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ORIGINAL RESEARCH ARTICLE

Water deficit responses of field-grown Pinot noir mediated by rootstock genotypes in a cool climate region

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ABSTRACT

Under the global warming scenario, water scarcity is expected to intensify in most grape-growing regions. The use of drought-tolerant rootstocks is considered a useful tool to mitigate the negative effects of soil water deficit on vine functioning. Differences in leaf gas exchange, plant water status, specific hydraulic conductivity in petioles ($K_{petiole}$), xylem vessel size and vegetative vigour of field-grown Pinot noir grafted onto five rootstocks (3309C, 101-14 MGt, Kober 5BB, Riparia Gloire de Montpellier, 41B MGt) were investigated during one season under water deficit in Switzerland. The water deficit was imposed by installing waterproof and non-reflecting plastic sheets on the soil from March to harvest (September) to avoid rainfall infiltration. Rootstocks had stronger effects on vine water status than on gas exchanges. During the grape ripening stage, vines grafted onto 41B MGt and 101-14 MGt were characterised by more severe water stress as shown by the lowest values of pre-dawn leaf (Ψ_{pd}), stem water potential (Ψ_{stem}) and water stress integral (S_{Ψ}), whereas 3309C and Kober 5BB rootstocks induced milder effects. Significant differences in photosynthesis (A), stomatal conductance (gs) and transpiration (E) were only observed between vines grafted onto 41B MGt and 3309C at later stages of ripening. Changes induced by rootstocks in shoot vigour, $K_{petiole}$ and the number and size of xylem vessels in petioles and stems were correlated to differential responses of Pinot noir to water deficit. The increased vegetative vigour induced by 3309C and Kober 5BB was associated with the highest $K_{petiole}$, xylem vessel size and a good plant water status of Pinot noir under low soil water availability. Kober 5BB induced the highest yield, probably due to the better vine water status, whereas vines grafted onto 41B MGt showed the lowest malic acid content and yeast assimilable nitrogen in berries.

KEYWORDS: Gas exchange, plant hydraulic, rootstocks, water stress, xylem anatomy

INTRODUCTION

Most of the world's non-irrigated vineyards may experience seasonal drought with a negative impact on vine vigour and yield potential depending on intensity, duration and rate of water deficit progression (Medrano *et al*., 2002; Costa *et al*., 2007; Chaves *et al*., 2010). It is well known that stomatal closure and consequent decline in photosynthesis due to restriction on CO_2 diffusion are among the earliest physiological processes driven by water deficit. Field-grown grapevine is considered drought resistant due to a large and deep root system associated with physiological drought avoidance mechanisms, such as efficient stomatal control of transpiration, xylem embolism and osmoregulation capacity (Chaves *et al*., 2010; Simonneau *et al*., 2017). Moreover, alterations in leaf area, root/shoot ratio, xylem vessel size and hydraulic conductivity have also been revealed as adaptive strategies by grapevine cultivars under low soil water availability (Gómez-del-Campo *et al*., 2003; Lovisolo *et al*., 2010; Gerzon *et al*., 2015; Dayer *et al*., 2017; Zufferey *et al*., 2017).

Considering that most vineyards worldwide are grafted mainly to cope with *Phylloxera* and also the possibility of water scarcity in the near future in some regions due to global warming (IPCC, 2008), the interest in drought tolerance induced by rootstocks has increased in viticulture (Serra *et al*., 2014; Ollat *et al*., 2016). This interest is supported by the fact that phenology, shoot growth, biomass accumulation and repartition, yield and grape quality of scion can be modified by rootstock genotype (Keller 2010; Kidman *et al*., 2013; Souza *et al*., 2015; Dias *et al*., 2017). Under low soil moisture, grapevine rootstock may affect scion growth and drought responses by controlling and adjusting the water supply to shoot transpiration demand through stomatal control, hydraulic conductivity and capacity of water extraction from the soil (Soar *et al*., 2006a; Soar *et al*., 2006b; Alsina *et al*., 2011; Tramontini *et al*., 2013). Therefore, rootstock selection could become an important tool for mitigating water deficit.

Although rootstock classifications based on drought tolerance exist and evidence for the contribution of rootstock to the genetic variability of water use efficiency has been reported (Carbonneau, 1985; Marguerit *et al*., 2012; Lovisolo *et al*., 2016, Ollat *et al*., 2016), field observations do not always confirm published classification, when based on potted experiments (Bauerle *et al*., 2008; Stevens *et al*., 2010; Chaves *et al*., 2016). This fact could be attributed to scion effects and the influence of soil type on root distribution (Smart *et al*., 2006; Tandonnet *et al*., 2010). The depth and distribution of the grapevine root system may vary according to the soil texture, water and nitrogen availability, soil salinity, vine spacing and climatic conditions (Smart *et al*., 2006; Koundouras *et al*., 2008). During periods of water deficit, high vigour and drought-resistant rootstocks may induce deep root production during the hot and dry summer (Alsina *et al*., 2011) or enhance root growth in more humid soil zone favouring water supply to the scion (Bauerle *et al*., 2008). On the other hand, Tramontini *et al*. (2013) showed that grapevine water deficit responses are not only dependent on the rootstock but also influenced by stomatal control of the scion cultivar.

The stem xylem tissue development can also be affected by the rootstock. Santarosa *et al*. (2016) showed that during the first growth cycle, the vegetative growth of Merlot and Cabernet Sauvignon grafted onto three different rootstocks was associated with a larger proportion of stem xylem vessels with greater mean diameters. Several authors have also shown that an increased vegetative vigour was linked to high hydraulic conductance induced by vigorous rootstocks in apple (Atkinson *et al*., 2003) and cherry (Gonçalves *et al*., 2005; Zoric´ *et al*., 2012) because of larger xylem vessels. If confirmed, the anatomical differences induced by rootstock in the stem xylem of the scion might improve water uptake and transport capacity.

The effects of rootstock on hydraulic conductivity and xylem vessel size of grapevine under water deficit remain unclear. This study reports how rootstocks differing in their effect on scion vigour, as previously reported by Spring *et al*. (2016) on the same experimental vineyard, affect gas exchanges, water relations, petiole hydraulic conductivity, xylem anatomy, yield and grape composition of field-grown Pinot noir under water deficit conditions. It provides a preliminary dissection of the main grapevine responses to water deficit in interaction with rootstock genotypes and feeds our understanding of the major mechanisms involved. Furthermore, it provides preliminary data about scion-rootstock interactions under cool climate viticulture, as most published experiments were carried out under warmer or potted conditions.

MATERIALS AND METHODS

1. Experimental site and plant material

This experiment was conducted in 2017 at the experimental vineyard of Agroscope at Leytron, Switzerland (46º10' N, 7º12' E; 485 m above sea level). The region's climate is continental, with 603 mm of annual rainfall and an average daily temperature of 10 ºC (average 1981–2010, Météo Suisse). Monthly mean temperatures and rainfalls during the experimental period (from March to August) and long-term average (1981–2010) are shown in Table 1 (Data from the nearest weather station, Sion, Switzerland, MétéoSuisse database). The soil is very stony (5 % clay, 15 % silt and 80 % gravel), highly calcareous (44–45 % of total lime content) and deep (> 2.5 m vine root depth) with a water-holding capacity estimated at 150 mm and a pH of 8.1–8.3 (Spring *et al*., 2016; Zufferey *et al*., 2017). The experimental plot was homogeneous and planted in 1995. Pinot noir (clone RAC 12) vines were grafted onto Kober 5BB (*V. riparia* x *V. berlandieri*), 3309C (*V. riparia* × *V. rupestris*), 101-14 MGt (*V. riparia x V. rupestris*), Riparia Gloire de Montpellier (Riparia Gloire) (*V. riparia*) and 41B MGt (*V. vinifera x V. berlandieri*). The vines were evaluated in a completely randomised block design with three replicates represented by 13 vines per block, as already described in Spring *et al*. (2016). The rootstocks analysed in the present experiment were chosen according to previous pruning weight data provided by Spring *et al*. (2016) for rain-fed Pinot noir vineyard in the Wallis county, with 41B MGt inducing the lowest vigour (about 250 g per vine), Riparia Gloire a medium vigour (about 350 g per vine), whereas 101-14 MGt, Kober 5BB and 3309C induced the highest vigour (about 500 g per vine). The grapevines were spaced 0.9 m between vines and 1.4 m between rows, trained in a simple Guyot system (6 vertical shoots positioned per vine) and north–south oriented. The vines were summer trimmed to maintain canopy height at 1.2 m above the first cordon for all combinations. The water deficit was imposed by the installation of waterproof and non-reflecting green plastic sheets on the soil (on both sides of the rows) from the beginning of March to harvest (September) to avoid rainfall infiltration. Due to the small size of the experimental plot, a control (irrigated or rainfed) treatment could not be established.

2. Gas exchange and water relations

The seasonal evolution of photosynthesis rate (*A*), stomatal conductance (gs) and transpiration (*E*) were evaluated using an infrared gas analyzer (LI-6400, LI-COR Inc., Lincoln, NE, USA). The measurements were taken weekly from the beginning of berry development (June) until harvest (September) on six mature and sunny exposed leaves (two leaves per block; one leaf per vine) in the morning (10h30–11h30) and in the afternoon (14h00–15h00). The *A* and gs values were used to calculate the instantaneous intrinsic water use efficiency (A/gs).

The vine water status was assessed by measuring pre-dawn leaf (Ψ_{pd}) and midday stem (Ψ_{stem}) water potentials (on the same day as gas exchange measurements) with a Scholander pressure chamber (Model 600, PMS Instruments, Albany, OR, USA). Ψ_{pd} was measured between 04h00 and 05h00 and Ψ_{stem} was performed during the afternoon (between 13h00 and 15h00) on six mature leaves (two leaves per block). Ψ_{stem} was measured on leaves covered with an opaque plastic bag at least one hour prior to measurement to stop transpiration. The leaf water potential is considered equivalent to the stem water potential when leaf transpiration was prevented (Choné *et al*., 2001). The Ψ_{pd} was used to calculate a water stress integral (S_Ψ) as proposed by Meyers (1988). The S_Ψ expresses the stress intensity of the vine by integrating the duration of water status below a maximum value of Ψ_{pd}. S_Ψ reflects the intensity and duration of water stress experienced by grafting combinations. It was estimated from t (eight) measurements of Ψ_{pd} at intervals of seven days using the formula:

$$S_\Psi = \sum_{i=0}^{i=t} \left(\Psi_{pd\ i,i+1} - c\right) n$$

where $\Psi_{pd\ i,\ i+1}$ is the mean Ψ_{pd} for any interval i, i+1, *c* is the maximum Ψ_{pd} measured during the study and n is the number of days in each interval. The maximum Ψ_{pd} value measured at the beginning of the experimental period was –0.16 MPa.

Specific hydraulic conductivity in petioles ($K_{petiole}$) was measured during the ripening period (from 14 August to 5 September) when Ψ_{pd} among treatments ranged from -0.27 to -0.48MPa, using a xylem embolism meter (XYL'EM, Bronkhorst Instructec, Montigny-les-Cormeilles, France) as described in Zufferey *et al*. (2017) and Dayer *et al*. (2017). The XYL'EM measures the water flow rate (F; mmol s^{-1}) entering the petiole of a cut leaf when exposed to a hydrostatic pressure (P, MPa) using a high-resolution liquid mass flow meter. Petioles were cut underwater and kept in darkness by covering the water-filled containers with aluminium foil. The excised petiole was attached to the XYL'EM apparatus and the initial hydraulic conductance (K_{init}), calculated by the F:ΔP ratio, was determined with a hydrostatic pressure gradient of approximately 3 to 4 kPa. For all measurements, distilled and degassed water was used as perfusion liquid. $K_{petiole}$ was calculated as $K_{petiole} = K_{init} \times L/LA$, where K_{init} is the initial hydraulic conductance (mmol s^{-1} MPa), L is the petiole length (m) and LA is the leaf blade area (m^2). The XYL'EM was interfaced with a computer to log the data automatically. The measurements were done on four fully expanded leaves per treatment collected from the middle third of primary shoots and immediately transported to the laboratory in water-filled containers covered with aluminium foil.

3. Petiole and stem xylem vessels anatomy

Five petioles and five stem segments per treatment were collected from adult leaves and stem segments were collected at the middle third of the primary shoot one week after the grape harvest. Each petiole and stem fragment was prepared according to Roland and Vian (1991). The sections were observed using a light microscope (Leica DMLB, Leica Microsystems, Heerbrugg, Switzerland). The cross-sectional area of the xylem vessel was measured using Image-Pro Plus software provided with a Leica DFC 490 FX camera. The frequency of vessels cross-sectional area was distributed in six categories for petiole (200–500 µm^2, 501–1000 µm^2, 1001–1500 µm^2, 1501–2000 µm^2, 2001–2500 µm^2, > 2500 µm^2) and stem (200–500 µm^2, 501–1000 µm^2, 1500–3000 µm^2, 3001–5000 µm^2, 5001–10,000 µm^2, >10,000 µm^2). For each biological replicate (three replicates per rootstock), twenty images were processed to calculate the frequency according to area classes. The frequency of vessels per category was expressed as a percentage of 720 vessels (minimal number of vessels observed for all cross-section images) per cross-section image.

4. Leaf and berry nitrogen status, shoot weight

The chlorophyll index was monitored on each replicate from June to September, using a portable N-tester (Yara, Nanterre, France) on 30 mature leaves per replicate from the median area of the canopy. This device measures leaf blade colour intensity positively correlated with nitrogen (N) status (van Leeuwen *et al*., 2000). Yest assimilable nitrogen (YAN) in berries was estimated by NIR spectroscopy (WineScan®, FOSS NIRSystems, USA). YAN provides information about the amount of N-compounds (ammonium ion and primary amino acids, excluding proteins) available for the yeast to use during fermentation (Verdenal *et al*., 2021). The shoot vigour was evaluated after leaf fall by measuring the weight of the penultimate shoot of the cane. Eight shoots per replicate (one shoot per vine) were collected, trimmed to one-meter length from the base and weighted. The results were expressed as grams per meter of shoot. They were well correlated with the full vine pruning weights reported by Spring *et al*. (2016).

5. Yield and berry composition

Yield data were collected on a per area (kg m^{-2}) basis using 13 vines per replicate. One hundred berries per replicate were used to extract juice to determine sugar content, titratable acidity, malic and tartaric acid concentration (expressed as gL^{-1}) using an infrared spectrophotometer (FOSS WineScanTM).

6. Carbon isotope composition

The carbon isotope composition ($\delta^{13}C$) was determined in the must collected at the end of the ripening period on 29th August as grape must appear to be the most sensitive water deficit indicator as compared to other organs (Souza *et al*., 2005a). Samples of 100 berries per replicate were taken randomly. The $\delta^{13}C$ values in must sugars were determined by elemental analysis-isotope ratio mass spectrometry (EA-IRMS) using a Carlo Erba 1108 Elemental Analyzer connected to a Thermo Fisher Scientific (Bremen, Germany) Delta V mass spectrometer. $\delta^{13}C$ was using the delta-values (δ) as

$$\delta^{13}C = [(R_{sa} - R_{st})/R_{st})]$$

where R_{sa} is the $^{13}C/^{12}C$ ratio of the sample and R_{st} is the $^{13}C/^{12}C$ ratio of the Vienna Pee Dee Belemnite (VPDB) standard. The $\delta^{13}C$ are presented as per mil (‰) versus VPDB.

7. Statistical Analyses

All data sets were subjected to analysis of variance (ANOVA) using TIBCO Statistica software (ver. 13.5.0.17, TIBCO Inc., Palo Alto, CA, USA). Seasonal rootstock effects on gas exchanges, Ψ_{pd}, Ψ_{stem}, and N tester data were compared with individual ANOVAs for each measurement day. For $K_{petiole}$, two way ANOVA was used with rootstock and date as the main factors. To satisfy the assumptions of normality, the data of $K_{petiole}$ were transformed by and the values were shown as the average of five dates as there were no statistical differences among dates and no significant interaction between date and rootstocks (Table 2). The means were separated by Fisher's LSD test when ANOVA indicated significant differences ($p < 0.05$). Linear regressions between the different physiological variables were determined using SigmaPlot software (version 12.0). Student's t-test was applied to compare the slopes of the curves obtained by the regression analysis for Ψ_{pd} and VPDL versus gs for the different rootstocks (Andrade and Estévez-Pérez, 2014). This test evaluated the null hypothesis that gs do not differ among rootstocks. Student's t-test was applied to the regression variances of the treatments weighted by the respective degrees of freedom to establish the experimental t (t_{exp}) value compared to the Student's t distribution table (t_{tab}). The number of degrees of freedom for t_{tab} was also estimated by applying the procedures described by Andrade and Estévez-Pérez (2014).

RESULTS

This study was conducted in 2017, a year considered warmer than the long-term records of temperature. The average monthly mean temperatures (Tmean) and amount of rainfall during the 2017 growing season were, respectively, 2.6 ºC and 21 mm higher than long-term averages (Table 1). The highest Tmean (around 22 ºC) were recorded from the fruit set (June) to the ripening stage (July–August), the period under higher rainfall (189 mm) as compared with values recorded from March to May (127 mm).

The water status of field-grown Pinot noir was significantly influenced by rootstocks, as shown by predawn (Ψ_{pd}) and stem (Ψ_{stem}) water potential measured throughout the season (Figure 1). Over the season, Ψ_{pd} of vines grafted onto Kober 5BB and 3309C was statistically higher mainly compared to values of Ψ_{pd} induced by 41B MGt and 101-14MGt rootstocks (Figure 1A) (Supplementary Table 1). The Ψ_{pd} decreased from -0.2 to -0.7 MPa for vines grafted on 41B MGt and 101-14MGt, whereas the highest values (around -0.4 MPa) were recorded for Kober 5BB and 3309C at the end of the season (247 DOY, Figure 1A). For the last three dates of measurement, Ψ_{pd} of vines grafted onto Riparia Gloire did not show significant differences when compared to 3309C

TABLE 1. Monthly mean temperatures (Tmean, °C) and rainfall (mm) in Leytron (Switzerland) during the experimental period in the 2017 growing season compared with long-term averages (1981–2010).

	2017		1981-2010	
Month	Tmean	Rainfall	Tmean	Rainfall
March	9.7	62	6.5	42
April	11.6	21	10.4	35
May	16.4	44	14.9	49
June	21.7	68	18.1	54
July	22.1	51	20.1	58
August	21.3	70	19.2	57

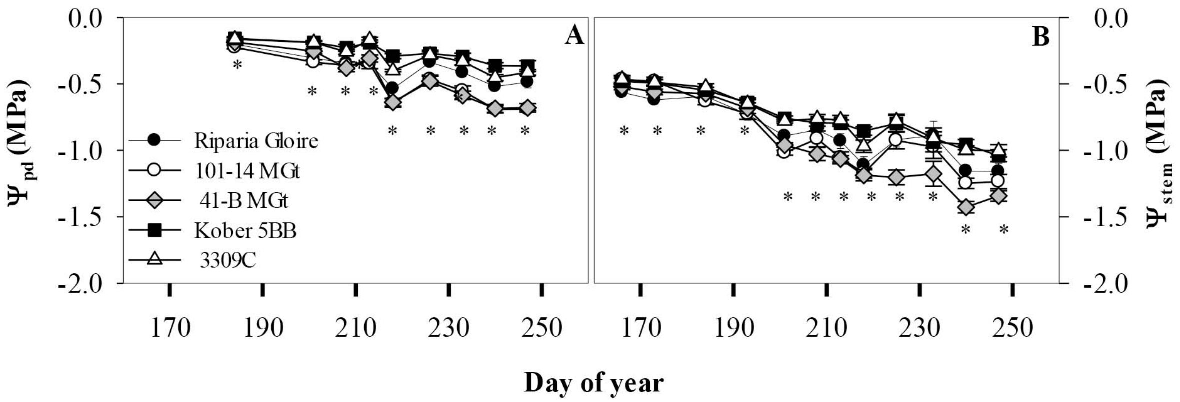

FIGURE 1. Seasonal pre-dawn water potential (A, Ψ_{pd}) and stem water potential (B, Ψ_{stem}) of field-grown Pinot noir grafted onto different rootstocks.

Values are means ± standard error (n = 6). The asterisks show significant differences among treatments at $p < 0.05$ by Fisher's LSD test.

TABLE 2. Integral water stress (S_Ψ) in leaves, specific hydraulic conductivity in petioles ($K_{petiole}$), and carbon isotope composition ($\delta^{13}C$) in must sugar of field-grown Pinot noir vines grafted onto different rootstocks.

Rootstock	S_Ψ (MPa d^{-1})	$K_{petiole}$ (mmol m^{-2} s^{-1} MPa^{-1})	$\delta^{13}C$ (‰)
41B MGt	17.41 a	5.31 c	–23.28 a
101-14 MGt	18.58 a	8.14 b	–24.24 a
Riparia Gloire	13.36 b	7.78 b	–23.7l a
3309C	7.78 c	12.22 a	–24.81 a
Kober 5BB	5.79 c	13.07 a	–24.18 a

Values are means of six replicates for S_Ψ four replicates for $K_{petiole}$ and 100 replicates for $\delta^{13}C$. Means followed by the same letter do not differ significantly at $p < 0.05$ by Fisher's LSD test. Values of $K_{petiole}$ are the average of five dates.

and it was significantly higher than 101-14MGt and 41B MGt and lower than Kober 5BB (Supplementary Table 1). Similarly to Ψ_{pd}, Ψ_{stem} was also significantly higher in vines grafted onto Kober 5BB and 3309C compared to 41B MGt and 101-14MGt (Figure 1B) (Supplementary Table 2). At the end of the season (last two weeks), the rootstocks were discriminated into three different significant groups: 41B MGt induced the lowest Ψ_{stem} (around -1.4 MPa), whereas the highest Ψ_{stem} values were recorded for Kober 5BB and 3309C (around -1.0 MPa) and the intermediate values for 101-14MGt and Riparia Gloire (around -1.20 MPa) (Supplementary Table 2).

Based on the values of the cumulative integral of predawn leaf water potential (S_Ψ, MPa d^{-1}), rootstocks could be significantly separated into three water deficit levels (Table 2). The lowest water deficit intensity was recorded for 3309C and Kober 5BB (6.8 MPa d^{-1}), the intermediate level for Riparia Gloire (13.4 MPa d^{-1}), whereas the highest water deficit intensity (16.5 MPa d^{-1}) was observed for vines grafted onto 101-14 MGt and 41B MGt.

During most days, photosynthesis (*A*), stomatal conductance (gs), transpiration (*E*) and intrinsic water use efficiency (*A*/gs) did not differ among rootstocks measured in both times (Figure 2) (Supplementary Tables 3, 4, 5 and 6). During the morning, *A* was significantly lower in vines grafted onto 41B MGt, 101-14 MGt and Riparia Gloire at 240 DOY only, whereas vines grafted onto 3309C and Kober 5BB showed higher *A* than other rootstocks at 233 and 240 DOY, during the afternoon (Figure 2A, E) (Supplementary Table 3). Vines grafted onto 41B MGt exhibited, during the afternoon, the largest *A* reduction at 225, 233 and 240 DOY mainly when compared to 3309C and Kober 5BB, but there were no significant differences among 41B MGt, 101-14 MGt and Riparia Gloire at 240 DOY. During the morning, gs was also significantly reduced for 41B MGt at 213, 225 and 240 DOY when compared to 3309C and Kober 5BB, but there were no significant differences when compared to 101-14 MGt and Riparia Gloire at 213 and 240 DOY (Figure 2B) (Supplementary Table 4). During the afternoon, at 225, 233 and 240 DOY, the largest differences in gs were observed between vines grafted onto 41B MGt and 3309C, whereas at 240 DOY, Kober 5BB and 3309C induced higher gs than other rootstocks (Figure 2F). Vines grafted onto 41B MGt, 101-14 MGt induced lower *E* than Kober 5BB and 3309C only at 240 DOY during the morning, whereas Riparia Gloire was statistically different only when compared to Kober 5BB (Figure 2C). During the afternoon, Kober 5BB and 3309C induced the highest *E* mainly when compared to 41B MGt at 233 and 240 DOY, but *E* was significantly higher in vines grafted onto Kober 5BB and 3309C at 240 DOY (Figure 2G) (Supplementary Table 5). In general, the largest differences

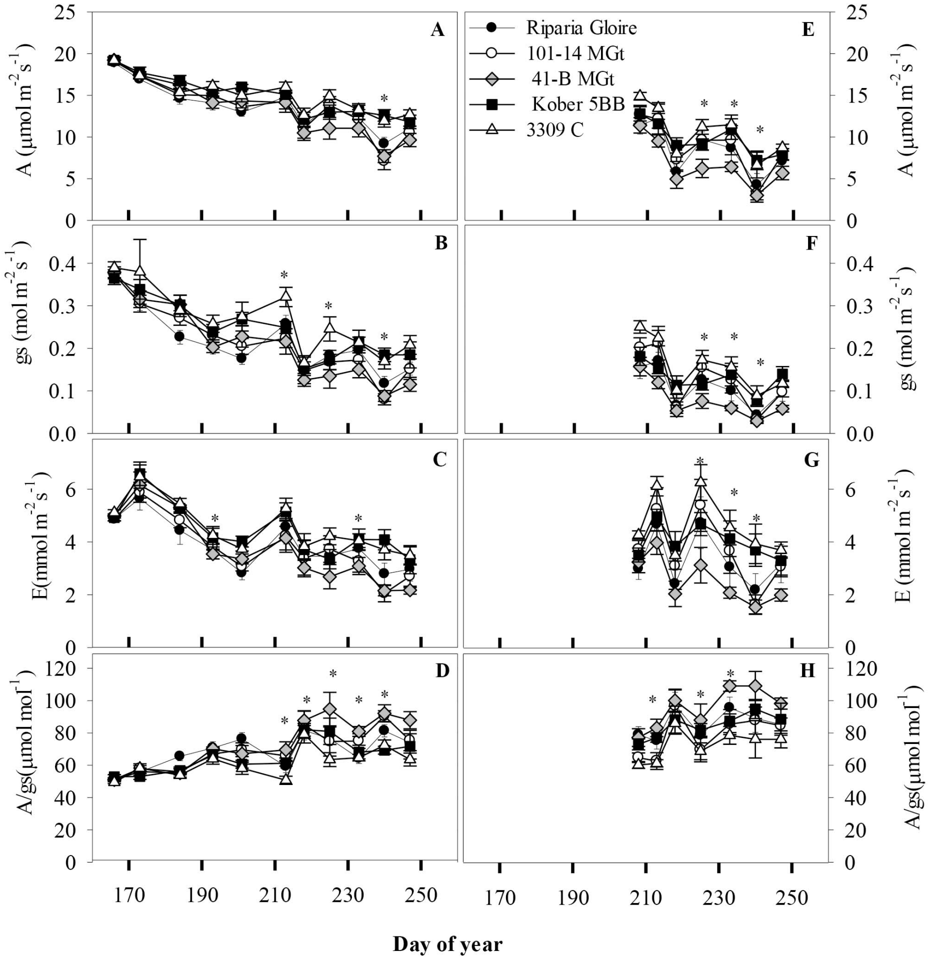

FIGURE 2. Seasonal trends of photosynthesis (A), stomatal conductance (gs), transpiration (E) and intrinsic water use efficiency (A/gs) of field-grown Pinot noir grafted onto different rootstocks during the morning (A,B,C,D) and afternoon (E,F,G,H).

Values are means ± standard error (n = 6). The asterisks show significant differences among treatments at $p < 0.05$ by Fisher's LSD test.

in *A*/gs were recorded between 41B MGt and 3309C (at 213, 225, 233 and 240DOY) during the morning (Figure 2D) (Supplementary Table 6). During the afternoon, 41B MGt induced the highest A/gs mainly when compared to 3309C and 101-14 MGt (Figure 2H). At harvest (on 247 DOY), there were no statistical differences among rootstocks for gas exchange variables.

Gs was significantly correlated with leaf water pressure deficit (VPDL) and Ψ_{pd} for every individual rootstock (except for Kober 5BB) (Figure 3A, B). However, there are no significant differences among rootstocks slopes ($t_{exp} < t_{tab}$) (Supplementary Table 7), indicating that stomatal sensitivity to VPDL and Ψ_{pd} were not influenced by rootstock genotype.

Seasonal water deficit progression did not influence the petiole specific hydraulic conductivity ($K_{petiole}$) for any grafting combinations and no interaction with the rootstocks could be observed (Table 2). The lowest $K_{petiole}$ was observed for vines grafted onto 41B MGt (5.3 mmol m^{-2} s^{-1} MPa^{-1}), the intermediate values for 101-14 MGt and Riparia Gloire (7.9 mmol m^{-2} s^{-1} MPa^{-1}) and the highest for 3309C and Kober 5BB (12.6 mmol m^{-2} s^{-1} MPa^{-1}) (Table 2).

The chlorophyll index was significantly lower in vines grafted on 41B MGt during most of the season, whereas there were no significant differences among other rootstocks (Figure 4). Although the vines grafted onto 3309C and Kober 5BB displays the highest shoot weight, the differences were only significant between vines grafted on Riparia Gloire and Kober 5BB and between 41BMGt on one side and 3309C and 5BB on the other side (Table 3). The highest yield (0.9 kg m^{-2}) was recorded for vines grafted onto Kober 5BB compared to the other rootstock combinations (0.7 kg m^{-2}). The lowest malic acid content and yeast available nitrogen (YAN) were observed in grapes from vines grafted onto 41B MGt (Table 3). There were no significant differences in sugars, total acidity and tartaric acid among rootstock combinations (Table 3).

Carbon isotope composition ($\delta^{13}C$) measured on sugars in must at ripeness ranged from -24.81 ‰ for vines grafted

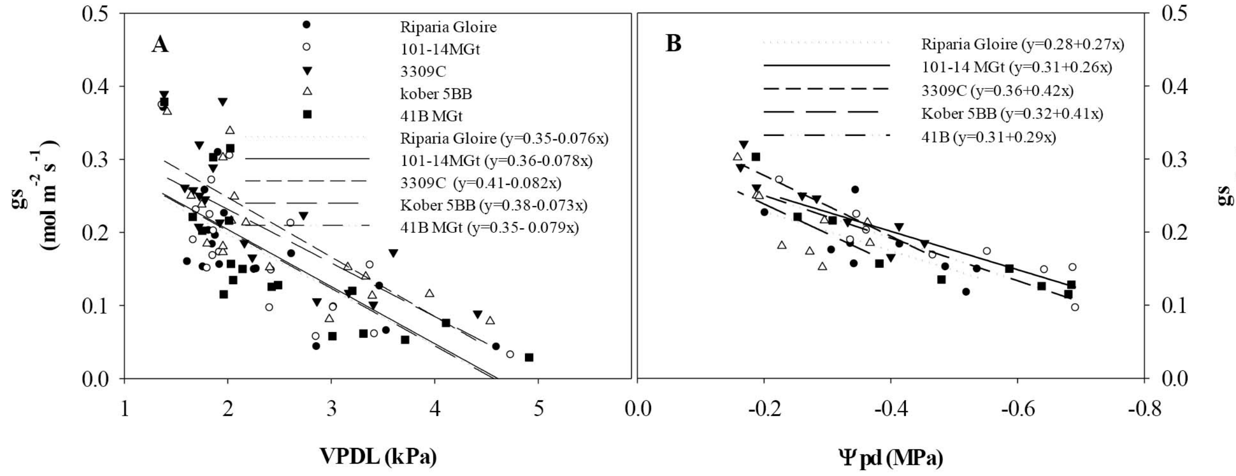

FIGURE 3. Rootstock effects on stomatal conductance response (gs) of Pinot noir to leaf to air vapour pressure deficit (VPDL) (A) and to predawn leaf water potential (Ψ_{pd}) (B).

Equations of liner regression of rootstocks are shown in each panel (n = 6).

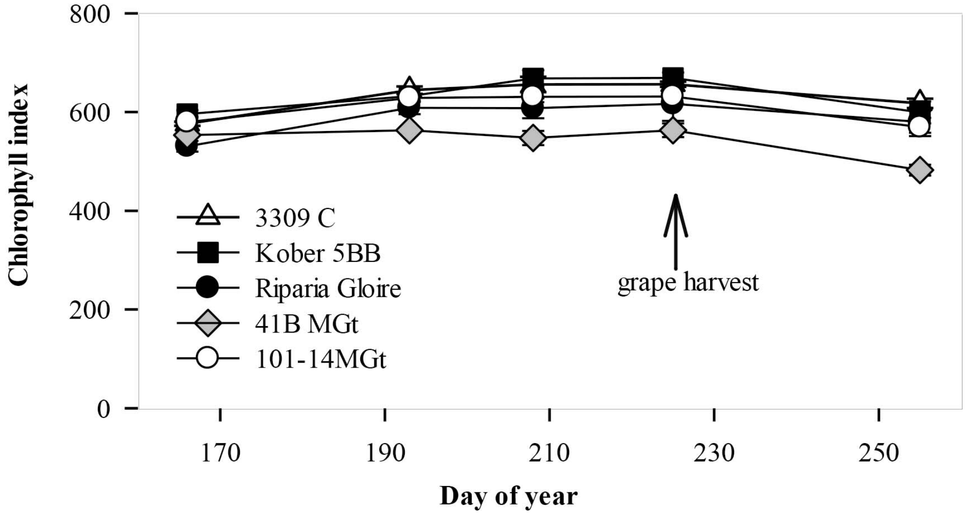

FIGURE 4. Chlorophyll index (N tester) in leaves of field-grown Pinot noir grafted onto different rootstocks.

Values are means ± standard error (n = 90).

onto 3309C to –23.28 ‰ for vines grafted onto 41B MGt (Table 2). The differences were not statistically significant ($P = 0.19$).

The anatomical analyses revealed that most xylem vessels had a cross-sectional area lower than 1000 µm^2, both stems and petioles (Tables 4 and 5). Petioles of Pinot noir grafted onto 41B MGt had the highest proportion of vessels with a cross-sectional area smaller than 500 µm^2 and the lowest proportion with a cross-sectional area higher than 1001 µm^2 (Table 4).

For most category sizes, there was no difference among 101-14 MGt, Riparia Gloire, Kober 5BB and 3309C. In the category size above 2500 µm^2, most differences were observed between vines grafted onto 41B MGt and Kober 5BB, but for a very low frequency.

Stems of vines grafted onto Riparia Gloire and 3309C were characterised by the highest and lowest frequency of small vessels (200-500 µm^2), respectively, whereas there was no significant difference for the other rootstocks (Table 5). The highest frequency of vessels number with a cross-section area higher than 10,000 µm^2 was observed for vines grafted onto 3309C and Kober 5BB compared to 41B MGt and Riparia Gloire. There was no similar pattern among rootstocks between smaller (200–500 µm^2) and larger stem vessels (> 10,000 µm^2) (Table 5).

TABLE 3. Shoot weight (g m^{-1} of shoot length) ($n = 18$), yield (kg m^{-2}) ($n = 13$), sugar content, acidity, tartaric acid, malic acid (g L^{-1}) and yeast assimilable nitrogen (YAN, mg N L^{-1}) ($n = 100$) in must grape of field-grown Pinot noir grafted onto different rootstocks.

Rootstock	Shoot weight	Yield	Sugar	Acidity	Tartaric acid	Malic acid	YAN
	(g m^{-1})	(kg m^{-2})	(g L^{-1})	(g L^{-1})	(g L^{-1})	(g L^{-1})	(mg N L^{-1})
41B MGt	40.7 c	0.7 b	240.3 a	6.8 a	6.4 a	1.9 b	107 b
Riparia Gloire	42.5 bc	0.6 b	232.6 a	7.2 a	6.3 a	2.6 a	175 a
101-14 MGt	49.2 abc	0.6 b	239.8 a	7.0 a	6.1 a	2.5 a	168 a
3309C	56.9 ab	0.7 b	237.8 a	7.2 a	6.4 a	2.6 a	198 a
Kober 5BB	63.7 a	0.9 a	241.7 a	7,3 a	6.5 a	2.6 a	173 a

Means followed by the same letter do not differ at $p < 0.05$ by Fisher's LSD test.

TABLE 4. Frequency (%) of vessels per cross-sectional area (µm^2) category in the petiole of field-grown Pinot noir grafted onto different rootstocks.

	Frequency (%) of vessels per cross-sectional area (µm^2) category					
	200–500	501–1000	1001–1500	1501–2000	2001–2500	> 2500
41B MGt	48.36 b	30.02 a	7.17 b	1.39 c	0.14 b	0.05 c
Riparia Gloire	38.67 a	31.92 a	14.42 a	5.25 b	1.59 ab	0.98 ab
101-14 MGt	39.35 a	30.14 a	13.19 a	6.11 ab	2.13 a	0.60 abc
3309C	37.92 a	32.04 a	14.86 a	6.25 ab	2.41 a	0.56 bc
Kober 5BB	36.30 a	31.57 a	15.42 a	6.81 a	2.27 a	2.45 a

Means followed by different letters in the same column differ significantly at $p < 0.05$ by Fisher's LSD test.

TABLE 5. Frequency (%) of vessels per cross-sectional area (µm^2) category in the stem of field-grown Pinot noir vines grafted onto different rootstocks.

	Frequency (%) of vessels per cross sectional area (µm^2) category					
	200–500	501–1000	1500–3000	3001–5000	5001–10,000	> 10,000
41B MGt	21.99 ab	27.67 a	12.39 a	9.74 a	14.61 a	8.36 cd
Riparia Gloire	32.50 b	25.04 a	9.12 a	7.63 a	10.28 a	7.04 d
101-14 MGt	20.83 a	24.32 a	13.24 a	10.93 a	14.44 a	11.62 bc
3309C	15.90 a	28.70 a	10.86 a	10.16 a	17.33 a	16.65 a
Kober 5BB	23.61 ab	23.10 a	12.64 a	9.31 a	14.03 a	14.21 ab

Means followed by different letters in the same column differ significantly at $p < 0.05$ by Fisher's LSD test.

An inverse correlation was found between S_Ψ and $K_{petiole}$ while shoot weight, petiole vessel size higher than 2000 µm^2 and stem vessel sizes higher than 10,000 µm^2 seemed not to be affected by S_Ψ (Table 6). On the other hand, increased shoot weight and petiole vessel size higher than 2000 µm^2 were associated with increased $K_{petiole}$. A/gs measured at harvest was also significantly correlated with $\delta^{13}C$ (Table 6).

DISCUSSION

The use of drought-tolerant rootstocks has been considered a low-cost adaptive strategy to cope with the decrease in soil water availability in viticultural areas predicted under the context of global change (Fraga *et al.*, 2013; van Leeuwen and Darriet, 2016). This study reports data recorded during one season regarding rootstock genotype effects on ecophysiological and agronomical responses to water deficit of field-grown Pinot noir under cool climatic conditions and how these effects could be related to vigour. In comparison to references, rainfalls were slightly higher during the spring and summer of 2017, but both seasons were considered the third warmest ones since records began in 1864 (MétéoSuisse database). This higher mean temperature recorded during the 2017 growing season associated with the water shortage imposed by soil coverage of the experimental plot with waterproof plastic, equivalent to the year 2009 situation (Spring *et al.*, 2016), contributed to simulating stressful climatic projections for a non-irrigated and well-established vineyard.

Studies regarding scion-rootstocks interactions under water deficit in cool-climate countries have not been well explored as most published data have been carried out under very hot and dry summer conditions such as usually

TABLE 6. Correlations between water stress integral (S_{Ψ}) and the average of specific hydraulic conductivity in petioles ($K_{petiole}$) with shoot weight, petiole vessel size > 2000 μm², stem vessel size > 10,000 μm² and correlation between isotopic carbon composition ($\delta^{13}C$) in must and intrinsic water efficiency (A/gs).

Variables	S_{Ψ} (Mpa d^{-1})			$K_{petiole}$ (mmol m^{-2} $s^{-1}MPa^{-1}$)		
	Equation Regression	R^2	P-value	Equation Regression	R^2	P-value
Kpetiole	y = 15.83-0.52x	0.82	0.03	-	-	-
Shoot weight	y = 68.09-1.41x	0.68	0.08	y = 23.97+2.86x	0.92	0.01
Petiole vessel size > 2000 μm²	y = 5.27-0.21x	0.54	0.16	y = 4.63+1.77x	0.78	0.04
Stem vessel size > 10,000 μm²	y = 17.53-0.47x	0.45	0.21		$\delta^{13}C$ (‰)	
				Equation Regression	R^2	P-value
A/gs				y = 417.05+14.24x	0.87	0.02

recorded in the Mediterranean (Koundouras *et al*., 2008; Romero *et al*., 2018) and Australian (Soar *et al*., 2006a; Stevens *et al*., 2010) vineyards. Moreover, most of these studies were carried out in deficit-irrigated vineyards. Our findings showed that, in a non-irrigated vineyard of a cool country such as Switzerland, invigorating rootstocks favoured the water supply to the scion and it was associated with some features such as increased xylem hydraulic conductivity, high nitrogen uptake and probably also due to larger root system.

The soil coverage used in this experiment promoted a progressive water deficit throughout the growing season, as revealed by the reduction in Ψ_{pd} and Ψ_{stem}. According to the literature, the water deficit intensity observed during the ripening period was classified from weak to moderate (Ψ_{pd} = -0.3 to -0.5 MPa; Ψ_{stem}= -0.9 to -1.1) and severe (Ψ_{pd} < -0.6 MPa; Ψ_{stem}=< -1.4 MPa) depending on rootstocks and physiological indicators of vine water status (Deloire *et al*., 2004; van Leeuwen *et al*., 2009). Based on Ψ_{stem} values, water deficit could be classified as severe for vines grafted onto 41B MGt, moderate for vines grafted onto 101-14 MGt and Riparia Gloire and weak for vines grafted onto 3309C and Kober 5BB. Based on Ψ_{pd} values, only two levels of water deficit could be defined with severe water deficit for 101-14 MGt and 41B MGt (Ψ_{pd} < -0.6 MPa) and mild (-0.3 to -0.5 MPa) for Riparia Gloire, Kober 5BB and 3309C, confirming that Ψ_{stem} is a more sensitive indicator of water status than Ψ_{pd} (Choné *et al*., 2001; Patakas *et al*., 2005; Souza *et al*., 2009). S_{Ψ} was shown to be a useful parameter for estimating and integrating water stress in different crops (Meyers, 1988), including grapevine (Souza *et al*., 2005a). Indeed in this study, S_{Ψ} followed similar patterns as Ψ_{stem} values for the different rootstock combinations. The S_{Ψ} values of Pinot noir grafted onto 3309C and Kober 5BB were similar to values found for Moscatel and Castelão cultivars under full irrigated conditions (100%ETc) in a Mediterranean vineyard during the driest year, while 41B MGt and 101-14MGt induced values near to deficit irrigated Castelão (50%ETc) as shown by Souza *et al*. (2005a). These results indicate that Pinot noir, even under a non-irrigated vineyard in a cool climate region, expressed a lower intensity of water stress than other field-grown cultivars under warmer and drier conditions.

Rootstocks had a more pronounced impact on vine water status than on the gas exchange of Pinot noir. The differences among rootstocks for gas exchange traits were hardly significant, except during the last weeks of the ripening period depending on variables and time of measurements. In general, at later stages of ripening, Kober 5BB and 3309C exhibited higher *A,* gs and *E,* mainly as compared to 41B MGt during the afternoon. *A*, gs and E decreased for all rootstocks from the onset (166DOY) to the last date of measurements (247DOY), with a more pronounced decrease before the 210DOY when Ψ_{pd} values were still above –0.4 MPa and Ψ_{stem} above –1.0 MPa. Nevertheless, differences among rootstocks appeared to be more pronounced afterwards when Ψ_{pd} and Ψ_{stem} further decreased. Grapevines have been considered resistant to drought mainly due to the early and efficient stomatal control to regulate water losses during mild to moderate water stress (Chaves *et al*., 2010). Even under water deficit characterised as severe (Ψ_{pd}=-0.7MPa) (240 and 247 DOY), the values of mid-morning gs were lower than 0.10 mol m^{-2} s^{-1} in vines grafted onto 41B MGt and 101-14 MGt only at 240DOY due to the higher VPDL (2.3 kPa). However, at 247 DOY, all rootstocks induced values of mid-morning gs higher than 0.10 mol m^{-2} s^{-1} when VPDL was lower (1.8 kPa), indicating still active gas exchanges and the absence of metabolic limitations of photosynthesis as previously suggested (Medrano *et al*., 2002).

Gs was significantly correlated with Ψ_{pd} (excepted for Kober 5BB) and with VPDL, suggesting control by both the soil water status (dependent on the rootstocks) and the evaporative demand of the atmosphere (independent of the rootstock) (Davies *et al*., 2002; Chaves *et al*., 2016). However, the relationships of gs versus VPDL and Ψ_{pd} suggested a similar stomatal response among rootstocks, even though it was previously shown that the sensitivity of gs to VPDL and Ψ_{pd} could be affected by the scion cultivar and rootstock (Soar *et al*., 2006a,b; Tramontini *et al*., 2013). In our study, in some few days, *A*, gs and *E* were higher for the more vigorous vines (higher stem weight) grafted on 3309C and Kober5BB, in agreement with the observations of Soar *et al*. (2006a), who reported, in an irrigated vineyard, that the most vigorous rootstocks such as Teleki 5C and Ramsey induced the highest gs in Syrah. According to these authors, rootstock effects on canopy gas exchange would be initially driven by differences in root capacity to extract soil water, mediated via dynamic variation in physiological-active leaf abscisic acid (ABA).

Along the water deficit progression, an increase in intrinsic water use efficiency (A/gs) was reported for all rootstocks, agreeing with previous authors (Souza *et al*., 2005a; Chaves *et al*., 2016; Zufferey *et al*., 2017). Leaf ageing along the growing season could also affect A/gs. The highest *A*/gs has been observed in vines grafted onto 41B MGt, mainly due to the lowest stomatal conductance induced by this rootstock during the days where significant differences among rootstocks were found. In contrast to gas exchange techniques that provide measurements of photosynthesis rates at a single point in time, $\delta^{13}C$ measured on must integrate the ratio of net photosynthesis to water transpired over the growing season. It has been shown to be a useful tool for evaluating vine water use efficiency over the ripening period (Gaudillère *et al*., 2002; Souza *et al*., 2005a; Zufferey *et al*., 2017). Although $\delta^{13}C$ was correlated with mid-morning A/gs measured at harvest, the rootstocks could not be discriminated by $\delta^{13}C$, probably due to the small range of $\delta^{13}C$ values among rootstocks (van Leeuwen *et al*., 2009). It could also be related to a short period of water deficit and small differences in the level of water stress intensity among rootstocks, while in other studies, the authors found larger differences in vine water status among water treatments (Souza *et al*., 2005a; Zufferey *et al*., 2017).

A pronounced effect of rootstock on $K_{petiole}$ of Pinot noir leaves was observed. The seasonal average of $K_{petiole}$ was closely correlated with S_Ψ, petiole xylem vessel frequency higher than 2000 µm^2 and shoot weight, suggesting a link between petiole hydraulic conductivity, petiole xylem anatomy, stem vigour and water status. $K_{petiole}$ was high for Kober 5BB and 3309C as compared to other rootstocks. 101-14 MGt was characterised by a higher $K_{petiole}$ and a greater frequency of xylem vessels with a larger cross sectional area in the petiole than 41B MGt. It was previously shown that petiole specific hydraulic conductivity ($K_{petiole}$) of grapevine cultivars could be affected by vine water status (Zufferey *et al*., 2017; Dayer *et al*., 2017) and our data show that it can also be affected by rootstock genotype, probably in a relationship with conferred vigour.

Shoot vigour was significantly correlated with the frequency of the largest vessels in the stem. It supports the findings reported by Santarosa *et al*. (2016) for grapevine and by Gonçalves *et al*. (2005) for sweet cherries, which show that vigorous rootstocks affect the size of scion xylem vessels. Atkinson *et al*. (2003) also reported that the stem hydraulic conductivity of grafted apple trees was increased by invigorating rootstock in relation to xylem anatomical changes. In peach, xylem vessel characteristics of the dwarfing rootstock genotypes appear to influence tree growth directly rather than through an effect on the xylem vessel size of the scion (Tombesi *et al*., 2010).

Although the negative relationship between shoot weight and S_Ψ was not significant, drought responses of field-grown Pinot noir appeared to vary according to differences in shoot vigour induced by rootstock over the growing season. Vines grafted onto high vigorous rootstocks (3309C and Kober 5BB) were characterised by higher Ψ_{pd} and Ψ_{stem} compared to 41B MGt, Riparia Gloire and 101-14 MGt, which induced a lower shoot weight and more negative Ψ_{pd} and Ψ_{stem}. These observations agree with those of Souza *et al*. (2009), who showed that vigorous rootstocks promoted water status and vegetative vigour of Syrah vines. They also agree with the results reported by Spring *et al*. (2016) under rainfed conditions, suggesting that rootstocks conferring a high vegetative vigour appear to limit the appearance of water deficit in vineyards.

The lowest shoot vigour measured in vines grafted onto 41B MGt was probably not explained by water deficit as the largest water status differences on Ψ_{pd} appeared only after the 2 August once shoot growth was largely completed and because of the absence of correlation between S_Ψ and shoot vigour. The absence of a relationship between S_Ψ and frequency of vessel sizes larger than 2000 µm^2 in petiole and larger than 10,000 µm^2 in stem also suggests that vascular anatomy was not affected by water deficit but most probably related to differences of vigour promoted by rootstocks. The reduced stem xylem vessel size observed for the less vigorous vines can decrease the shoot hydraulic conductivity and the efficiency of the water flux in vine shoots under a soil water restriction, as demonstrated by Pagay *et al*. (2016).

The lowest chlorophyll index values measured in vines grafted onto 41B MGt at the beginning of the season, under not-yet limiting water conditions, indicate that shoot vigour was also reduced due to low nitrogen uptake rather than water limitation. This is also in agreement with the lower yeast assimilable nitrogen of grape must observed for this rootstock. It is well known that nitrogen is one of the most important nutrients for vegetative development because it is a component of proteins, enzymes, chlorophyll and nucleic acids (Taiz and Zeiger, 2004) and, therefore, under low N supply, vine shoot growth can be compromised (Keller, 2010; van Leeuwen *et al*., 2018). As root system development is correlated with vegetative vigour (Southey and Archer, 1988), it may be hypothesised that a reduced root system development for the vines grafted onto 41MGt may have impaired both nitrogen and water uptake. In addition, the negative effect of water deficit on nitrogen uptake and content in leaves and berries could have further limited YAN content in grape must of vines grafted onto 41B MGt (Spring *et al*., 2012; Zufferey *et al*., 2017). Low vigour may also explain the lowest malic acid content for this combination, probably due to an increased berry exposure to sunlight, favouring malic acid degradation by rising in temperature as shown by other authors (Souza *et al*., 2005b; Santos *et al*., 2007; Zufferey *et al*., 2017). A direct effect of drought level on malic acid content has also been reported occasionally (Kizildeniz *et al*., 2018).

There is no accordance in the literature regarding the degree of drought tolerance among these five rootstocks ranging from low to highly drought-sensitive depending on scions and experimental conditions (Serra *et al*., 2014; Ollat *et al*., 2016; Spring *et al*., 2016). Under semi-arid conditions, Romero *et al*. (2018) reported that Monastrell vines grafted onto 41B MGt exhibited a lower vigour and yield than vines grafted onto 1103P, without any differences in vine water status. It was also shown that Sultana vines grafted onto 41B MGt grown in pots showed a reduced root system development and shoot biomass in comparison to vines grafted onto Kober 5BB (Yıldırım *et al*., 2018). Our field study brings additional data to show that of the responses to water restriction of Pinot noir vines were affected by the vigour conferred by rootstocks. Although 41B MGt was supposed to be the most lime tolerant rootstock among the five studied, it did not perform well in these alkaline conditions. Kober 5BB and 3309 provided the slowest water deficit progression throughout the growing season, probably due to a better water supply supported by a larger and deeper root system. From a practical point of view, our one-year data contribute to support that the use of vigorous rootstocks could avoid the need for supplemental irrigation in vineyards during the periods of water scarcity. Nevertheless, experiments should be replicated to identify the most relevant traits to characterise drought responses induced by different rootstocks under field conditions to be used in breeding programs for a cool climate region.

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