Vineyard water relations in a karstic area: deep roots and irrigation management

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ABSTRACT

Ongoing variations in rainfall and temperature regimes affect the physiology and productivity of grapevines, calling for irrigation in drought-prone areas. During vintage 2015, we monitored plants water status and indirectly assessed rooting depth and exploited water sources (oxygen isotope analyses) in a mature *Vitis vinifera* cv. Malvasia Istriana vineyard on red soils ("terra rossa") developed on highly permeable carbonate rocks. We also investigated effects of topsoil irrigation or late summer rains on plant water status and yield. Under the harsh summer environmental conditions of 2015, the plant water status was overall favorable (moderate water deficit) and never reached critical levels, suggesting that irrigation was not mandatory. Leaf conductance to water vapor (g_L) measured in July decreased by about 70% compared to spring, while minimum leaf water potential (Ψ_{min}) dropped by only 16%, suggesting an isohydric behavior of the cultivar (strict stomatal control of transpiration). Both Ψ_{min} and g_L reached a minimum in July (peak of drought), and returned to pre-drought values in late summer. Rainfalls or supplemental irrigation (about 40 mm) promoted prompt recovery of plant water status. Irrigation treatments or occasional summer rainfalls can influence the water status of plants, although roots have access to deep water sources. In fact, the isotopic composition of xylem sap was similar to that of soil water sampled in a nearby deep cave, supporting the hypothesis that deep soil is the main water source for grapevines in karstic areas during summertime. Deficit irrigation, based on careful evaluation of physiological indicators of plant water status, might be an effective strategy for promoting sustainable viticulture, and a rationale use of water resources in karstic ecosystems.

1. Introduction

Grapevine (*Vitis vinifera* L.) is a crop widely cultivated in many countries (Lovisolo et al., 2010; Costa et al., 2016). Several vineyards regions are characterized by seasonal drought, imposing significant constraints on yield and quality. Rising global temperatures coupled to prolonged droughts (IPCC, 2014) have already negatively affected plants' growth and production in both natural and agricultural ecosystems (Marx et al., 2017; Nardini et al., 2014; Potopová et al., 2017; Tripathi et al., 2016). The projected increase in frequency/severity of anomalous drought events (IPCC, 2014) calls for adaptation of viticulture to climate change, by using drought-tolerant rootstocks/cultivars and suitable agronomic practices (Costa et al., 2016; Ferlito et al., 2014; Herrera et al., 2015; Koundouras et al., 2008; Lopes et al., 2011). Vineyards are traditionally rain-fed in the Mediterranean area, although irrigation practices are increasing to guarantee stable yield production, while in many other regions viticulture can thrive only when irrigation is available (Costa et al., 2016; Lovisolo et al., 2010).

Drought responses of grapevine have been investigated from a physiological and molecular point of view to select more resistant varieties/genotypes (Acevedo-Opazo et al., 2010; Bota et al., 2016; Chaves et al., 2010; Medrano et al., 2015; Tombesi et al., 2014). In general, grapevine responses to drought are influenced by the environment in which the plants grow (Hochberg et al., 2017), but are also partly cultivar-dependent, with some of them displaying relatively high resistance/resilience to environmental stress (Chaves et al., 2010;

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Medrano et al., 2015; Tombesi et al., 2014). In particular, cultivars differ in physiological traits which are at the base of their potential resistance to drought, i.e. osmoregulation, water use efficiency, vulnerability to xylem embolism, and stomatal response to water deficit (Bota et al., 2016; Chaves et al., 2010; Medrano et al., 2015; Tombesi et al., 2015). Hence, water use strategies of grapevine were suggested to range from perfect isohydry (strict stomatal control) to anisohydry (reduced stomatal control), although recent studies call for a revision of this terminology (Hochberg et al., 2017; Nardini et al., 2018; Schultz and Stoll, 2010; Tombesi et al., 2014).

Optimization of water use in arid-prone areas is the key to prevent wasting of water resources (Acevedo-Opazo et al., 2010; Chaves et al., 2010; Fernández and Cuevas, 2010; Tripathi et al., 2016). Deficit irrigation approaches significantly reduce the "water footprint" of agriculture, and in particular of vineyards (Chaves et al., 2010; Schultz and Stoll, 2010). Different physiological indicators can be used to assess plant water status and regulate water delivery, including soil water content/potential, plant stem diameter variation, sap flow, thermal and visible imaging (Brillante et al., 2016; Fernández and Cuevas, 2010; Lopes et al., 2011). However, the most reliable parameters to quantify plant water stress are pre-dawn, minimum, and stem water potential (Ψ_{pd}, Ψ_{min}, and Ψ_{stem}, respectively), as well as stomatal conductance to water vapor (Acevedo-Opazo et al., 2010; Fernández and Cuevas, 2010; Flexas et al., 2002; Medrano et al., 2015; van Leeuwen et al., 2009). Deficit irrigation based on water potential measurements has emerged as a strategy allowing grapevines to withstand water shortage with non-significant decreases of yield, and positive impacts on fruit and wine quality (Chaves et al., 2010; dos Santos et al., 2003; Girona et al., 2006; van Leeuwen et al., 2009). As an example, Acevedo-Opazo et al. (2010) reported that a regulated mild water stress ($\Psi_{min} = -1.3$ MPa) in Cabernet Sauvignon vines leads to 13% increase in skin to pulp ratio (compared to well-watered plants) and to significant increments in soluble solids and anthocyanins, without affecting pruning weight but assuring about 90% water saving. These results are in accordance with those reported by other authors, suggesting that moderate water deficit exerts direct and/or indirect effects on bunch development with consequent higher content of polyphenols (anthocyanins, flavonols, tannins), stilbenes, carotenoids, and terpenoids (Herrera et al., 2015; Medrano et al., 2015; Sivilotti et al., 2005; van Leeuwen et al., 2009).

The effectiveness of irrigation strategies in improving plant water status and productivity depends on a combination of plant-, climate- and soil-related factors. In particular, root hydraulic properties and distribution in the soil are fundamental traits influencing both plant water relations, and plant responses to rain events or irrigation treatments. Soil structure, stoniness, and the depth of the water table significantly influence root growth, while the genotype has relatively little influence (Deloire et al., 2004). However, different rootstocks can partially influence water supply to the plants, making mandatory the correct selection of rootstocks adapted to local climate and soil type (Deloire et al., 2004; Koundouras et al., 2008; Nardini et al., 2006). Grapevine root systems have been studied in a range of climates (Mediterranean, humid continental, subtropic) and soil textures (loam, clay, sand), revealing that approximately 80% of roots lies within the upper 1 m (Celette et al., 2005; Smart et al., 2006). The few studies addressing maximum rooting depth suggested that *V. vinifera* roots can reach depths of more than 6 m. However, even deeper rooting patterns cannot be excluded in water limited environments (Smart et al., 2006). Significant gaps remain in our understanding of rooting depth and water relations of grapevines growing on shallow soils overlying fractured bedrock, mainly due to experimental difficulties limiting the use of the "profile wall method" based on excavation (Smart et al., 2006). However, limestone environments subjected to marked moisture stress are relatively frequent across European wine-producing regions (FAO, 1981). In karstic ecosystems, plants can develop deep roots growing through rock cracks and fissures often filled with clay pockets, that might represent important water sources (Estrada-Medina et al., 2013a,b; McElrone et al., 2004; Nardini et al., 2016; Querejeta et al., 2006; Schwinning, 2010). It is not clear whether grapevine can also adopt a similar strategy, and how this eventually relates to the effectiveness of irrigation strategies in such substrates. Hence, considering the ongoing climate changes and the economic importance of viticulture in limestone-dominated regions, information on vines rooting depth is fundamental for future irrigation scheduling, and water management.

This study was carried out in the Classical Karst (NE Italy), an area which experienced an anomalous summer drought in 2012 (+2.3 °C and −50% rains compared to the historical mean) leading to important losses of wine production, and posing a new threat to local agriculture. The loss of yield and plant mortality were mainly a consequence of scarcely developed irrigation systems and practices, not based on actual plants water needs. We monitored grapevine water status over a growing season, indirectly assessed rooting depth and estimated which water sources are exploited by plants in a mature karstic vineyard. We hypothesized that a deep rooting system enables plants to thrive under summer harsh environmental conditions. Furthermore, we investigated effects of irrigation of top soil on plant water status and yield.

2. Materials and methods

2.1. Study site and plant material

The research was carried out in a commercial vineyard in NE Italy (6 km from the town of Trieste, 45° 44′ 10″ N, 13° 45′ 2″ E; 290 m a.s.l) during the 2015 growing season. The area is located in the Classical Karst, a plateau extending between Italy and Slovenia dominated by carbonate rocks (mainly Cretaceous limestone and dolostome; Jurkovšek et al., 2016), covered by few centimeters of red karst loam ("terra rossa", red soil, carbonate and flysch product; Lenaz et al., 1996; Mrak and Repe, 2004). The climate is semi-Mediterranean, with strong continental influences, warm and dry summers, and mild winters. The average annual temperature is 13 °C, and yearly rainfall is 1385 mm, with less than 200 mm falling in July-August (www.osmer.fvg.it, 1992–2017). The effects of relatively high precipitation on natural vegetation and crops are however contrasted by high permeability of the substrate (Mrak and Repe, 2004).

The studied cultivar was *V. vinifera* cv. Malvasia Istriana, a local white wine variety largely cultivated in Croatia, Slovenia and Italy. In the Classical Karst, "Malvasia Istriana" is of high economic importance as one of the leading wine varieties (AIS, 2010; Bianchi et al., 2008). A mature 25-years-old vineyard of about 0.1 ha with grapevines grafted on SO4 rootstock was selected. The planting density was 5000 plants per hectare, with vines spaced 1 m and 2 m within and between rows, respectively. The row orientation was NW-SE. Annual pruning was performed in late winter by leaving three canes per plant, while during spring the shoots were trained to trellis (wires). According to traditional practices, some summer leaf removal was performed as part of canopy management. The substrate consisted of about 40 cm deep red soil laying on fractured carbonate bedrock. The bedrock consists in dolostones and limestones, and is widely and deeply karstified (Zini et al., 2015). The underground karst features mainly consists in karstified vertical fractures which can be empty or filled with soil. According to local cultural practices, the soil was tilled to a depth of 20 cm two times during the growing season. Throughout the study period, air temperature (T_{air}) and relative humidity (RH) were recorded on hourly basis, using two data loggers (EasyLog-USB-2, Lascar Electronics Inc., Salisbury, UK) installed at 1.5 m height, facing north, and partially shielded with aluminum foil to prevent over-heating. Average midday daily T_{air} and RH (11:00–14:00, solar time) were used to calculate maximum vapor pressure deficit, as VPD = $E_0 \times$ (1–RH), where E_0 is the saturated vapor pressure at any definite T_{air}. The daily reference evapotranspiration (ET_0) was calculated with the Penman-Monteith equation (Snyder and Eching, 2007). Rainfall data were obtained from the nearby Sgonico weather station (www.osmer.fvg.it). In May 2015, two 40 cm deep holes were dug between two pairs of neighboring vines (50 cm from the trunk base), the soil was sampled for further analysis (see following sections) and a pre-calibrated (see below) soil moisture content sensor (WC, EC-5, Decagon Devices Inc.) was installed in each hole (45° angle).

The study vineyard was selected on the basis of its proximity (about 150 m) to a cave (Caverna Monte Vides, www.catastogrotte.fvg.it), with easy access for deep soil sampling and measurements of water isotopic composition (see following sections). In fact, due to karstic terrain and limestone base it was not possible to find proper bedrocks/fractures under the vineyard, therefore groundwater sampling was performed in the nearby cave which extends for 32 m and reaches a depth of about 7 m below soil surface.

At the peak of the summer aridity, according to traditional management practices, two supplemental irrigations were applied in a sub-area of the study vineyard (IR, about 50% of vineyard), while the other half of the vineyard was kept non-irrigated (N-IR). Surface drip irrigation (about 3 l per hour per plant) was applied during the nights of 17–18th July and 31st July providing 400 $m^3 ha^{-1}$ and 200 $m^3 ha^{-1}$ of water, respectively (Fig. 1). The irrigation volumes were in agreement with those generally applied in other semi-arid wine-producing regions (Acevedo-Opazo et al., 2010; Vaz et al., 2016; Greer, 2017). Approximately 25% of the vineyard area was considered for soil sampling and physiological measurements, while samples for isotopic analyses were collected from randomly selected vines growing over the whole vineyard.

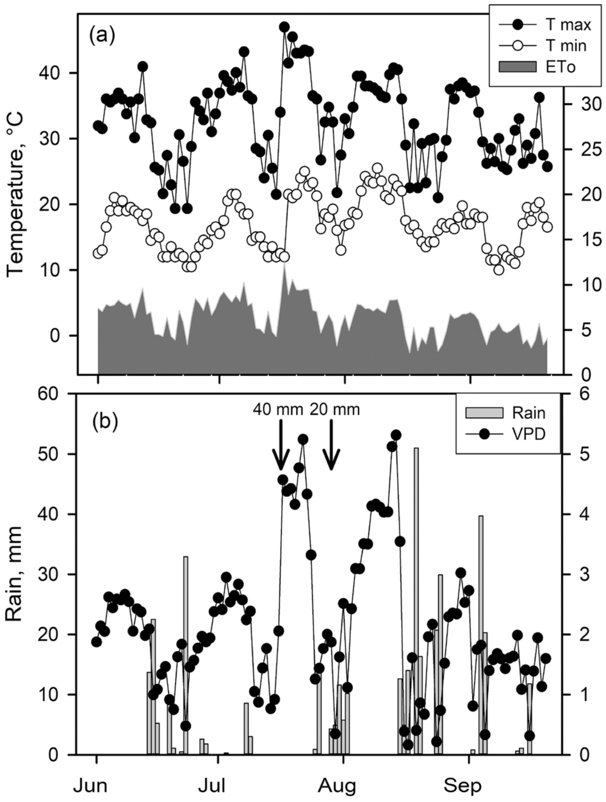

Fig. 1. a) Daily maximum (T_{max}, closed circles) and minimum (T_{min}, open circles) air temperature, and daily reference evapotranspiration (ET_0, dark grey area); b) rainfalls (grey bars) and midday vapor pressure deficit (VPD, closed circles) as recorded from June to September 2015 in the study vineyard. Arrows indicate the two irrigation treatments applied during the nights of 17–18th July and 31st July.

2.2. Soil moisture release curves and bulk density

To characterize the water relations of the local red soil, soil bulk density and the relationships between water content (WC) and water potential (Ψ_{soil}, moisture release curves; Savi et al., 2014) were measured on soil samples obtained from the vineyard. Due to frequent and decades-long tilling, the soil profile of the karstic vineyards is considered highly homogeneous. To verify this hypothesis, two shallow (0–10 cm) and two deep (30–40 cm) soil samples were collected, about 50 m apart of each other. Four sub-samples (about 1 l each) were gently, but abundantly watered to field capacity (SWC, saturated water content). Sample holders were filled with a few grams of rehydrated soil, and Ψ_{soil} (WP-4 dewpoint hygrometer, Decagon Devices Inc, Pullman) and fresh weight (FW) were sequentially measured during progressive soil bench-dehydration. After complete oven-drying (48 h at 45 °C) samples were re-weighed to obtain their dry weight (DW). WC was calculated as (FW-DW)/DW, and plotted versus the corresponding Ψ_{soil} values. A regression curve function was used to interpolate the theoretical volume of available water (AWC, calculated as SWC − WC at $\Psi = -1.5$ MPa; Lambers et al., 2008).

The soil moisture content sensors (see above) were pre-calibrated by installing them in pots with a known amount of substrate at field capacity (about 1 l). Pots were maintained in the laboratory and soil was progressively air-dehydrated. Substrate WC was periodically measured (see above) and related to the volumetric water content (v/v) recorded by sensors. The regression line was used to convert values of v/v in WC, and subsequently in Ψ_{soil} using the moisture release curve function. Finally, five oven dried soil samples (DW) of about 20 g each were tightly wrapped in parafilm. The sample volume (V) was measured with the water displacement method (Hughes, 2005), and the dry bulk density estimated as: DW/V.

2.3. Seasonal changes in plant water status and stomatal conductance

To quantify plant water status, and assess the effects of irrigation treatments, pre-dawn (Ψ_{pd}) and minimum leaf water potential (Ψ_{min}), as well as leaf conductance to water vapor (g_L), were measured from June to September 2015 on selected sunny days (PPFD in the central hours of the day > 1500 $\mu mol\, m^{-2} s^{-1}$). On 11th June, 7th, 17th and 21st July, 5th August, and 1st September, fully expanded, undamaged leaves were collected from the south-exposed part of the crown of at least five plants (non-irrigated, N-IR). Leaves were sampled between 4:00 and 5:00 (Ψ_{pd}) and between 11:00 and 13:00 solar time (Ψ_{min}, two leaves per individual), wrapped in cling film, sealed in plastic bags containing a piece of wet paper and transported to the laboratory in a cool bag. The water potential was measured with a pressure chamber (mod. 1505D, PMS Instruments Company, Albany, USA) within two hours after sampling. On the same days, midday g_L and leaf surface temperature (T_{leaf}) were measured on at least two leaves per individual (including leaves used for Ψ_{min} measurements) with a steady state porometer (mod. SC-1, Decagon Devices Inc, Pullman, USA), and an infrared thermometer (mod. 805, Testo SE & Co. KgAa, Lenzkirch, Germay). On 21st July, 5th August, and 1st September (after irrigation treatments, see above) all measurements were performed on at least five irrigated vines (IR), as well. Measurements were taken from the middle rows of the irrigated sub-area of the vineyard to reduce border effects.

2.4. Oxygen isotope composition

To identify water sources exploited by grapevines and estimate rooting depth, the oxygen isotope composition ($\delta^{18}O$; Lubis et al., 2014; Phillips and Gregg, 2001) of rain, deep soil water, irrigation water, and xylem sap was measured from June to September 2015. On 7th and 21st July, 5th August, and 1st September, healthy stems were sampled from three to five randomly selected vines (N-IR). Bark and leaves were immediately removed; the stem was cut in 2–3 cm long pieces and sealed in plastic bags. On 21st July, 5th August, and 1st September (after irrigation treatments, see above) sampling was performed on irrigated vines (IR), as well. Rainfall was collected in the periods 11th June − 7th July and 21st July – 1st September, using a rain-gauge equipped with anti-evaporation paraffin oil layer. Samples of deep soil were collected on 7th July and 1st September in the nearby cave (see above), at an estimated depth of about 7 m below soil surface, and sealed in plastic bags. All samples were transported to the laboratory in a refrigerated bag and stored frozen at −20 °C. A cryogenic vacuum distillation line was used to extract water from stem and soil samples, while avoiding isotopic fractionation (Orlowski et al., 2013). Water samples were treated with active charcoal (0.1 mg ml^{-1}), filtered at 0.25 µm, and their oxygen isotope composition ($\delta^{18}O$) was measured with isotope-ratio mass spectrometry (Delta Plus Advantage, Thermo Fisher Scientific, Waltham, USA, for details see Nardini et al., 2016).

2.5. *Yield related parameters*

In order to study the effects of irrigation on vine productivity, yield measurements were performed at the date of harvest (19th September 2015). Fruits from five IR and five N-IR plants were harvested and total yield weight and berries diameter were recorded. Berries diameter was measured on at least eight berries for four clusters per plant (for a total of 160 berries per treatment) using a digital caliper (IP54, Shenzhen Pride Instrument Inc, Shenzhen, China). The average berries diameter per cluster and per plant was calculated.

2.6. *Statistical analysis*

Data were statistically analyzed using SigmaPlot v13 (Systat Software Inc, Chicago, USA). Data normality and homoscedasticity were assessed and statistically significant differences were defined with Student's *t*-test and One-way analysis of variance (ANOVA) followed by Pairwise multiple comparisons (Holm-Sidak method). The level of significance was set at $P = 0.05$. Means ± standard error (SEM) are reported ($n = 5$).

3. Results

Daily maximum and minimum air temperature, precipitation, ET_0, and midday VPD recorded in the study vineyard from 1st June to 20th September 2015 are reported in Fig. 1. Summer rainfalls did not differ significantly from those of the 1992–2017 reference period (www.osmer.fvg.it) and averaged about 300 mm (June–August). On the other hand, the spring season (March-May) was drier than normal, with a precipitation anomaly of about −40% (−120 mm). Compared to the historical data, mean T_{air} in 2015 was by about 0.5 °C and 1.7 °C higher in spring and summer, respectively, with absolute maximum temperatures above 40 °C recorded by the data loggers in the study vineyard in July (Fig. 1a). VPD varied consistently during the growing season, with the lowest and the highest values corresponding to 5.3 kPa and 0.2 kPa, respectively.

Fig. 2 shows the relationship between WC and Ψ_{soil} as measured for superficial and deep red karst loam (two samples per depth). Saturated water content (SWC) and theoretical volume of available water (AWC) did not differ between the two depths, hence data were averaged. The SWC of the red soil was 0.34 ± 0.002 g g^{-1}, while the AWC was 0.16 ± 0.006 g g^{-1}. The soil dry bulk density was 1360 ± 9 kg m^{-3}.

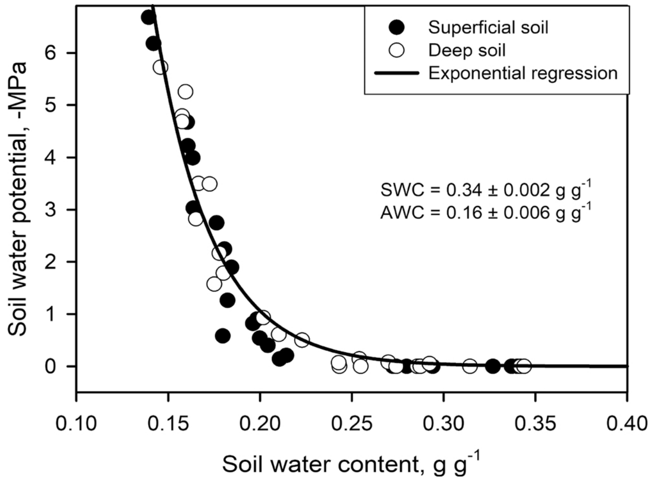

Fig. 2. Relationship between water potential (Ψ_{soil}) and water content (WC) as measured for superficial (closed circles) and deep (open circles) local karst loam (red soil). The regression curve is expressed by the following function: $y = a \times \exp(-b \times x)$. Coefficients: $a = 640.0$, $b = 32.0$, $R^2 = 0.94$. Saturated water content (SWC) and theoretical volume of water available to plants (AWC) are also reported.

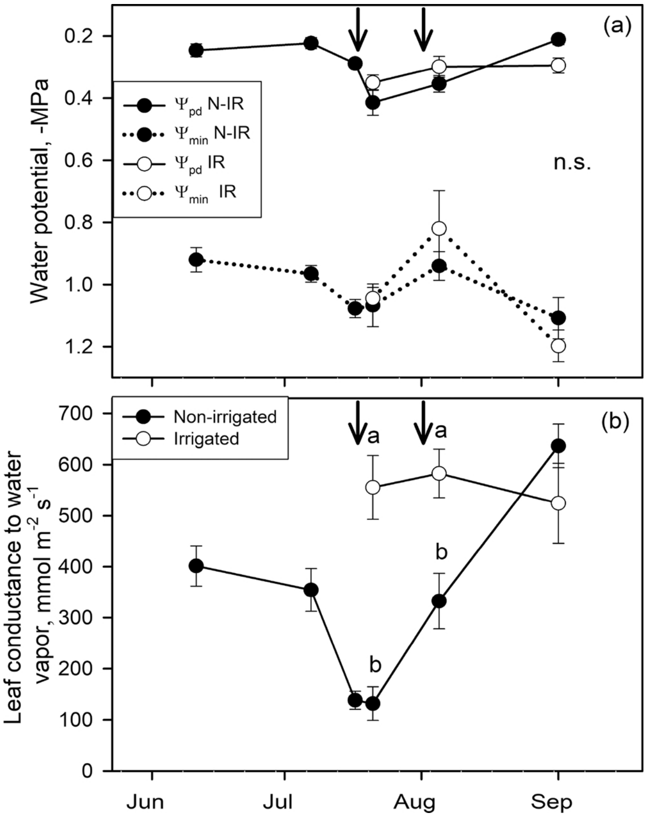

Fig. 3. Seasonal changes of pre-dawn (Ψ_{pd}) and minimum (Ψ_{min}) leaf water potential (a) and leaf conductance to water vapor (g_L, b) as measured in non irrigated (N-IR, closed circles) and irrigated (IR, open circles) vines from June to September 2015. n.s. indicates the lack of significant differences between experimental treatments. Different letters indicate significant differences between N-IR and IR plants (Student's *t*-test, $P < 0.05$). Arrows indicate the two irrigation treatments applied during the nights of 17–18th July and 31st July. Error bars represent the SEM ($n = 5$).

In late spring (June) and at the beginning of summer, grapevines had a favorable water status as Ψ_{pd} and Ψ_{min} were around −0.2 and −0.9 MPa, respectively (Fig. 3a). The high soil water availability ($\Psi_{soil} = -0.1$ MPa, WC = 0.27 g g^{-1}) was reflected in high g_L, which averaged about 380 mmol $m^{-2} s^{-1}$ (Fig. 3b). After the first week of July, Ψ_{pd} and Ψ_{min} progressively decreased reaching the lowest values of −0.4 MPa and −1.10 MPa (N-IR plants), respectively. At the peak of the summer aridity ($\Psi_{soil} < -2.5$ MPa, WC < 0.17 g g^{-1}), a significant stomatal closure was observed (minimum values = 130 mmol $m^{-2} s^{-1}$), likely causing T_{leaf} to increase up to 37 °C

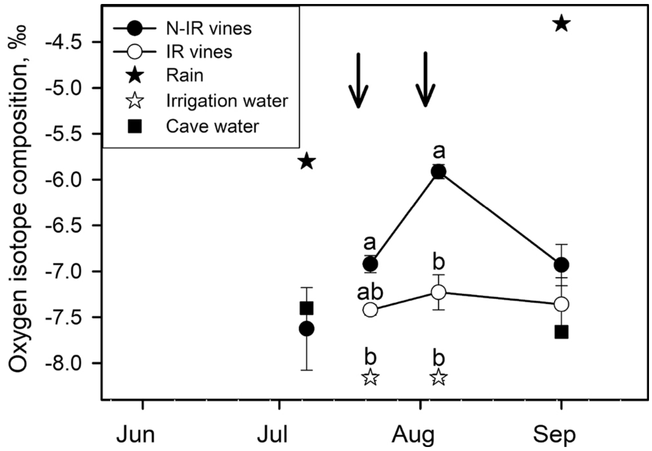

Fig. 4. Oxygen isotope composition ($\delta^{18}O$, reported against the V-SMOW standard) as measured from June to September 2015 in xylem sap extracted from non irrigated (N-IR, closed circles) and irrigated (IR, open circles) vines, rainfalls (closed stars), irrigation water (opened stars), and deep cave water sampled at 7 m below soil surface (closed squares). Different letters indicate significant differences among irrigation water, N-IR and IR plants (One-way ANOVA, $P < 0.05$). Arrows indicate the two irrigation treatments applied during the nights of 17–18th July and 31st July. Error bars represent the SEM ($n = 5$).

(pre-drought values = 33 °C, data not shown). After the irrigation treatment, on 21st July no differences ($P > 0.05$) were observed between IR and N-IR vines for both Ψ_{pd} and Ψ_{min}. On the contrary, g_L of IR vines was about 300% higher, while T_{leaf} was about 4 °C lower compared to N-IR plants ($P < 0.05$). However, after late-summer rainfalls (50 mm), Ψ_{pd} and T_{leaf} of N-IR vines promptly returned to pre-drought values, while g_L reached the seasonal maximum peak of about 600 mmol m^{-2} s^{-1}.

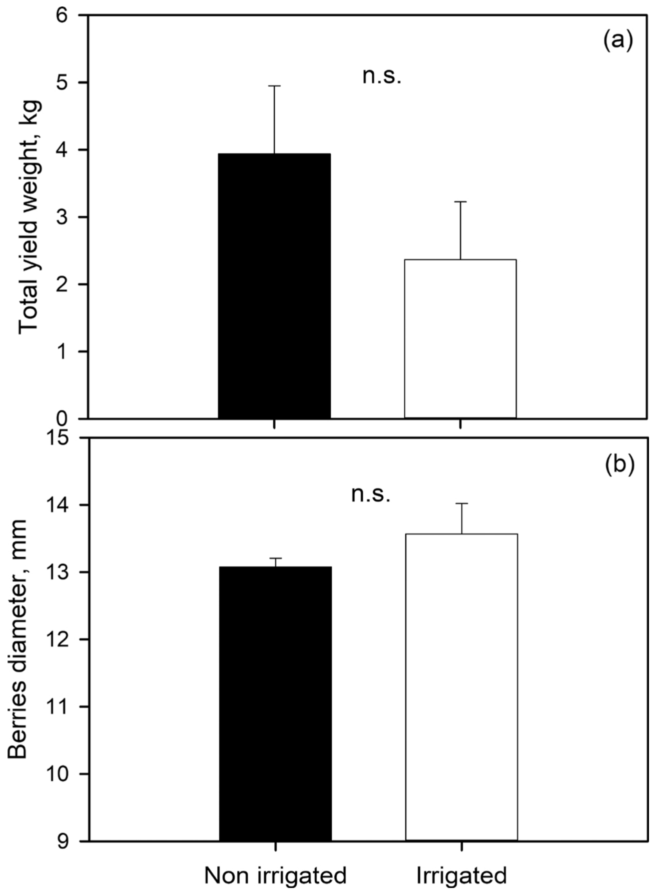

Fig. 5. Total yield weight (a) and berries diameter (b) as measured in September 2015 in non irrigated (N-IR, black bars) and irrigated (IR, white bars) vines. n.s. indicates the lack of significant differences betwe.

The oxygen isotopic composition ($\delta^{18}O$) of rainfall ranged between −5.78‰ in the period June-early July, and −4.33‰ in late July-August, while that of deep water extracted from cave soil was more negative, i.e. about −7.40‰ on 7th July and −7.66‰ on 1st September. The isotopic composition of vines' xylem sap (N-IR) was similar to that of deep water both in July and in September (about −7.10‰), while it increased to −5.91 ± 0.31‰ at the beginning of August, reaching values similar to those of late summer rainfalls. After two irrigation treatments, IR vines had significantly lower $\delta^{18}O$ compared to N-IR ones (−7.42 ± 0.17‰ and −5.91 ± 0.31‰, respectively) with values closer to that of irrigation water (−8.16 ± 0.05‰).

Yield related parameters measured at grape harvest are summarized in Fig. 5. Total yield weight was 3.9 ± 1.1 kg in N-IR and 2.4 ± 0.9 kg in IR vines. Mean berries diameter was by 4% higher in IR vines compared to N-IR (13.6 ± 0.5 mm *vs* 13.1 0.1 mm), but high variability of data masked eventual significant differences.

4. Discussion

Grapevine in the Classical Karst has been traditionally cultivated under rain-fed conditions, but increasing aridity is prompting for the use of irrigation. Our study describes physiological responses to drought of a local vine variety in the complex karst soil, and provides useful insights into possible irrigation strategies to sustain production while avoiding inappropriate consumption of water resources.

The water content at field capacity (SWC) of local red soil was 0.34 g g^{-1} (31% in volume), i.e. within the range reported for natural sandy, loamy, and clay soils (10–40% in volume; Lambers et al., 2008; Acevedo-Opazo et al., 2010; Munitz et al., 2017). The benefits of relatively high SWC was however counterbalanced by the still high amount of water retained at $\Psi_{soil} = -1.5$ MPa, i.e. the permanent wilting point (Estrada-Medina et al., 2013a; Lambers et al., 2008). As a consequence, the theoretical total amount of water available to plants turned out to be only about 18% in volume (0.16 g g^{-1}). Overall, our results are in agreement with those obtained for top karstic soils in Mexico, where SWC and AWC of 0.37 g g^{-1} and 0.15 g g^{-1}, respectively, have been reported (Estrada-Medina et al., 2013a). Considering the shallow soil profile of the study vineyard (about 40 cm), it can be concluded that its potential water storage (average soil volume × AWC, Estrada-Medina et al., 2013a) was limited to about 700 m^3 ha^{-1}. Nevertheless, deep rooting apparently allowed vines in the area to avoid severe water stress during periods with high temperatures and lack of rainfall. In fact, N-IR plants showed a relatively favorable water status throughout the entire growing season, with Ψ_{pd} and Ψ_{min} in the range of −0.2 and −0.4 MPa, and −0.9 and −1.1 MPa, respectively. These values suggest that irrigation was not necessary according to standard protocols. Measurements of leaf water potential are widely accepted as one of the most suitable and accurate physiological proxies to estimate vine water needs (Brillante et al., 2016; Fernández and Cuevas, 2010; Schultz and Stoll, 2010; van Leeuwen et al., 2009). Water deficit can lead to a wide range of effects in different vine cultivars, as a function of intensity and of critical phenological stages characterized by high drought sensitivity (Chaves et al., 2010; Ferlito et al., 2014; Girona et al., 2006; Munitz et al., 2017). According to the literature, from flowering (mid-May in the study vineyard) to *veraison* (onset of ripening, beginning of August), moderate water deficit ($\Psi_{pd\,>}$ −0.4 MPa) is favorable and promotes the control of vigor without affecting berries biochemistry (Deloire et al., 2004, van Leeuwen et al., 2009).

At the onset of summer drought, high temperatures and VPD (Fig. 1) increased the evaporative demand, leading to a gradual decline of plant water status. At midday, stomatal aperture was reduced

($130\ \mathrm{mmol\,m^{-2}\,s^{-1}}$) compared to spring values ($400\ \mathrm{mmol\,m^{-2}\,s^{-1}}$), thus limiting transpiration and preventing major drops in Ψ_{min}, but causing T_{leaf} to rise up to 37 °C. Fast stomatal closure under drought has been frequently reported for grapevine, suggesting a marked isohydric behavior, which is considered advantageous under dry conditions (Bota et al., 2016; Chaves et al., 2010; Lovisolo et al., 2010). According to the positive correlation between stomatal conductance and net photosynthesis, as reported by Flexas et al. (2002), a 70% reduction of g_L (as observed in our study) indicates a moderate water stress suffered by plants, which increases the water use efficiency and optimizes grape quality (Chaves et al., 2010; Deloire et al., 2004; Lovisolo et al., 2010). Although T_{leaf} reached substantially high values, no signs of wilting, necrosis and/or accelerated leaf senescence were observed. Furthermore, T_{leaf} values were in agreement with values reported for non-irrigated vines by other authors, ranging between 30 °C and 44 °C (Chaves et al., 2010; Greer, 2017; Schultz and Stoll, 2010). This temperature range, even when coupled to drought stress, does not cause permanent reduction of photosynthetic efficiency and carbon acquisition (Greer, 2017; Lovisolo et al., 2010; Maroco et al., 2002).

Although Ψ_{soil} measured at 40 cm depth dropped below -2.4 MPa, the minimum seasonal values of Ψ_{pd}, Ψ_{min}, and g_L recorded in N-IR plants were higher than those reported as critical for several vine cultivars, i.e. < -0.6 MPa, < -1.3 MPa, and $50\ \mathrm{mmol\,m^{-2}\,s^{-1}}$, respectively (Deloire et al., 2004; Flexas et al., 2002; Lovisolo et al., 2010; van Leeuwen et al., 2009). The discrepancy between soil water potential and plant water status at pre-dawn suggests that roots had grown to deep fractures in the karstic substrate. This hypothesis is supported by the analysis of isotopic composition of xylem sap collected both in pre- and post-drought surveys, which was similar to that of cave soil water, sampled at about 7 m depth (Fig. 4). $\delta^{18}O$ of xylem sap is a mixture of isotopic signatures of water absorbed from different sources by functional roots, giving information about the depth of the soil from which the plant is absorbing water (Estrada-Medina et al., 2013b; Lubis et al., 2014; Orlowski et al., 2013; Querejeta et al., 2006). The complex structure of the karstic substrate makes difficult to estimate the precise rooting depth. However, we can speculate that at least a small amount of vines' roots absorbed deep water which may represent a fundamental resource in these areas, especially during the dry season. According to a mixing model analysis (Phillips and Gregg, 2001; http://www.epa.gov/wed/pages/models.htm), at the peak of the drought (21st July), the contribution of deep water to plant xylem sap (N-IR vines) was about 75%, while rain water contributed by about 25%. In order to thrive under dry conditions, vines have evolved a highly branched root system, which develops both horizontally and vertically, with most of the roots lying in the upper 1–3 m (broad range of soil environments considered; Acevedo-Opazo et al., 2010; Celette et al., 2005; Girona et al., 2006; Smart et al., 2006). However, soil thickness and water storage capacity, porosity of the underlying bedrock, as well as the depth of stable water resources are crucial factors influencing root vertical growth (Deloire et al., 2004; Schenk and Jackson, 2002; Smart et al., 2006). As a consequence, it has been documented that grapevine roots can reach depths of 6–7 m, similar to those estimated in our study vineyard (Smart et al., 2006).

Although physiological measurements suggested an overall favorable plant water status throughout the growing season, two irrigation treatments were applied to a sub-area of the vineyard, following the wine-growers experience-based assessment of plants' water needs (Fig. 1). Interestingly, after the first treatment, Ψ_{pd} and Ψ_{min} of IR plants were not statistically different from those of N-IR ones, while g_L was by about 300% higher ($P < 0.001$, Fig. 2), suggesting the absorption of irrigation water, and a resulting switch to a water-spending behavior. This hypothesis was strengthened by similar values of $\delta^{18}O$ measured in IR plants and irrigation water (Fig. 4). Assuming the reduced top soil water content at the peak of the summer season ($0.17\ \mathrm{g\,g^{-1}}$, 50% of the SWC), the irrigation water ($400\ \mathrm{m^3\,ha^{-1}}$) would theoretically saturate only the upper 20–30 cm of substrate. However, during vineyard tilling and soil sampling (see Section 2.1), we could not detect the presence of mature or fine roots in the upper 40 cm of soil. Isotope analysis performed after the second supplemental irrigation further confirmed access to irrigation water by highlighting significantly lower $\delta^{18}O$ in IR vines compared to N-IR ones (Fig. 4). On the other hand, the increase of $\delta^{18}O$ in N-IR plants after late summer thunderstorms revealed possible uptake of rainfall water. In fact, the contribution of deep water to xylem sap decreased to 34%, while that of the rain increased to 66%. Our method of superficial root assessment was limited to a restricted area of the vineyard, hence we cannot completely exclude the presence of roots in the upper soil layers. Thus, the quick recovery of plant water status after irrigation (IR plants) and late summer thunderstorms (N-IR plants, Fig. 3) could be promoted by a small, but extremely important, fraction of roots laying in the top 40 cm of soil. However, we can also hypothesize that the abundant cracks forming on the red soil during dehydration might facilitate water infiltration to the deeper rooting zone (bedrock, cracks and fissures). In our opinion, both phenomena promoted plant recovery upon stress relief, and might play important roles in grapevine survival in limestone environments.

The water status of vines has a significant influence on berry growth and development (Deloire et al., 2004). However, the two irrigation treatments did not influence significantly the total yield weight and berries diameter in our study vineyard, in accordance with previous studies (Acevedo-Opazo et al., 2010; dos Santos et al., 2003; Girona et al., 2006). Hence, taking into consideration the relatively favorable water status of N-IR plants throughout the entire growing season, we can conclude that the irrigation treatments, performed according to traditional management practices, were not necessary and, if avoided, might have led to water saving in a typically water-limited environment.

5. Conclusion

Our data provides the first assessment of the rooting depth of grapevine grown on shallow soils overlying limestone bedrock, which are generally missing in the literature. Our results support the hypothesis of deep rooting patterns, which ensured favorable plant water status during harsh summer conditions, hence making irrigation not necessary during the study year. Furthermore, our results demonstrate the feasibility of the development of precision irrigation methods in karstic areas under future global-change-type droughts. In fact, vines apparently maintain the ability to absorb water from shallow soil layers (irrigation water, summer rains), while still largely relying on deep water sources. The amount of water and the frequency of irrigation during the growing season should be adjusted by monitoring the plant water status. However, in the hypothetical case of irrigation needs in semi-arid wine-producing regions characterized by a highly permeable substrate, an irrigation volume of about $200\ \mathrm{m^3\,ha^{-1}}$ might be appropriate to start the treatment. To achieve a more sustainable viticulture, adoption of soil management practices favoring water infiltration to deeper horizons might also be useful.

Delcarations of interest

None.

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