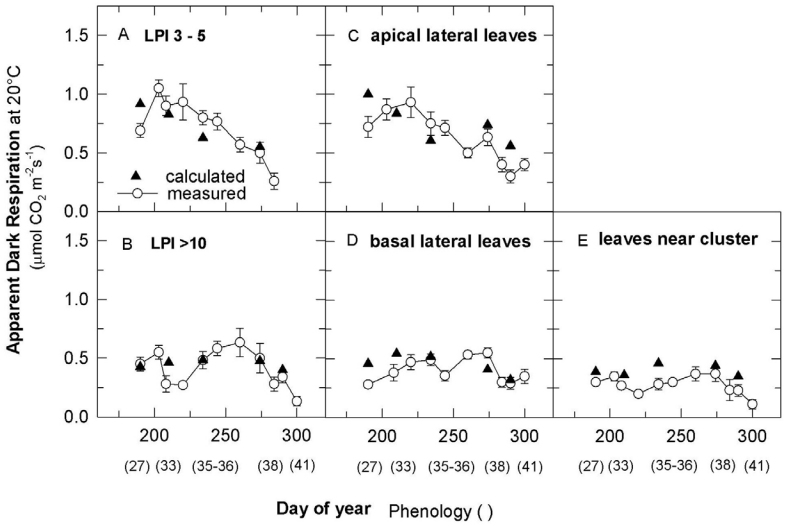

Fig. 7: Seasonal evolution of calculated and measured apparent dark respiration values at a leaf temperature of 20 °C on leaves of different ages from primary (LPI 3-5, > 10, leaves near cluster) and secondary shoots (apical and lateral leaves). Measured data (circles) are presented. Model values (triangles) were calculated according to equation (2). 'Chasselas', Pully (CH).

A simulation of R_D values by using equation 1 gave satisfactory results for the different leaf types and age groups, even though the temperature was the single varying parameter that was taken into consideration by the model. A slight over-estimation of the R_D in apical leaves on lateral shoots was noted by the model in comparison with the measurements. The R_D estimations for adult leaves were remarkably good. At a constant leaf temperature of 20 °C, the R_D rates that were measured in young primary and lateral leaves (Fig. 7 A, C) were greatest at the start of the plant growth period (stages 27-33) during the time of rapid berry and vegetation development. Afterwards, the R_D decreased regularly over the season. In adult leaves on primary shoots, a decrease in the R_D was observed between phenological stages 33 and 35, followed by an increase in respiratory activity during the ripening phase (stages 36-37) (Fig. 7 B, D). At the end of the season, the R_D decreased considerably. Furthermore, in the leaves inserted in opposite clusters (Fig. 7 E), the R_D values were found to be more or less constant throughout the season, and they were the lowest of the plant canopy. Basal leaves on lateral shoots showed R_D values that were comparable with those from leaves on primary shoots (Fig. 7 D).

Discussion

Effects of the temperature, leaf age and phenology: The R_D was found to be largely dependent on the temperature and leaf age, particularly during the vegetative growth period (stages 27-36). A rise in leaf temperature was accompanied by an increase in R_D in the leaves on primary and lateral shoots. Temperature is a major factor in CO_2 losses through R_D as has been demonstrated in numerous plants (Gary 1989, Atkin *et al*. 2000b, Wright *et al*. 2006), including grapevines (Schultz 2003, Palliotti and Cartechini 2005, Schultz and Stoll 2010, Escalona *et al*. 2012, Hochberg *et al*. 2015). During the rapid plant growth phase, young primary and lateral leaves presented the highest R_D rates of all the leaves in the plant canopy, particularly when the temperatures were above 20 °C. The R_D of young developing leaves on primary shoots (LPI < 10) was 2 to 3 times greater than that of adult leaves (LPI > 10). The dissimilation of young leaves on lateral shoots gave R_D rates that were 3 to 4 times greater than those of adult leaves on lateral shoots. Once the growth had stopped, the effect of the leaf age on the R_D was less noticeable.

Meristematic tissues, such as the apices of shoots and roots, very young growing leaves and flowers, generally showed much greater respiratory activity than older plant parts in which a low respiration maintenance level prevailed (Keller 2010). The observed reduction in R_D in young leaves, after plant growth had ceased, most likely reflects the slowing down of the so-called growth respiration (McCree 1970). The R_D changes observed during leaf ontogenesis have been linked to the reduced capacity of the cytochrome pathway, a decrease in glycolysis and leaf soluble sugar concentrations (Azcon-Bieto 1983, Gary 1989, Reddy *et al.* 1991, Noguchi and Terashima 1997), and an acclimation to ambient temperatures (Körner and Larcher 1988, Atkin *et al*. 2000b).

During ripening (stages 36-38), the highest R_D rates were recorded on the basal leaves of lateral shoots, which also presented the greatest photosynthetic activity of all the leaves in the canopy (Zufferey *et al*. 2000). Furthermore, many studies have shown that the R_D rate (as maintenance respiration) correlates positively with the daily canopy or leaf photosynthetic capacity (Azcon-Bieto and Osmond 1983, Atkin *et al*. 2006, Wertin and Teskey 2008, Lewis *et al*. 2011, Slot *et al*., 2013). Our data on the R_D activity in primary and lateral shoots also corroborates the observations made by Schultz (1989; 1991) on the 'Riesling' cultivar in all respects. Previous research (Bosian 1968, Kriedman 1968, Rühl *et al*. 1981, Düring 1988) into various cultivars and the respiratory activity of adult leaves, as measured at approximately 20 °C, has confirmed R_D values that were recorded under similar conditions to those of the

present study. At the end of the plant growth period (stages > 38), a sharp decrease in R_D was observed, independent of the leaf type and age. This finding can be explained in part by the senescence of leaf tissues, and also by plant acclimation to lower temperatures at this time of the year. Moreover, R_D has been associated with leaf nitrogen content (TURNBULL *et al.* 2003, ATKIN *et al*. 2008, REICH *et al*. 2008) which, at the end of the season, may present limiting values for CO_2 net exchanges (ZUFFEREY 2000) because a very high proportion of leaf nitrogen is recycled and exported from leaves into reserve organs during senescence (LÖHNERTZ *et al*. 1989). Acclimating to lower temperatures may well be linked to changes in the cell membrane configuration, leading to an increase in the membrane permeability of the chloroplast, according to BERRY and BJÖRKMAN (1980). In addition to the changes in carbon substrate supply towards the end of the season, the acclimation may also reflect the changing respiratory requirements associated with maintenance (in other words, to the maintenance of the solute gradients, membrane transport and repair of damaged proteins, LAMBERS *et al*. 1998, AMTHOR 2000) or in the phloem loading of senescent leaves on primary and lateral shoots (BOUMA *et al*. 1995). According to ATKIN *et al*. (2000a), the decreased R_D (in darkness) associated with low temperatures could be the result of a lowered carbon supply in the mitochondria and/or variations in mitochondria electron transport (reduced demand in ATP at low temperatures). In this way, key enzyme activities controlling the supply of substrates in the mitochondria (pyruvate dehydrogenase, NAD^+-malic enzyme) would be reduced at low temperatures and could also explain the inhibition of R_D in low canopy light conditions, as observed on shade leaves compared with sun leaves (SCHULTZ 1991).

In conclusion, the R_D response to temperature was especially marked in June (stages 25-27) during the time of rapid plant growth. The Q_{10} values (the factor by which the R_D increases for each 10 °C increase in temperature) reached 2.1 at the beginning of the season, stabilized at approximately 2.0 in the summer (July-August) and decreased during ripening to 1.5 in our study. Seasonal Q_{10} values were calculated at 1.9 for stem, 1.77 for cluster and 1.66 for leaf on potted Cabernet Sauvignon grapevines (PONI *et al*. 2006). High Q_{10} values signify intense physiological and metabolic activity, particularly when the energy requirements for growth are great. During ripening, SCHULTZ (1991) noted that the increase observed in the Q_{10} values (> 3.0) occurred when the photosynthetic activity was high and plant growth was often close to zero. This Q_{10} increase could be linked to rapid berry growth and to the supply of sugars that was exported from leaves to fruit during this period. The lack of agreement on Q_{10} values may be explained by differences in experimental conditions, especially growth temperature and plant acclimation. Q_{10} values do, however, differ according to species (LARIGAUDERIE and KÖRNER 1995, SLOT *et al*. 2013), and they are influenced by the metabolic status of tissues and growth conditions (ATKIN *et al*. 2000a). For example, the highest Q_{10} values were calculated in plants with significant soluble sugar concentrations (BERRY and RAISON 1981, QUICK *et al*. 1992, HÜVE *et al*. 2012).

Effect of water stress: Plant water status, as assessed by measuring the predawn leaf water potential (Ψ_{PD}), affects the R_D activity. The present results clearly indicate that water shortages lead to a decrease in the R_D, particularly when temperatures are high. Various studies have indeed demonstrated that water availability is an important factor in R_D (GALMES *et al*. 2007, AYUB *et al*. 2011) in relation to the temperature (OW *et al*. 2010). Numerous studies have shown that night-R_D (as measured at ambient temperatures) decreases as water stress rises in different plants (FLEXAS *et al*. 2005, 2006, ATKIN and MACHEREL 2009) including grapevines (SCHULTZ 1996, DE SOUZA *et al*. 2003, GOMEZ-DEL-CAMPO *et al*. 2004, ESCALONA *et al*., 2012). Under water stress, the response seems to be related with the plant species and the plant organ investigated (FLEXAS *et al*. 2005) and the plant acclimation. Nonetheless, SCHULTZ and STOLL (2010) point out how little information on respiratory responses of grapevines to environmental factors there is. Question to know if the photosynthesis is more sensitive to severe water stress than respiration remains open for grapevines in comparison with other plants (ATKIN and MACHEREL 2009).

According to AYUB *et al*. (2011), drought may bring about lowered R_D rates in different ways, namely, by reducing the supply of carbon substrates to the mitochondria (similar to the result of reduced photosynthesis); by reducing the demand in respiratory requirements to support cell metabolism; and by reducing the number, structure and composition of mitochondria to support cell metabolism (ATKIN and MACHEREL 2009). Other studies have furthermore shown that the starch concentration in leaves rose under water stress (AYUB *et al*. 2011), which would indicate slowing C-export, suggesting a decrease in the energy demands (ATP) associated with the phloem loading of sugars, which may in turn account for the lower R_D rates. Water stress, depending on its intensity and duration, may also lead to a reduction in the leaf nitrogen (N) content. Because low N contents lead to a reduction in the energetic demands in ATP associated with protein turnover, it is in turn linked to reduced R_D (REICH *et al*. 2008, LEWIS *et al*. 2011). In the present study, however, the small drop in leaf N observed in grapevines that were subjected to water stress (results not presented) does not allow for a confirmation that the N factor played an important role in the reduced respiratory activity of stressed leaves.

Nocturnal evolution of R_D: The nocturnal evolution of R_D was primarily dependent on the temperature and leaf age. The R_D was highest as night began to fall, regardless of the leaf age, and it decreased gradually throughout the night to reach the lowest R_D rates just before dawn. The higher temperatures were measured at nightfall, in comparison with those measured at the end of the night, which provides a partial explanation of the greater R_D values observed as night fell. In the present study, higher starch and soluble sugar contents in leaves were also observed at nightfall than at the end of the night (results not presented). Various studies have demonstrated that global respiration over the nocturnal period could be positively correlated with the photosynthetic activity of the previous day (AZCON-BIETO and OSMOND 1983, NOGU-

chi and Terashima 1997, Atkin *et al*. 2000a). The relation established between leaf photosynthesis and the R_D rate is likely modulated by the soluble carbohydrates and starch accumulated in the leaves during the previous period of daylight (Noguchi and Terashima 1997). Starch degradation is a catabolic process, and along with C-export from leaves during the night, it causes a decrease in leaf carbohydrate content, thus leading to a decrease in R_D over the nocturnal period (Reddy *et al*. 1991). These results would suggest that nocturnal R_D rates are limited in some cases by carbohydrate concentrations (primarily soluble carbohydrates) (Lewis *et al*. 2011). Under controlled environmental conditions, experiments have shown that high levels of carbon substrates are associated with high R_D rates (Atkin *et al*. 2000b). The limited availability of sugars before dawn and/or the energy requirements of leaves (the loading of assimilates in the phloem) would explain the progressive decrease in the R_D. The addition of glucose to leaf tissues brings about an increase in carbon substrates and glycolysis enzymes, which stimulate the R_D (Stitt *et al*. 1990, Geigenberger and Stitt 1991). However, different studies performed under field conditions have not shown any interaction between diurnal canopy light interception and the nocturnal evolution of carbohydrate contents or R_D rates (Atkin *et al*. 2000a, McCutchan and Monson 2001).

The limiting threshold values of carbon substrates for R_D are, as yet, unknown. In addition, the spatial and biochemical partitioning and allocation of sugars inside and between the leaf tissues and cell compartments show the complexity in the relation between leaf carbohydrates and their use by the R_D.

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