Plant and Leaf Physiological Responses to Water Stress in Potted ‘Vignoles’ Grapevine

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Abstract. Several studies have investigated water relationships in grapevines, but the responses to water limitation on individual leaves developed in different shoot positions are scarce in the literature. To begin to fill in this gap, we examined the adaptive responses of vines at the leaf level to varying amounts of water stress using young hybrid ‘Vignoles’ in a controlled growth chamber. We found that the reduction in water availability to 40% of daily evapotranspiration limited shoot and leaf growth, affecting leaf number, shoot elongation, and leaf area. After 2 days of water stress we observed young developing leaves (nodes six to eight from the shoot apex) to have drastically reduced stomatal conductance (gs, about 20 mmol H2O/m2s) and net photosynthesis (Pn, 2 μmol CO2/m2/s). On the 4th day Pn in mature leaves (nodes 9 to 12 from the shoot apex) fell to values below 2 μmol CO2/m2/s. After 6 days, both Pn and gs stabilized at lower values with fluctuations related only to leaf position along the shoot axis. Young leaves revealed substantial enrichment of carbon-13 (13C) and high water-use efficiency suggesting a higher and faster adaptive capacity to water shortage conditions as compared with mature leaves.

Not much is known about the influence of leaf position on photosynthesis in water-stressed leaves. We do know that stomatal control of water loss is an early plant response to water deficit under field conditions (Chaves, 1991; Comin and Massacci, 1996) and that it contributes to a temporary reduction of leaf carbon fixation (Liu et al., 1978; Naor et al., 1994; Schultz, 2003). However, it has been reported for grapevines in the field that irreversible effects on photosynthesis occur when stomata are close during water deprivation and leaves are exposed to direct, high irradiance light for long periods of time (Medrano et al., 2003; Palliotti et al., 2008; Schultz, 1996; Silvestroni et al., 2005). Multiple summer stresses, such as high temperature, radiation regime, and vapor pressure deficit, often exacerbate this process (Cornic, 1994). Furthermore, young immature leaves (10- to 15-d old) are often more susceptible to photoinhibition than mature leaves (Iacono and Sommer, 1996).

A grapevine canopy consists of leaves of different ages and positions that are subjected to variable light intensities during the growing season (Hunter and Visser, 1988). The leaf’s position in the canopy is the primary influence governing its photosynthetic output (Boardman, 1977). Initially as the leaf expands, it is sustained by imported carbon and accomplishes little Pn. Under optimal conditions, it will reach its maximum photosynthetic activity and ambient carbon dioxide (CO2) concentration at or slightly before full expansion (Allwedd et al., 1982). The rate of Pn then steadily declines as it ages toward senescence (Intieri et al., 1992; Nicotra et al., 2003; Poni and Intieri, 1996). Grape leaf Pn is specifically known to peak ≈30 to 40 d after unfolding and to decline thereafter (Intieri et al., 1992; Kriedmann et al., 1970; Poni and Intieri, 1996).

In plant species using the C3 form of photosynthesis, such as grapevine, both the environmental and internal factors that affect photosynthetic capacity and gs are related to carbon isotope discrimination (Δ13C) via their impact on the ratio between intercellular (Ci) and atmospheric (Ca) concentrations of CO2. In contrast to using gas exchange techniques that provide measurements of Pn rates at a single point in time, the Δ13C method offers the advantage of integrating physiological responses of plants to environmental stress (Brugnoli and Farquhar, 2000; Farquhar et al., 1982). The correlation between Δ13C and C/ Ci has been confirmed many times in different species and environmental conditions (Farquhar et al., 1989). Furthermore, the reduction in gs is associated with an optimization of intrinsic water-use efficiency (WUEi, the ratio of Pn to transpiration, ET). An indicator of long-term regulation of carbon assimilation under drought conditions (Bota et al., 2001; Cifre et al., 2005). The isotopic analysis of recently formed starch and sugar offers an estimate of the daily physiological response and, therefore, Δ13C related to short-term soil water availability. While the analysis of isotopic composition in leaf tissue represents not only C/ Ci and WUEi of the growing season, it also reflects the previous year’s carbon assimilation and allocation. Thus, Δ13C is related to long-term environmental changes (Brugnoli and Farquhar, 2000; de Souza et al., 2005; Gaudillère et al., 2002). Throughout the entire plant, the carbon isotope composition (δ13C) is dominated by CO2 assimilation and its diffusion into leaves via internal partitioning and the metabolism of primary assimilates produces differences in δ13C among plant tissues (Brugnoli and Farquhar, 2000; de Souza et al., 2005; Gleixner et al., 1993; Le Roux-Swarthout and Martin, 2001; Leavitt and Long, 1985).

Although the adaptive physiological changes of field-grown grapevines grown under water stress are well-known, less is known of grapevine performance under a more complex situation of simultaneous stress (e.g., water scarcity, excess light, and heat) even though it may be a more common occurrence in the summer and for lengthy periods. A preliminary container study in a controlled growth chamber is a useful model, whereby we can readily induce, control, and specifically study water stress alone or in tandem. The objective of this work was to evaluate the effects of water stress on the morphological and physiological responses of young ‘Vignoles’ potted grapevines in relation to leaf position and age and, subsequently, their ability to discriminate natural carbon isotope during photosynthesis.

Materials and Methods

Plant material and experimental conditions.
We carried out this trial in the Department of Horticulture of Michigan State University (East Lansing, MI) on young grapevines of the French–American hybrid ‘Vignoles’ (Ravat 51), a cross between ‘Seibel 6905’ and ‘Pinot noir’ (Galet, 1979). One-year-old vines were grown in 4-L black polyethylene containers filled with a 2 sand : 3 peat : 5 soil (by volume) mixture. We potted the vines on 10 May and placed them in a greenhouse under ambient light, temperature, and relative humidity. Vines

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were well watered and fertilized, and each shoot was staked at the beginning of the experiment. Three weeks before the application of the water-stress treatments, 12 vines, each with one primary shoot between 80 and 100 cm in length and 20 to 25 leaves, were transferred into a controlled growth chamber (model PGV36; Conviron, Winnipeg, Canada). Any senescent leaves in the basal node positions were removed. We followed by categorizing the remaining leaves into three classes by age: old (46–60 d), mature (31–45 d), and young (15–30 d), according to their position on the basal, medial, and distal parts of the shoot starting about from node 5 or 6 cm then to the apex including the last unfolding leaf. Water stress was applied for a period of 18 d, during which the growth chamber was set to a daily cycle of 25 °C for 16 h (daytime) followed by 18 °C for 8 h (nighttime). The photon flux density during each day was measured at 800 μmol·m⁻²·s⁻¹ (halogen lamps) and the relative humidity averaged 50%.

We divided vines into two groups of six pots each to compare the effects of two different watering treatments: 1) full irrigation (well-watered vines: WW-vines), with daily manual irrigation to restore soil moisture lost by evapotranspiration and to maintain the soil at field capacity; and 2) limited irrigation (water-stressed vines: WS-vines), watered daily to replace only 40% (about) of the WW-vines daily evapotranspiration. The water supply for each treatment was determined daily by weighing each pot using an electronic balance (SB 32001 Delta Range; Mettler-Toledo Inc., Columbus, OH). Differences between daily successive irrigations and daily pot weight for each vine were used as the basis for calculations of the amount of water used by the vine as reported in Lavrenčič et al. (2007). Each vine in both treatments was considered a replicate. We made measurements corresponding to control treatments on the first day of the experiment when all of the plants had been fully watered.

Vine morphology. At the end of the experiment, we measured shoot lengths and counted numbers of leaves in all vines. In addition, leaf area per shoot was measured using a leaf area meter (LI-COR Portable Area Meter model LI-3000; LI-COR Environmental, Lincoln, NE).

Gas exchange. Net photosynthesis, gs, and Ci at saturating light (1000 μmol photon/m²·s) were measured using a CIRAS-2 portable photosynthesis system (PP Systems Version 2.02; Amesbury, MA). Readings were taken on a leaf area of 2.5 cm² when steady state conditions in gas exchange were achieved (≈2 min). The CO₂ concentration (375 μmol·mol⁻¹) and leaf temperature (30 °C) inside the leaf cuvette were controlled by the CIRAS-2. Grapevine photosynthetic capacity was determined on the leaf at every node position in the morning (between 0900 and 1200 h) at 1 d intervals from the beginning through day 12 of the experiment. The WUEi, μmol CO₂/mol H₂O was calculated as a ratio of Pₚ/gs.

Photosynthetic pigment analysis. Total chlorophyll a and b chlorophyll contents were determined on two leaf discs (total area = 2.3 cm²) taken from each leaf in the distal, medial, and basal shoot positions in both treatments. Discs were weighed and ground in a small mortar using a pestle with ≈2 mL of 80% acetone and then transferred to homogeneous tubes with an additional 2 to 3 mL of 80% acetone. Next, we transferred the substance to prechilled centrifuge tubes and re-frigerated each for 10 min in the dark to precipitate proteins. The supernatant was then repeatedly decanted into prechilled graduated tubes until the green color was absent. Pigment concentrations of the extract were calculated by applying the Amon equation (Amon, 1949) to absorbance values recorded at 663, 645, and 470 nm, respectively, using a spectrophotometer (Model ultraviolet-1601; Shimadzu Corporation, Kyoto, Japan).

Carbon isotope discrimination in dry leaf matter. Leaves for the determination of carbon isotope composition (δ¹³C) were sampled from distal, medial, and basal positions of the shoots at the end of the experiment. Leaves were oven dried at 85 °C for 72 h. Dried leaf samples were then ground into a fine homogeneous powder from which 1-mg subsamples were withdrawn for carbon isotope analysis. Carbon isotope content was measured using a continuous flow isotope ratio mass spectrometer (GC-MS; PDZ Europa 20–20 Mass Spectrometer and ANCA-GSL Sample Combustion Unit, PDZ Europa, Sandbach, UK). The δ¹³C was expressed as: δ¹³C = [(Rs – Rb)/Rb] × 1000, where Rs is the ratio ¹³C/¹²C of the sample and Rb is the ¹³C/¹²C of the Pee Dee Belemnite standard (Farquhar et al., 1989). Carbon isotope values were converted to discrimination values by the use of two equations:

\[ \Delta = (\delta a - \delta p)/(1 + \delta p) \]

where δp refers to the isotopic composition of the leaf tissue and δa refers to the isotopic composition of the CO₂ in the atmosphere (−8%), and

\[ \Delta = a + (b - a) \times (Ci/Ca) \]

where the Δ¹³C value of C₃ plants is related to the Ci and Ca ratio based on the model.

It was possible to calculate the theoretical values of δ¹³C using the carbon isotope discrimination formula proposed by Farquhar et al. (1989). The Ci values, taken during Pₚ measurements, and the Ca value, ≈375 μmol·mol⁻¹, were inserted in Eq. [2]. Then, using Eq. [1] it was possible to calculate the theoretical values of leaf carbon isotope composition (δp) where: δp = (δa − Δ)/(Δ + 1) with the objective to generate information on isotope signals during the water-stress period. Calculations were made when all vines were well-irrigated (day 0) and at the 6 and 12 d points from the onset of water stress on leaves from the distal, medial, and basal shoot positions.

Statistical analysis. Results were tested for homogeneity of variance and subjected to analysis of variance (ANOVA) and mean separation test (Student–Newman–Keuls for P = 0.05) was performed using Statgraphics software (version XV; Statpoint Incorporated, Herndon, VA). We used Sigma Plot software (version 10.0; SPSS Chicago, IL) to obtain linear regressions. The effects of water-stress treatments were tested by one-way ANOVA and means separation was calculated by applying the Student–Newman–Keuls test at P = 0.05 level. In the graphics data are shown as mean values ±SE.

Results

Before the water stress application, with vines well-watered, the daily evapotranspiration (ET_daily) was similar for both treatments, ≈0.3 L/d, a value which the WW-vines continued to stay near throughout the trial period (Fig. 1). The ET_daily of WS-vines gradually declined to a minimum of ≈0.13 L/d. From...
the sixth day of the trial, WS-vines maintained a rate of ≈0.15 L/d until the end of the experiment. Vine biometry was closely correlated to water limitation (Table 1). Compared with WW-vines, WS-vines had final shoot length (−57%), leaf number (−46%), and leaf area (−71%) negatively impacted by the water limitation. Internode length and individual leaf area were also reduced by ≈20% and 46%, respectively.

Before the water stress treatments, Pn values were about 5 μmol CO₂/m²/s in both WW-vines and WS-vines. At day 4 post-treatment, the mean Pn values of all WS-vines leaves were significantly reduced to 2 μmol CO₂/m²/s, a figure which remained near constant until the end of the trial (Fig. 2A). In WW-vines, the mean Pn values of all leaves were maintained between 4.5 and 6 μmol CO₂/m²/s during the experimental period (Fig. 2A).

At the beginning of the experiment, when all vines were well watered, gs was similar in both treatments at values near 50 mmol H₂O/m²/s (Fig. 2B). After 4 d of water stress, gs values in WW-vines were maintained between 50 and 60 mmol H₂O/m²/s until the end of the experiment showing a constant water status. The low-water availability affected gs, which was significantly reduced after 2 d of water limitation by ≈30 mmol H₂O/m²/s and reached a minimum value of 20 mmol H₂O/m²/s on day 6 (Fig. 2B).

On the second day of water stress, Ci in WS-vines were reduced to values lower than 180 μmol·mol⁻¹ from the initial values just below 200 μmol·mol⁻¹ in both treatments (Fig. 2C). After 4 d of water stress, Ci in WW-vines increased sharply reaching a peak of ≈200 μmol CO₂/mol and then leveling off at 190 μmol CO₂/mol, slightly higher than WW-vines. Only on day 12 were Ci in the WS-vines characterized by lower values than in the WW-vines (Fig. 2C).

The WUEi values were similar in both treatments, with all vines well irrigated, ≈100 μmol CO₂/mol H₂O (Fig. 2D). The rate of Pn, which is closely associated with the stomata restrictions, decreased in parallel to the reduction in gs and a close relationship was found between gs and Pn (Fig. 3).

Treatment did not affect total chlorophyll concentration (Chl a+b) or chlorophyll a:b ratio (Table 2). The Chl a+b per unit area of the young leaves in the distal positions of WS-vines were 3.4% less than those of WW-vines in the same locations, but without significant differences between treatments. Also, in mature leaves positioned in the medial portion of the shoot, the Chl a+b per unit area were similar between the two treatments, but Chl a+b content observed in WW-vines was 9.8% lower than WS-vines (Table 2). In old stressed leaves, the total chlorophyll concentration per unit area was 5.2% lower than those in WW-vines. Both Chl a+b and Chl a:b ratios were similar in different leaf positions in both WW- and WS-treatments (Table 2).

The physiological responses of leaves according to their position along the shoot axis shows that initially when all the vines were well watered the Pn, gs, and Ci values were quite similar independent of leaf position (Fig. 4). In WS-vines, after only 2 d of limited water supply, Pn and gs significantly decreased especially in distal and medial positions (i.e., from 5 to 13). The young leaves occupying the distal nodes were most affected by the low-water availability as evidenced by the lowered gs of ≈20 mmol H₂O/m²/s and Pn below 3 μmol CO₂/m²/s. The Ci values in these leaves were a little higher than others ranging from 150 to 200 μmol·mol⁻¹ (Fig. 4).

On the fourth day of the water-stress treatment, mature leaves showed further reduction in Pn reaching the minimum of ≈1.7 μmol CO₂/m²/s without limiting gs or Ci (Δ40 mmol H₂O/m²/s and Pn below 2 μmol CO₂/m²/s, respectively). From the sixth day of water stress onward the persistent water shortage led to a general lowering of Pn (about 2 μmol CO₂/m²/s) and gs (≈20 mmol H₂O/m²/s) without marked fluctuations according to leaf position (Fig. 4). In the measurements conducted at 8, 10, and 12 d of water shortage, no significant variations in Pn and gs by leaf position were observed and Ci were maintained at over 150 μmol·mol⁻¹ (data not shown).

We calculated the values of δ¹³C by shoot position from the data obtained in the gas exchange measurements taken at 0, 6, and 12 d of water shortage (Table 3). After 6 d of water stress, the newly developed young leaves in the distal positions had significantly reduced discrimination capacity. The theoretical values of δ¹³C had risen to −21.9% from −24.4% measured at the beginning of the trial (Table 3). The prolonging of the water shortage for 12 d did not change the discriminatory capacity in the mature leaves, which remained quite stable during the water-stress period and similar to that in WW-vines. However, at the end of experiment the old leaves in the basal node positions, with an average age between 50 and 80 d, showed the lowest values of theoretical δ¹³C maintaining high discrimination capacity, namely the lowest water-use efficiency regardless of water availability (Table 3). The ¹³C analysis of leaf tissues at the end of the 18 d water-stress period provided information concerning the composition of carbohydrates present in the leaves, including both structural (those transferred from the reserve organs or formed largely before the trial) and those assimilated during the trial period. The leaf tissues of WW-vines were less rich in ¹³C compared with those of

Table 1. Influence of water stress on vegetative growth of pot-grown ‘Vignoles’ grapevines as compared with well-watered control. Measurements taken at the end of the experiment on the apical shoot portion bearing four growing leaves at trial onset.

| Treatment | Shoot length** | Internode length** | Leaf size** | Leaf count** | Leaf area** |
|-----------|----------------|-------------------|-------------|--------------|-------------|
| WW-vines  | 76.0 a          | 7.04 a            | 91.2 a      | 10.8 a       | 1028.0 a    |
| WS-vines  | 32.9 b          | 5.67 b            | 51.4 b      | 5.8 b        | 298.3 b     |

**Mean per shoot calculated using Student–Newman–Keuls test.

*Significance (*): significant at P = 0.05.
Carbon isotope concentration levels decreased from young leaves (≈–24%) to mature and old leaves (≈–26%), confirming the values found using the δ13C calculation (Table 3).

**Discussion**

Water shortage and the consequent reduction in the water flow through the plant negatively affect vine growth (Matthews et al., 1987; Shellie, 2006). The reduction of shoot length and leaf area of the vines is one of the most evident responses to water stress to reduce transpiration and, therefore, the loss of water (Taiz and Zeiger, 2002). Drought directly inhibits cell division and expansion (Zhu, 2002) and the reduction in growth is generally considered the most sensitive process under water stress conditions (Boyer, 1970; Hsiao, 1973). This outcome could be interpreted as an adaptive response to limit transpiring surface area. Moreover, slower growth allows plants to divert assimilate energy into protective molecules to combat stress and/or to maintain root growth and improve water uptake (Chaves et al., 2010). In grapevine, drought events occurring during the development of vine structures dampens vine growth (Palliotti et al., 2008, 2014; Poni et al., 1993). According to Palliotti et al. (2014), the limitation in the total leaf area per vine, assessed also in this study, subjected to water limitation was caused by fewer leaves per vine (–46%) and smaller leaf size (–29%), which correspond to an average of –43.8 cm2 per leaf.

The measured δ13C values were very low in both treatments before the experiment and during the water-stress period. However, the photosynthetic activity of WS-vines was higher (5 to 6 μmol CO2/m2/s) than that of WW-vines (2 μmol CO2/m2/s). Water-stressed vines showed δ13C values lower than 50 mmol H2O/m2/s after 2 d of water stress and remained low throughout the entire trial period. The values of WUEi obtained in this study confirm that in drought-adapted crops, grapevines have very high WUEi (Bota et al., 2001; Moriana et al., 2002).

According to Cifre et al. (2005), the δ13C values measured in our experiment indicate severe water stress and the occurrence of nonstomatal limitation to Pn. In such a situation, besides a steeper reduction in Pn, the WUEi decreases and the Ci steeply increases (Cifre et al., 2005; Düring, 1987; Flexas et al., 2002). In this experiment, when gS reached minimum values (20 mmol H2O/m2/s) at day six, water stress was intensified and a decrease in WUEi was found because of a higher reduction of gS than Pn. In this phase, Ci values were maintained around 190 μmol CO2/m2 until day 12, which showed lower values than WW-vines suggesting that CO2 availability in the chloroplast could not be regulated by gs. In contrast, although gs values were very low, an increase in WUEi and a strong reduction of C1 (below 180 μmol·mol−1) were observed after 2 d of water stress, suggesting that vines were being affected and the decrease in C1 indicates the stomatal limitations dominate, irrespective of any metabolic impairment (Flexas and Medrano, 2002). This discrepancy in gs values, as an integrative parameter for the degree of water stress, could be explained by very low gs values also in WW-vines sometimes exceeding 60 mmol H2O/m2/s.

The hypothesis that the low photosynthetic activity was due predominantly to stomatal limitations was confirmed by the close curvilinear correlation (R2 = 0.8) found between gs and Pn (Fig. 3) in the study vines. This finding suggests that the reduction of Pn registered in the WS-vines was due mainly to the stomata closure, consistent with reports for other varieties grown in the field (Cifre et al., 2005; Flexas et al., 1998; Palliotti et al., 2007). In addition, the total chlorophyll values determined at the end of the experiment in leaves from distal, medial, and basal shoot positions were similar in both treatments and in the same leaf position. Contrary to field experiments (Palliotti et al., 2008, 2014) and greenhouse work (Bertamini et al., 2006; Perez-Martin et al., 2009), the water deprivation in our experiments did not modify photosynthetic pigment concentrations, likely due to the lack of oxidative stress generally caused by conditions of high light and temperature.

Different Pn values among leaves in different positions along the shoot characterized leaf gas-exchange measurements in the water stress treatment. The evolution of Pn values is closely correlated to the discrimination of both 13CO2 atmospheric and 13C in leaf tissue (Farquhar et al., 1982, 1989). Theoretical δ13C calculations produced different 13C values among young, mature, and senescent leaves along the shoot suggesting a different Δ13C. According to gas exchange values, young leaves in the distal shoot positions suffered the effects of water shortage immediately (2 d from onset of water stress) and the first response was an immediate reduction in gs and, as a consequence, a rapid reduction in Pn activity. In this phase, gs was low in relation to Rubisco capacity, Δ13C was regulated by diffusion, and Δ13C was reduced as underlined by theoretical δ13C values. Mature leaves in the medial positions of the shoot suffered the water limitation after 4 d from onset of water stress; they had Pn values between those of young and old stressed leaves, and were characterized by intermediate theoretical δ13C values (≈–24%). Old leaves in the basal positions did not appear to be affected by water limitation, as underlined by low theoretical δ13C values (close to –26%). According to theoretical δ13C values and low Ci values (Table 3), indicative of decreased Ci/Ci ratios, only after 12 d of water stress did the theoretical δ13C values increase to –24%, suggesting a reduction of Δ13C.

**Table 1. Total chlorophyll concentration (Chl a+b) and Chl a/b ratio determined at the end of the trial (after 18 d of water stress) of pot-grown ‘Vignoles’ grapevines as compared with the well-watered control.**

| Treatment | Chl (a+b) (mg/dm2) | Chl (a/b) (mg/dm2) |
|-----------|------------------|-------------------|
| WW-vines⁴ | 3.8              | 3.7               |
| WS-vines⁵ | 3.7              | 4.1               |
| Significance | NS               | NS                |

⁴Leaf position: D = distal; M = medial; B = basal.
⁵Vines watered daily with 100% of daily evapotranspiration.
⁶Vines watered with 40% of daily evapotranspiration of control vines.
⁷Significance: significant at P = 0.05.

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**Fig. 3. Relationship between stomatal conductance (gs) and net photosynthesis (Pn) in pot-grown ‘Vignoles’ grapevines subjected to water stress [40% of daily well-watered vines (WW-vines) evapotranspiration, water-stressed vines (WS-vines)] and well-watered (100% of daily evapotranspiration, WW-vines). The best-fit adjustment is shown as a third-order nonlinear regression, Modified Hyperbola III.**
Measures of carbon isotope concentration in the dry leaf matter of WS-vines were lower, demonstrating the water treatment effect. The $\delta^{13}$C measured on leaves along the subject shoots confirms that young stressed leaves in the distal positions suffered greatest from the effects of water limitation as characterized by a substantial enrichment of $\delta^{13}$C (maximum $\delta^{13}$C values) followed by lower $\delta^{13}$C. These leaves quickly adapted to water stress and showed a greater WUEi, according to the inverse relationship between $\delta^{13}$C and water-use efficiency by Farquhar et al. (1982, 1989).

Mature and old stressed leaves were subjected less to water deprivation effects revealing an intermediate degree of water stress and resulting in a lower sensitivity and less rapid adaptability, as demonstrated by the lowest values of $\delta^{13}$C (~26.0% and ~26.1%, respectively) and suggesting a lower WUEi and higher discrimination capacity compared with atmospheric $^{13}$CO$_2$.

**Conclusions**

The application of water stress, without excessive temperature and light, reduced both evapotranspiration and shoot growth, which suggests an adaptive response that reduces transpiration by leaf area limitation and, consequently, water loss. The low-water availability negatively influenced $g_s$ and induced a significant decrease in $P_n$ associated with stomata closure. The close relationship between $g_s$ and $P_n$ ($R^2 = 0.8$) and unmodified photosynthetic pigment concentrations suggest that: 1) the vines were subjected to moderate water stress despite the low $g_s$ values of around 20 mmol H$_2$O/m$^2$/s and, 2) the impact on stressed leaves was ameliorated by stomata closure, considered the main factor responsible for the decrease in the net transpiration.

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**Table 3. Influence of water stress on theoretical carbon isotope composition (theoretical $\delta^{13}$C) calculated at the trial onset, when all vines were well watered (day 0), after 6 and 12 d of water stress (day 6 and day 12). Measured carbon isotope composition (measured $\delta^{13}$C) determined at the end of the trial (after 18 d of water stress) of pot-grown ‘Vignoles’ grapevines as compared with the well-watered control.**

| Leaves | Theoretical $\delta^{13}$C (%) | Measured $\delta^{13}$C (%) |
|--------|-------------------------------|---------------------------|
|        | Day 0 | Day 6 | Day 12 | Day 18 | Day 0 | Day 6 | Day 12 | Day 18 |
| WW-vines |  | | | | | | | |
| WS-vines |  | | | | | | | |
| D | $-25.7^b$ | $-24.4^a$ | NS | | $-23.0^a$ | $-21.9^a$ | NS | | $-22.9^a$ | $-21.7^a$ | NS | | $-25.1^a$ | $-24.3^a$ | * |
| M | $-23.9^a$ | $-24.3^a$ | NS | | $-23.3^a$ | $-23.9^a$ | NS | | $-24.4^a$ | $-24.2^a$ | NS | | $-26.4^b$ | $-26.0^b$ | NS |
| B | $-25.5^b$ | $-24.9^a$ | NS | | $-24.7^b$ | $-25.4^b$ | NS | | $-25.2^a$ | $-24.3^a$ | NS | | $-26.8^b$ | $-26.1^b$ | * |

Significance: * Significant at $P = 0.05$.

*Vines watered daily with 100% of daily evapotranspiration.

*Vines watered daily with 40% of daily control vines evapotranspiration.

*Means separated within columns by Student-Newman-Keuls test.

*Leaf position: D = distal; M = medial; B = basal.
photons.

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The young stressed leaves on the distal portion of the shoot showed a high capacity to adapt to water limitation through quick stomata closure, a lowering of the discrimination of atmospheric 13CO2 and an increase of the WUEi. The mature and old leaves at the medial and basal shoot positions, respectively, appeared to adapt more slowly to water stress by maintaining high discrimina-
tory capacity to 13CO2 without concurrent increases in the WUEi.

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HortScience Vol. 50(10) October 2015 1497