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ORIGINAL RESEARCH ARTICLE

Maximum stomatal conductance rather than stomatal sensitivity to drought differentiates the PIWI grapevine cultivar Souvignier gris from Muscaris and Donauriesling

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ABSTRACT

Pioneering grapevines, also referred to as "PIWIs" (after the German expression "*pilzwiderstandsfähig*" = fungus resistant), represent an interesting alternative to classic cultivars in several wine regions. Their resistance to some fungal diseases places them among the potential solutions for sustainability, especially under organic production systems. However, little is known about their response to abiotic stressors, such as water stress under drought conditions. Here we studied the response of three PIWI cultivars (Donauriesling, Muscaris, Souvignier gris, and Riesling or Grüner Veltliner *Vitis vinifera* cultivars as comparison; all plants without fruit) to drought under semi-controlled conditions over two consecutive seasons. While in season 2020, we imposed a single dry-down and re-watering cycle inside a glasshouse, during season 2021, we subjected the vines to three cycles of dehydration and re-watering under field conditions with the aid of a rain shelter. Along the experiments, we monitored leaf gas exchange and water potential during soil dehydration and characterised leaf area development and some key leaf hydraulic traits. Despite different conditions in our two study seasons, we found connective significances in some key parameters. Under well-watered conditions, Souvignier gris had the highest rates of stomatal conductance, whereas Muscaris exhibited the most conservative water use behaviour. When under drought, Souvignier gris showed an apparent less tight stomatal control than the other PIWIs in coordination with a lower (more negative) osmotic potential and turgor loss point. Our results suggest that Muscaris (and Donauriesling showed similar behaviour) might be more suitable for non-irrigated conditions than Souvignier gris. However, further research on berry composition and open field is needed to upscale our results and address the water management of PIWI vineyards.

KEYWORDS: water stress, intrinsic water use efficiency, stomatal regulation, pioneering grapevines, sustainable viticulture

INTRODUCTION

Fungus-resistant grapevine cultivars, also named "PIWIs" (short form for German "*pilzwiderstandsfähige Rebsorten*" = fungus-resistant grapevine cultivars), were developed by multiple backcrossing of interspecific hybrids (the cross-breeding of *Vitis vinifera* L. and North American and Asian *Vitis* species) with *Vitis vinifera* (Montaigne *et al*., 2016; Pedneault and Provost, 2016; Töpfer and Trapp, 2022). The advantage of these new genotypes is to interlink the resistance of most American species to fungal diseases (mainly *Erysiphe necator, Plasmopara viticola*) while retaining the high fruit quality characteristics for the vinification of *Vitis vinifera* cultivars (Hofmann *et al*., 2014; Fischer-Colbrie *et al*., 2015). In addition to the expediency of fungus resistance, there are economic advantages since the seasonal treatments of plant protection can be decreased and subsequently lead to lower annual doses of pesticides and fuel (Pedneault and Provost, 2016; Pertot *et al*., 2017). As winegrower's decisions towards sustainable management strongly depend on national and regional regulations (Chen *et al.*, 2022), and the European Commission aims to halve the use of pesticides by 2030 according to the "Farm to Fork" strategy (European Commission, 2020), the demand for robust new cultivars with low susceptibility to pathogens is raising. However, while the screening of resistance against pathogens and pests is part of the breeding and selection program (Reynolds, 2015; Merdinoglu *et al*., 2018), there is limited information on the agronomic performance of PIWIs in different climates (Casanova-Gascón *et al*., 2019) and on their response to abiotic stresses (e.g., drought).

Extensive world viticultural regions are located in areas characterised by warm and dry summers, often managed in rainfed conditions, with periods of drought and heatwaves (Moriondo *et al*., 2013). Moreover, droughts are further forecasted to increase due to climate change (Schultz and Stoll, 2009; Cook *et al*., 2018), and irrigation may not be sustainable in several regions (Costa et *al*., 2016; Gambetta *et al*., 2020). Stomatal regulation is considered a proxy to describe how grapevines respond to drought stress (Medrano *et al*., 2002; Chaves *et al*., 2010). Since stomata are responsible for CO_2 intake, their regulation is of great importance in terms of photosynthesis and water use efficiency (Schultz and Stoll, 2009; Medrano *et al*., 2018). There are biochemical (mainly driven by the plant hormone abscisic acid) and hydraulic mechanisms involved in the stomatal reaction to drought stress which are hard to disentangle (Buckley, 2019; Gambetta *et al*., 2020). Although *V. vinifera* cultivars may respond differently to drought, some exhibiting a tighter or a looser stomatal control in response to water deficit (Tombesi *et al*., 2014; Hochberg *et al*., 2016; Dayer *et al.*, 2020; Levin *et al.,* 2019), it is not yet clear to what extent such differences result from genetical or environmental factors or the combination of both (Van Leeuwen *et al*., 2019; Gambetta *et al*., 2020). Furthermore, to the best of our knowledge, no study has assessed the stomatal regulation of grapevine PIWIs nor compared it against *V. vinifera* cultivars. In this study, we set out to investigate the response to the drought of three PIWI grape cultivars of commercial importance in Europe, focusing on their stomatal regulation dynamics during soil dehydration.

MATERIALS AND METHODS

1. Plant material and study site

The study was carried out in the glasshouse and semi-controlled field facilities of BOKU UFT (Tulln, Austria) during the years 2020 and 2021. All experiments were conducted on one-year-old grapevines of Donauriesling ("D"; Riesling × Fr 589-54 [Seyve Villard 12-481 × Freiburg 153- 39 (Pinot Gris × Weißer Gutedel)]), Muscaris ("M"; Solaris × Gelber Muskateller), and Souvignier gris ("S"; Seyval Blanc × Zaehringer), all grafted on SO4 (in 2020) or Kober 5BB (in 2021) rootstocks (both *Vitis* interspecific crossings from *Berlandieri Resseguier* × *Riparia*), acquired from a local nursery and potted in 7 L plastic pots filled with commercial potting media supplemented with 20 % perlite (water holding capacity 0.6 v/v). In both seasons, plants of *Vitis vinifera* (cvs. Riesling "R" and Grüner Veltliner "G" in 2020 and 2021, respectively) were included in the trials (Figure 1).

For the first experiment in 2020, a total of 64 plants (n = 16 per cultivars D, M, S, and R) were potted on the 18th April and randomly arranged within 6 rows inside the glasshouse. Plants were allowed to develop one single shoot and were grown without water limitations for 80 days (establishment period) until each vine reached a height of about 1.80 m; all lateral shoots were removed. The plants were watered daily by drip irrigation (two drippers per pot) until dripping was observed from the bottom of the pot (on average between 1.5–2 L per day). In season 2021, a total of 160 plants of the same PIWI genotypes and Grüner Veltliner as V. vinifera cultivar (n = 40 per cultivar D, M, S and G) were potted on the 8th May and arranged within three rows (nort-south orientation) under filed conditions in blocks of 10 plants per cultivar each. The three rows were covered by a rain shelter (transparent plastic roof of 4 m height and opened at the sides as similarly described in Herrera *et al.,* 2015). The vines were grown for 86 days without water limitations by daily irrigating them (one dripper per pot) to field capacity until dripping was observed from the bottom of the pot (on average between 2–2.5 L per day); growth was not restricted, and laterals were not removed. Fertilisation was performed by adding 10 g of EntecVino® in each pot towards the end of May.

2. Environmental conditions

Air temperature and relative humidity were recorded hourly during study periods using a data logger (UT330B, Uni-trend Technology, Hong Kong) in the glasshouse in season 2020 and the local weather station of IWOB vineyard in 2021 (positioned 50 m apart from the study site); temperature and humidity data were used to calculate the vapour pressure deficit during the central hours of the day (between 10–14 h; VPD_{max}). The main climate parameters were followed over both years in both experimental set-ups: Records of climate

data in season 2020 showed an average temperature of 19 °C during the experiment (between DOY 189 and 212) in the glasshouse, with maximum temperatures reaching 33 °C during the drought period and at the end of the experiment (Supplementary Figure 1A). The daily minimum temperature was relatively stable at about 13 °C. The vapour pressure deficit during the central hours of the day (VPD_{max}) was, on average, 2.04 kPa during the experiment, reaching peak values of 2.96 kPa, 2.36 kPa, and 2.45 kPa on DOY 192, DOY 197, and DOY 202, respectively. In 2021, the average temperature between DOY 214 and DOY 261 in the semi-controlled field was 17.6 °C, while increasing temperatures in the first days of the experiment. In the duration of the experiment, temperatures peaked at 29.2 °C on DOY 222 and 32.3 °C on DOY 227. The average maximum and minimum temperatures were 24 °C and 12 °C, respectively. The vapour pressure deficit during the central hours of the day (VPD_{max}) was, on average, 1.32 kPa, with peak values of 2.61 kPa on DOY 227 and 2.52 kPa on DOY 252 (Supplementary Figure 1B).

3. Irrigation treatments

In season 2020, on July 7th (day of the year DOY 189; after the 80 days of establishment period), two treatments were randomly established in half of the plants (i.e., eight vines per cultivar): (i) water-stressed "ws", where irrigation drippers were removed from the pots for 13 days; (ii) well-watered controls "ww", where daily irrigation was maintained to field capacity as before. After 13 days without irrigation (DOY 202), all vines of the ws treatment were manually re-watered to saturation, and thereafter daily irrigation was resumed to the same levels of control. Gas exchange after re-watering was monitored for the successive 10 days (until DOY 212) (Figure 1A).

In season 2021, half of the plants were always maintained under well-watered conditions by daily irrigating the plants just as during the establishment period. The second half of the plants (i.e., 20 vines per cultivar) were exposed to three cycles of drought (water-stressed "ws"; water-stressed plants remained the same throughout all three cycles) by removing the irrigation drippers from the pots for 8 days; after each drought cycle, all vines were re-watered and maintained under "ww" conditions until the next drought cycle. The first ws cycle was imposed on DOY 214 (August 2nd; after 86 days of establishment period) and lasted until DOY 221. The second ws cycle started at DOY 242 until DOY 249, and the third ws cycle occurred from DOY 253 to DOY 260 (Figure 1B).

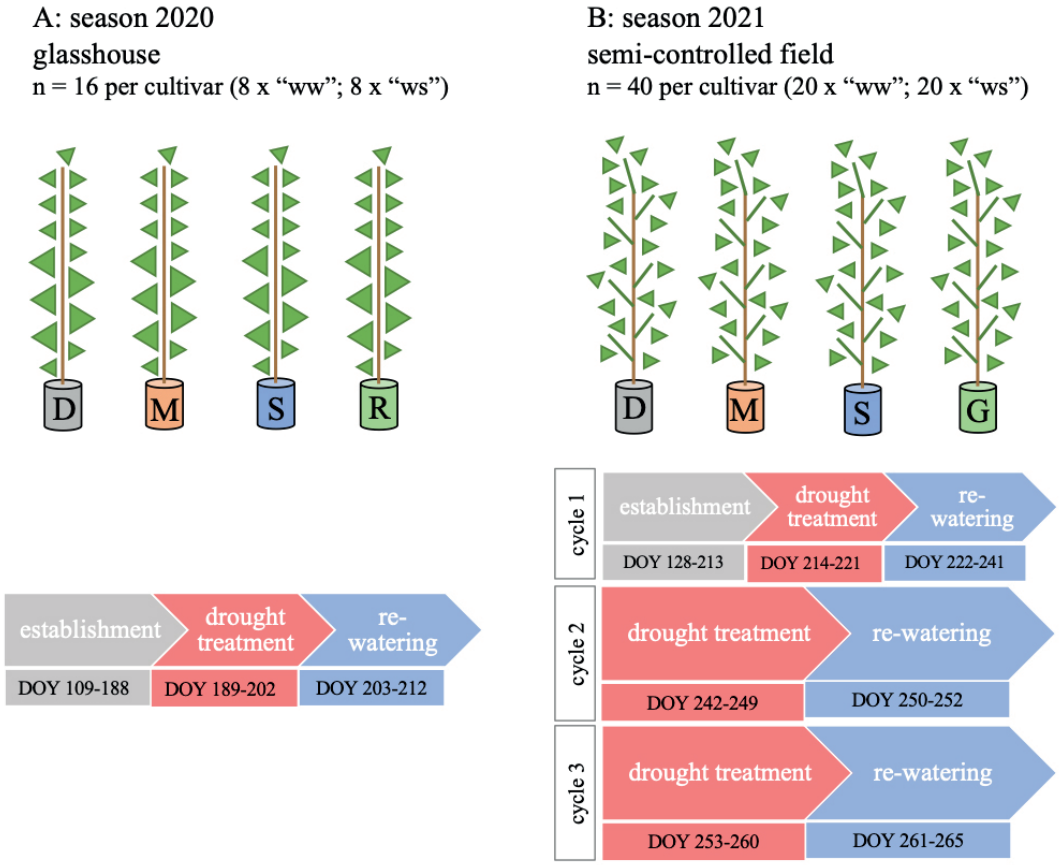


FIGURE 1. Experimental set-up of the two trials in 2020 (A) and 2021 (B).

2020: laterals were removed; 2021: no growth restrictions were imposed. Treatments were applied on half of the plants in both seasons: D, Donauriesling; M, Muscaris; S, Souvignier gris; R, Riesling/G, Grüner Veltliner; ww, well-watered; ws, water-stressed.

4. Leaf area measurements

In both seasons, leaf area was measured following the protocol by Herrera *et al.* (2021), starting on DOY 189 (2020) and DOY 214 (2021), where the leaf length of all the leaves of four vines per cultivar was measured. Another measurement was taken of leaves from three vines per cultivar and treatment at the end of the experiments. In 2020, at the end of the experiment (DOY 212), 15 leaves per cultivar were collected, and the leaf length and area were determined by image analysis using the ImageJ software; the data was used to compute a regression between leaf length and single leaf area per each cultivar. The equation was then used to calculate the leaf area per plant at DOY 189 and 212. Since in season 2021 plants were grown without restrictions (laterals and small leaves included), leaf area was determined by using a leaf area meter (LI-3100 Area Meter, LI-COR. Inc. Lincoln. Nebraska USA); we matched both data sets (season 2020 and 2021) to compute an average regression per cultivar (Supplementary Table 1); at the end of the experiment in season 2021, 10 main leaves (fully expanded) per cultivar were collected and measured to compare the average single leaf area of both seasons.

5. Gas exchange and water potential

Stomatal conductance (g_s), transpiration (E), and leaf net assimilation rate (A_N) were measured regularly using an infrared gas analyser (LC-ProSD, ADC Bio Scientific, UK). The measurements were done between 09:30 and 11:30 h (local time) on three (in 2020) or four (in 2021) plants per cultivar and treatment (using a healthy and sun-exposed leaf from the main shoot). The instrument was set to a constant airflow of 200 µmol s^{-1} and saturating light conditions (1500 µmol $m^{-2}s^{-1}$), while temperature, humidity, and CO_2 (400–450 ppm) were set to ambient conditions.

Stem water potential (Ψ_{stem}) was measured at midday (between 12:00–14:00 h, local time) on one mature leaf per vine (same plants as used for gas exchange analysis) with a Scholander pressure chamber (PMS 600, PMS Instrument Company, USA) as described in Levin (2019).

6. Leaf pressure–volume curves

In season 2020, at the end of the experiment (DOY 213), five leaves per cultivar (only well-watered control plants) were sampled in the morning (08:00 h) for the construction of pressure–volume curves. The bench dry method was used, as described in Dayer *et al.* (2020), which consists of plotting the reciprocal of leaf water potential (–1 / Ψ) against the relative water content (RWC). The RWC of the leaf was calculated as RWC = (fresh weight – dry weight) / (full turgor weight – dry weight) × 100. From the pressure–volume curves, the leaf water potential at the turgor loss point (Ψ_{tlp}), the osmotic potential at full turgor (π_{100}), and the modulus of elasticity (ε) were calculated as described in Dayer *et al*. (2020).

7. Statistics

The statistical evaluation of the results was carried out using the software SPSS Statistics (version 26, IBM Corp.). One plant was considered a biological replicate. All results were tested for normality and homogeneity of variance prior to being subjected to F-test ($p < 0.05$) in a two-way ANOVA (results of vegetative growth, gas exchange, and stem water potential; main factors were treatment and cultivar and their interaction) or one-way ANOVA (results of leaf size and pressure–volume curves; factor was cultivar). Post-hoc analyses were performed using the Tukey-HSD test ($p < 0.05$). All figures were created using SigmaPlot v14 (Inpixon, CA, USA) or ggplot2 package (Wickham, 2016) in R.

RESULTS

1. Vegetative growth

Despite different conditions (experimental set-ups, environmental conditions, rootstocks) within the two experiments, results show connective significances in some vegetative growth parameters (Figure 2, Table 1).

As observed in Figure 2, Muscaris (M) was the cultivar with the largest leaves (average area of single leaves on the main shoot of ca. 150 cm^2 across seasons), followed by Souvignier gris (S) (ca. 130 cm^2) and Donauriesling (D) (ca. 95 cm^2). Such differences translated into M recording a higher total leaf area per plant than the other cultivars only in 2020, while in 2021, all cultivars showed similar values of total leaf area per plant (Table 1). In detail, in 2020, before the imposition of drought treatments (DOY 189), the plant leaf area recorded was 3680.3, 2536.9, and 2898.6 cm^2 $vine^{-1}$ in M, S, and D, respectively; after 23 days of experiments (at DOY 212), M, S, and D recorded (average across treatments) 4996.7, 3394.5, and 3896.2 cm^2 $vine^{-1}$, respectively. As expected, drought treatments limited growth in all cultivars that resulted in leaf areas of ws vines being, on average, 15 % smaller than ww ones. Similar growth restriction was observed in 2021 after the three cycles of ws treatments (DOY 261). Statistical interactions of treatment × cultivar were not significant ($p > 0.05$) in both seasons (Table 1).

2. Water potential and gas exchange

According to our expectations, drought imposition determined a decrease in stem water potential (Ψ_{stem}), and values recorded

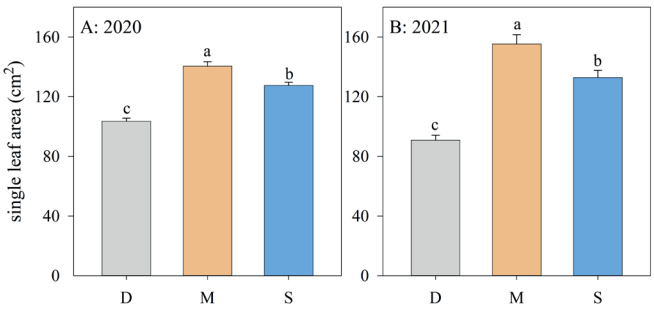


FIGURE 2. Single leaf area (average area of a single leaf on the main shoot) per cultivar in seasons 2020 (A) and 2021 (B).

Values are averages per cultivar + standard error of the mean (n > 15 and n = 10 in 2020 and 2021, respectively) sampled during the experiments (DOY 189 and DOY 214 in 2020 and 2021, respectively). Different letters identify significant differences between cultivars after a Tukey-HSD post-hoc test ($p < 0.05$).

TABLE 1. Leaf area (cm^2 $vine^{-1}$) at the beginning and end of experiments in 2020 and 2021.

	2020		2021	
	DOY 189	DOY 212	DOY 214	DOY 261
Cultivar	***	***	ns	ns
Muscaris	3680.3 a	4996.7 a	3581.2	6486.2
Donauriesling	2536.9 b	3394.5 b	3568.6	5691.8
Souvignier gris	2898.6 b	3896.2 b	3237.4	7120.9
Riesling / G. Veltliner	2658.6 b	4067.0 b	3456.4	6904.7
Treatment		***		*
ww		4359.7		6969.1
ws		3737.0		6132.6
Cultivar × Treatment		ns		ns

Values are the average of four (at the beginning; DOY 189 and 214) and three (at the end; DOY 212 and 261) vines per cultivar and treatment combination. Statistics are reported after one-way ANOVA (DOY 189 and 214) and two-way ANOVA (DOY 212 and 261) with cultivar and irrigation treatment as main factors and its interaction: ***, *, ns, state $p < 0.001$, $p < 0.05$, and $p > 0.05$, respectively. Different letters denote significant differences ($p < 0.05$) among cultivars after a Tukey-HSD post-hoc test.

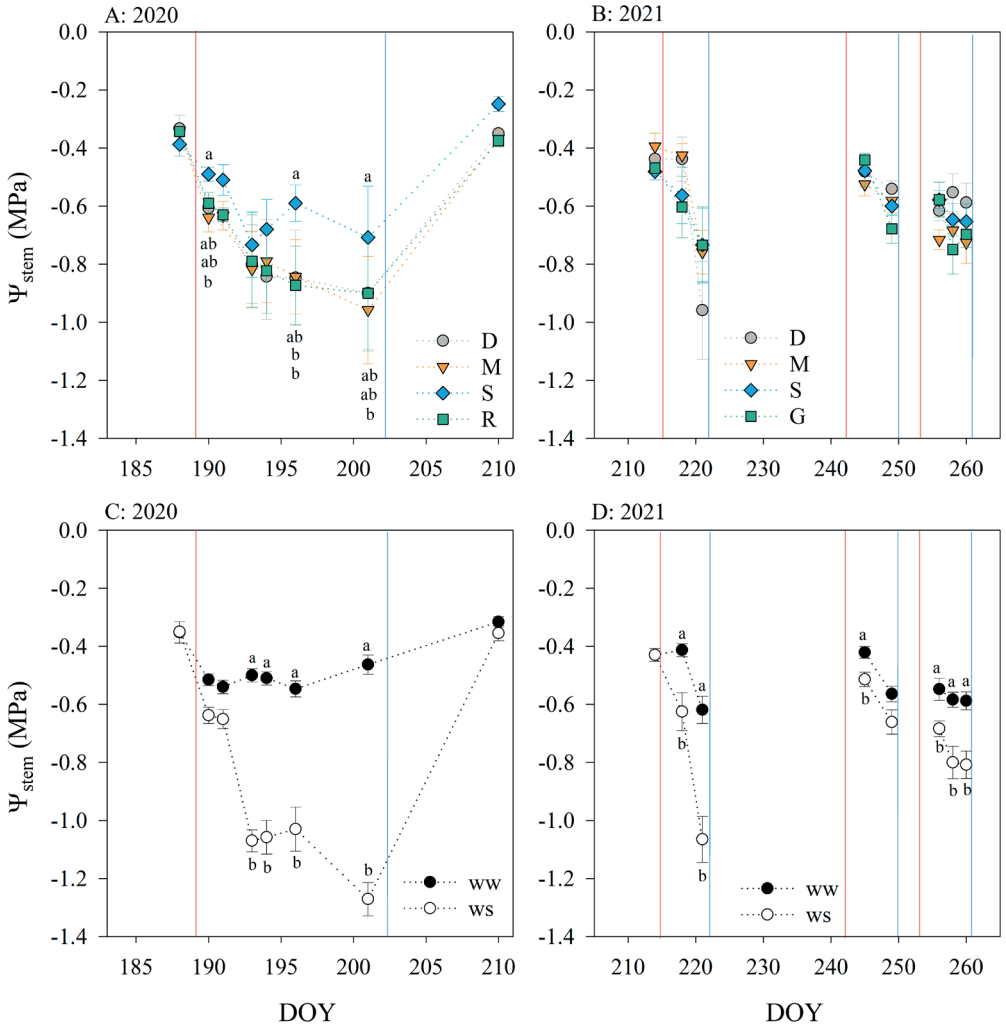


FIGURE 3. Midday stem water potential (Ψ_{stem} in MPa) in season 2020 (A,C) and 2021 (B,D).

Vertical lines indicate the imposition of drought (red) and re-watering (blue) treatment. The figures represent a two-way ANOVA analysis with cultivars and treatments as main factors; therefore, values are presented as averages ± standard error per cultivar (A, 2020: n = 6; B, 2021: n = 8) or per treatment (C, 2020: n = 12; D, 2021: n = 16) on each date (DOY, day of the year). Different letters denote significant differences ($p < 0.05$) between the main factor cultivar (in A and B) or treatment (in C and D). No significant interactions were recorded between main factors at any date of measurement ($p > 0.05$). D, Donauriesling; M, Muscaris; S, Souvignier gris; R, Riesling/G, Grüner Veltliner; ww, well-watered; ws, water-stressed.

for the *V. vinifera* cultivars were not significantly different from those from the tested PIWI cultivars in both seasons (Figure 3, Supplementary Figure 2). In 2020, it is notable that the average Ψ_{stem} of Souvignier gris (S) vines recorded significantly higher values as compared to all other cultivars on DOY 189, 196, and 202 after irrigation withholding (Figure 3A). The Ψ_{stem} of well-watered control vines ranged between –0.32 and –0.57 MPa without significant differences between cultivars (Figure 3C). Starting from equal Ψ_{stem} values of –0.35 MPa at DOY 189, water-stressed vines of all cultivars showed a decrease in Ψ_{stem} that reached the lowest values at the peak of drought stress on DOY 202, ranging from –1.10 MPa (in S) to –1.35 MPa (in M) (Supplementary Figure 2). During all three cycles in 2021, drought treatment decreased the average Ψ_{stem} of all cultivars (Figure 3B). Speaking of statistical significances, in the first cycle, the minimum of Ψ_{stem} was at –0.62 and –1.07 MPa for averaged ww and ws vines, respectively (Figure 3D); during the second cycle, significant change in the Ψ_{stem} was only shown in Grüner Veltliner (G) vines (DOY 248) (Supplementary Figure 2 right column), while in the third cycle the Ψ_{stem} of the ws averages decreased to –0.81 MPa as compared to the –0.58 MPa of the ww vines (Figure 3D).

The tested cultivars in our study exhibited differences in stomatal conductance (g_s) both under well-watered conditions and under drought (Figure 4 and Supplementary Figure 3). When under drought, S exhibited higher rates of g_s as compared to the other PIWIs. Particularly in season 2020, S showed a slower stomatal closure than M and D (Figure 4A and Supplementary Figure 3 left column); indeed, while M and D reached values of $g_s < 0.05$ mol m^{-2} s^{-1} (considered a severe stress for grapevines according to Medrano *et al.,* 2002) already after three days of irrigation withholding, S reached similar g_s values only after six days without irrigation. In season 2021, during each cycle of drought, S never reached g_s values below 0.05 mol m^{-2} s^{-1} and had statistically higher g_s values ($p < 0.05$) than the other cultivars during most of the experimental time (Figure 4B and Supplementary Figure 3 right column). Under well-watered conditions, S recorded the highest maximum stomatal conductance among PIWIs in both seasons ($g_{s\,max}$ of 0.343 and 0.248 mol m^{-2} s^{-1} in 2020 and 2021, respectively), surpassed only by R in 2020 ($g_{s\,max}$ = 0.360 mol m^{-2} s^{-1}) (Supplementary Figure 3). On the other hand, M was consistently the cultivar with the lowest maximum stomatal conductance ($g_{s\,max}$ of 0.238 and 0.168 mol m^{-2} s^{-1} in 2020 and 2021, respectively), closely followed by D (g_s of 0.317 and 0.163 mol m^{-2} s^{-1} in 2020 and 2021, respectively).

Re-watering in 2020 at DOY 203 resulted in stomata reopening of ws plants that did not reach the values of ww controls until DOY 208 (Figure 4C). In 2021, re-watering after the first drought cycle lasted for 20 days, and, even if not monitored, it is assumed that ws vines reached similar g_s values as the ww ones. Indeed, there are no significative differences between ww and ws at the beginning of the second cycle at DOY 242 (Figure 4D); between the second and third cycles of drought, re-watering of two days did not produce a significant effect in g_s (Figure 4D).

Transpiration (E) and net assimilation (A_N) values are presented in Supplementary Figures 4 and 5. Both parameters resembled the stomatal conductance (g_s) behaviour previously described. After a two-way ANOVA analysis with cultivars and treatments as main factors, the results showed that the averaged transpiration rates (E) of M and D decreased faster and reached lower values than S shortly after the drought imposition in both seasons. For example in season 2020, values of E in M and D were significantly lower than in S already at DOY 190 (one day after irrigation withholding) (M = 3.15, D = 3.39 and S = 4.59 mol m-2 s-1; Supplementary Figure 4A). Similarly, in 2021 at DOY 217 (three days after irrigation withholding) E values of M and D were as well statistically lower than S (M = 1.33, D = 1.72 and S = 2.03 mmol m^{-2} s^{-1}; Supplementary Figure 4B).

Drought stress resulted in lower assimilation rates (A_N), especially in season 2020, where averaged A_N values in ws plants were below 7 µmol $m^{-2}s^{-1}$ at all dates after drought imposition (Supplementary Figure 5C). The statistical analysis of different PIWI cultivars during drought showed higher A_N values in S as compared to D and M (e.g., averaged A_N in season 2020 at DOY 194: S = 7.71, D = 5.64 and M = 5.05 µmol $m^{-2}s^{-1}$; Supplementary Figure 5A), despite in season 2021 fewer differences in cultivars were apparent (Supplementary Figure 5B).

3. Leaf pressure–volume parameters

Analysis of leaf pressure–volume relations (performed only in 2020) showed that the water potential at the turgor loss point (Ψ_{tlp}) and the osmotic potential at full turgor (π_{100}) were more negative (by 0.2 MPa) in S as compared with the other tested cultivars (Table 2). The modulus of elasticity (Ɛ) did not differ among cultivars and was, on average, 10.7 MPa.

TABLE 2. Pressure–volume curves were used to calculate the water potential at turgor loss (Ψ_{tlp}), the osmotic potential at full turgor (π_{100}), and the modulus of elasticity (Ɛ).

	Donauriesling	Muscaris	Souvignier gris
Ψ_{tlp} (MPa)	–1.36 ± 0.02 b	–1.34 ± 0.03 b	–1.53 ± 0.03 a
π_{100} (MPa)	–1.10 ± 0.03 b	–1.06 ± 0.03 b	–1.31 ± 0.03 a
Ɛ (MPa)	12.4 ± 0.71 a	10.7 ± 1.33 a	9.08 ± 0.40 a

Values are averages ± standard error (n = 5). Within each row, different letters indicate statistical differences between cultivars determined by the Tukey-HSD post-hoc test ($p < 0.05$).

DISCUSSION

The results obtained in this study allowed the comparison of the water use and response to drought of three commercial PIWI cultivars. We found that Souvignier gris (S) recorded

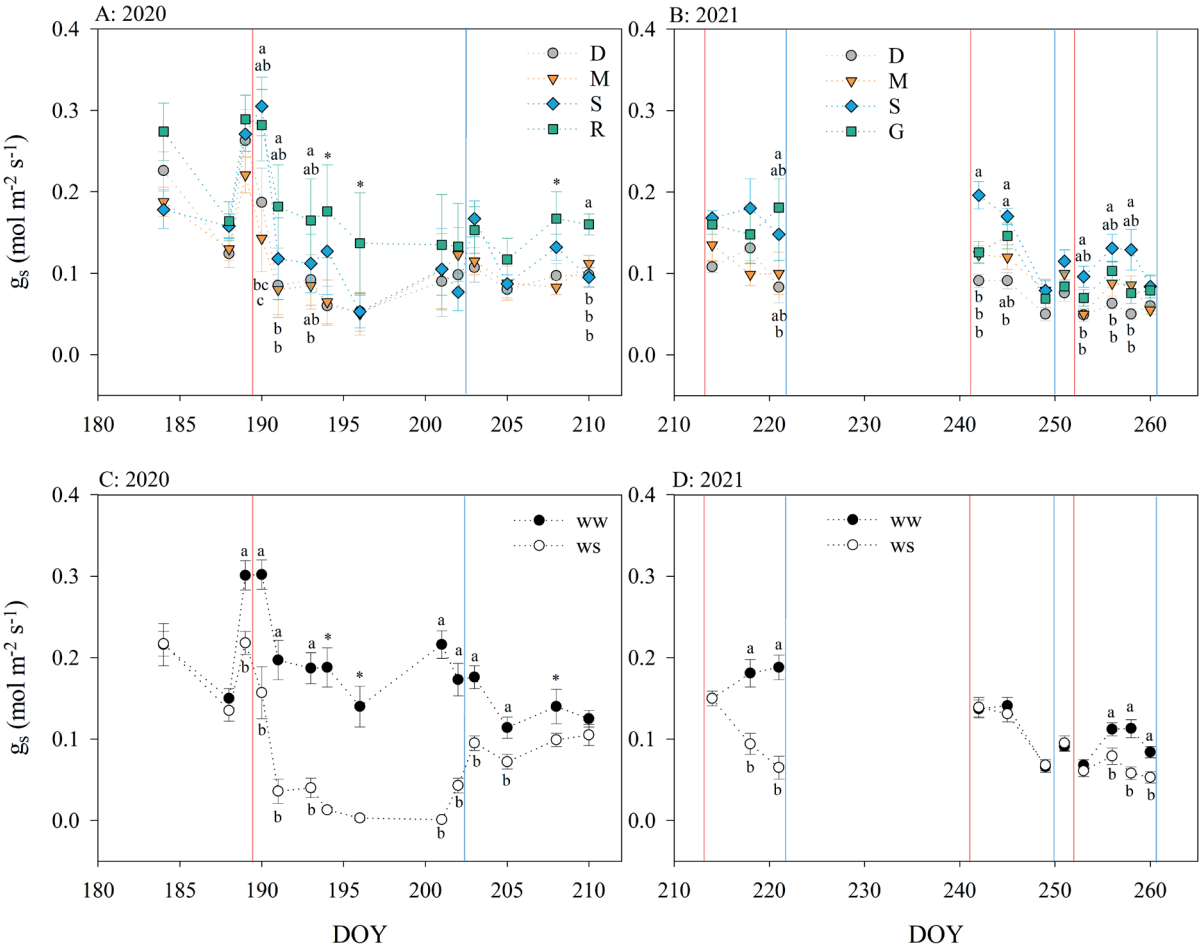


FIGURE 4. Stomatal conductance (g_s in mol m^{-2} s^{-1}) measured in 2020 (A,C) and 2021 (B,D).

Vertical lines indicate the imposition of drought (red) and re-watering (blue) treatment. The figures represent a two-way ANOVA analysis with cultivars and treatments as main factors; therefore, values are presented as averages ± standard error per cultivar (A, 2020: n = 6; B, 2021: n = 8) or per treatment (C, 2020: n = 12; D, 2021: n = 16) on each date (DOY, day of the year). No interaction between main factors was observed for almost any date of observation. In the figure, different letters denote significant differences ($p < 0.05$) between the main factor cultivar (A, B) or treatment (C, D); eventual significant interactions are represented with an asterisk (*) between the main factors cultivar and treatment ($p < 0.05$): No differences among cultivars were recorded within the ws treatment, but within the ww treatment Souvignier gris and Riesling were significantly higher than Donauriesling and Muscaris. D, Donauriesling; M, Muscaris; S, Souvignier gris; R, Riesling/G, Grüner Veltliner; ww, well-watered; ws, water-stressed.

the highest maximum stomatal conductance ($g_{s\ max}$) values among the PIWI cultivars tested (Figure 4), and it was also the one with the lowest (most negative) turgor loss point (Ψ_{tlp}) and osmotic potential at full turgor (π_{100}) as compared to Muscaris (M) and Donauriesling (D) (Table 2). Dayer *et al.* (2020) showed that these leaf traits ($g_{s\ max}$,Ψ_{tlp}, π_{100}) are coordinated within grapevine cultivars in a way that when maximum water use (represented in our case with $g_{s\ max}$) increases, leaf hydraulic traits allow the leaf to operate across a wider range of water potentials (i.e., by exhibiting a more negative Ψ_{tlp} and π_{100}). In our case, this may signify that S would provide increased resilience against drought events. However, under the experimental conditions in our study, S did not reach lower water potentials than M and D despite maintaining higher stomatal conductance (and thus a consequent faster soil water depletion), highlighting the difficulty of inferring plant behaviour from single leaf measurements as other factors also play an important role such as the canopy size and the variability among leaves. For instance, in 2020, M was the cultivar with the lowest $g_{s\ max}$ but also the one with the largest canopy (and therefore a bigger evaporative surface as compared to S), a combination of factors that could explain a faster depletion of soil water, in turn resulting in a lower midday water potential than S. Furthermore, despite the differences observed among cultivars when plotting g_s or Ψ_{stem} against time, our data showed that the water potential ranges for stomatal closure did not differ among cultivar as all of them reached $g_s < 0.05$ mol m^{-2} s^{-1} between Ψ_{stem} of –0.8 and –0.9 MPa (Figure 5); such results are comparable to what is reported in similar studies on potted grapevine cultivars such as Merlot (Hochberg *et al.,* 2016), Grenache and Syrah (Dayer *et al.,* 2020), or Montepulciano (Tombesi *et al.,* 2014). Therefore, the different drought stress degrees experienced by the PIWI cultivars in our study were more related to the different dynamics of water use by transpiration (and thus the time needed to deplete the

soil water reservoir and reach the critical Ψ_{stem} for stomatal closure) than to an intrinsic difference in their stomatal sensitivity to drought.

A closer look at the cultivar's gas exchange under well-watered conditions provides a useful picture of their water use behaviour. As shown in Supplementary Figure 6, differences in g_s between cultivars existed only at Ψ_{stem} > –0.75 MPa, with S, exhibiting higher stomatal conductance than M and D. In particular, M was the most conservative cultivar among the PIWIs studied in terms of stomatal conductance (i.e., M recorded $g_{s\ max}$ values that were lower by 30 % than the values measured for S) and transpiration. Our results suggest that M and D might be more suitable for non-irrigated conditions than S as they would deplete the soil water reservoir slower, while S, by maintaining higher transpiration rates longer, could face more intense drought stress situations and, therefore, some irrigation strategy would be needed, consistent with the idea of a "stress distance" introduced by Gambetta *et al*. (2020).

Interestingly, from the $A_N \sim g_s$ relationship (Figure 6), it is worth noting that M consistently showed lower A_N values than the other PIWIs at g_s > 0.15 mol m^{-2} s^{-1}, while D and S recorded largely similar A_N rates across a wide range of g_s values. The $A_N \sim g_s$ curve (Figure 6) showed a plateau at maximum assimilation rates of 14 µmol m^{-2} s^{-1} for S and D (consistent with values frequently reported in grapevines; Medrano *et al.,* 2002; Bota *et al.,* 2005; Bota *et al.,* 2016), while M reached an assimilation rate plateau between 10 to 12 µmol m^{-2} s^{-1}, suggesting a lower intrinsic water use efficiency (WUEi) at the leaf level and under well-watered conditions for M as compared to S and D leaves. However, as discussed before, M presented larger leaves and a bigger canopy than the other cultivars, a fact that could compensate for a lower WUEi. It remains difficult to upscale single-leaf WUEi to whole-plant WUE (Medrano *et al.,* 2015; Tomás *et al.,* 2014), and more studies are needed to further explore this trait, including yield components and total biomass production.

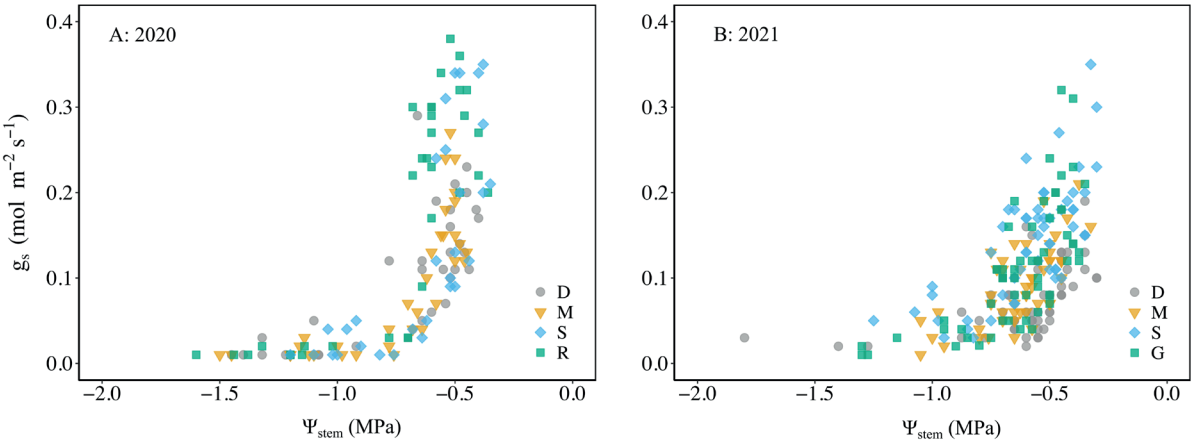


FIGURE 5. Relationship between stem water potential (Ψ_{stem} in MPa) and stomatal conductance (g_s in mol m^{-2} s^{-1}) during the entire experiment in seasons 2020 (A) and 2021 (B).

D, Donauriesling; M, Muscaris; S, Souvignier gris; R, Riesling/G, Grüner Veltliner.

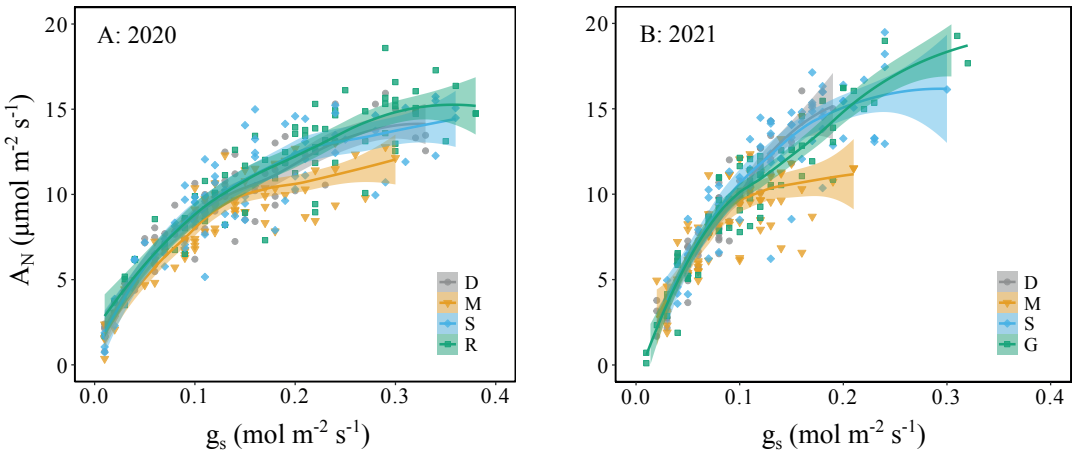


FIGURE 6. Relationship between stomatal conductance (g_s in mol m^{-2} s^{-1}) and net assimilation rate (AN in µmol m^{-2} s^{-1}) during the entire experiment in seasons 2020 (A) and 2021 (B).

Data were fit using a LOESS function. Shadows represent the confidence interval. D, Donauriesling; M, Muscaris; S, Souvignier gris; R, Riesling / G, Grüner Veltliner.

The results of this study provide important hints on the water relations of three PIWI cultivars, but we cannot exclude that under natural settings, the plant response to drought would differ from what was observed in young potted vines. Firstly, the stomatal closure thresholds would be different since, as suggested by Sorek *et al*. (2021) and Herrera *et al*. (2022), a seasonal osmotic adjustment in the leaves of grapevines can shift their sensitivity to drought towards lower (more negative) water potentials. In this regard, an open question remains whether different grapevine genotypes can osmotically adjust at different rates, perhaps contributing at least in part to the differences in stomata sensitivity to drought among grapevine cultivars often reported in the literature (Lavoie-Lamoureux *et al.,* 2017). Secondly, under field conditions, a large soil root explored volume (as normally observed in vineyards) would result in a longer time needed to dehydrate the soil (Gambetta *et al.,* 2020). Both factors combined would mean more time for the plants to react and acclimate to water deficit conditions (Hochberg *et al.,* 2017), an aspect still poorly explored in general for grapevines and even more in PIWIs. Finally, we shall not forget that important research questions concerning the impact of drought on the yield and fruit composition of PIWIs are to be approached to have a complete picture and be able to provide viticulturists and oenologists with the necessary information to manage PIWIs under dryer climatic conditions.

CONCLUSION

Taken together, the results of this study showed more conservative water use (i.e., lower maximum stomatal conductance values) of Donauriesling (D) and Muscaris (M) as compared to Souvignier gris (S) under no water limitations. Under mild-drought conditions, S maintained higher rates of gas exchange compared to D and M, but such differences vanished with the intensification of drought. While several important points are still to be studied (grape berry composition), we stress that our experiment produced similar results under greenhouse and semi-controlled field conditions and, therefore, under highly heterogeneous environments. Therefore, the study outputs might be generalised towards a hypothesis-driven validation at the field level to fully address best water management practices for PIWI vineyards.

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REFERENCES

Bota, J., Flexas, J., & Medrano, H., (2005). Genetic variability of photosynthesis and water use in Balearic grapevine cultivars. *Annals of Applied Biology*, *138*(3), 353-361. https://doi.org/10.1111/j.1744-7348.2001.tb00120.x

Bota, J., Tomás, M., Flexas, J., Medrano, H., & Escalona, J. M. (2016). Differences among grapevine cultivars in their stomatal behavior and water use efficiency under progressive water stress, *Agricultural Water Management*, *164*(1), 91-99, https://doi.org/10.1016/j.agwat.2015.07.016

Buckley, T. N. (2019). How do stomata respond to water status? *New Phytologist*, *224*, 21-36. https://doi.org/10.1111/nph.15899

Casanova-Gascón, J., Ferrer-Martín, C., Bernad-Eustaquio, A., Elbaile-Mur, A., Ayuso-Rodríguez, J. M., Torres-Sánchez, S., Jarne-Casasús, A., Martín-Ramos, P. (2019). Behavior of Vine Varieties Resistant to Fungal Diseases in the Somontano Region. *Agronomy, 9*(11), 738. https://doi.org/10.3390/agronomy9110738

Chaves, M. M., Zarrouk, O., Francisco, R., Costa, J. M., Santos, T., Regalado, A. P., Rodrigues, M. L., & Lopes, C. M. (2010). Grapevine under deficit irrigation: hints from physiological and molecular data. *Annals of Botany*, *105*(5), 661–676. https://doi.org/10.1093/aob/mcq030

Chen, Y., Herrera, R. A., Benitez, E., Hoffmann, C., Möth, S., Paredes, D., Plaas, E., Popescu, D., Rascher, S., Rusch, A., Sandor, M., Tolle, P., Willemen, L., Winter, S., & Schwarz, N. (2022). Winegrowers' decision-making: A pan-European perspective on pesticide use and inter-row management, *Journal of Rural Studies*, *94*, 37-53, https://doi.org/10.1016/j.jrurstud.2022.05.021

Cook, B.I., Mankin, J. S. & Anchukaitis, K.J. (2018). Climate Change and Drought: From Past to Future. *Current Climate Change Reports,* 4, 164–179. https://doi.org/10.1007/s40641-018-0093-2

Costa, J. M., Vaz, M., Escalona, J., Egipto, R., Lopes, C., Medrano, H., & Chaves, M. M. (2016). Modern viticulture in southern Europe: Vulnerabilities and strategies for adaptation to water scarcity. *Agricultural Water Management*, *164*, 5-18. https://doi.org/10.1016/j.agwat.2015.08.021

Dayer, S., Herrera, J. C., Dai, Z., Burlett, R., Lamarque, L. J., Delzon, S., Bortolami, G., Cochard, H., & Gambetta, G. A. (2020). The sequence and thresholds of leaf hydraulic traits underlying grapevine varietal differences in drought tolerance. *Journal of Experimental Botany*, *71*(14), 4333–4344. https://doi.org/10.1093/jxb/eraa186

European Commission (2020). A Farm to Fork Strategy - for a fair, healthy and environmentally-friendly food system. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52020DC0381

Fischer-Colbrie, P., Gross, P., Hluchy, M., Hofmann, U., Pleininger, S. & Stolz, M. (2015). Atlas der Krankheiten, Schädlinge und Nützlinge im Obst- und Weinbau: Mit umweltschonenden Strategien für gesunde Kulturen. Leopold Stocker Verlag, Graz.

Gambetta, G. A., Herrera, J. C., Dayer, S., Feng, Q., Hochberg, U., & Castellarin, S. D. (2020). The physiology of drought stress in grapevine: Towards an integrative definition of drought tolerance. *Journal of Experimental Botany*, *71*(16), 4658–4676. https://doi.org/10.1093/jxb/eraa245

Herrera, J. C., Bucchetti, B., Sabbatini, P., Comuzzo, P., Zulini, L., Vecchione, A., Peterlunger, E., & Castellarin, S. (2015). Water deficit and severe trimming effect on Merlot. *Australian Journal of Grape and Wine Research*, *21*, 254-265. https://doi.org/10.1111/ajgw.12143

Herrera, J. C., Calderan, A., Gambetta, G. A., Peterlunger, E., Forneck, A., Sivilotti, P., & Hochberg, U. (2022). Stomatal responses in grapevine become increasingly more tolerant to low water potentials throughout the growing season. *The Plant Journal*, *109*(4), 804-815. https://doi.org/10.1111/tpj.15591

Herrera, J.C., Savi, T., Mattocks, J., De Berardinis, F., Scheffknecht, S., Hietz, P. et al. (2021). Container volume affects drought experiments in grapevines: Insights on xylem anatomy and time of dehydration. *Physiologia Plantarum*, *173*(4), 2181– 2190. https://doi.org/10.1111/ppl.13567

Hochberg, U., Herrera, J. C., Cochard, H., & Badel, E. (2016). Short-time xylem relaxation results in reliable quantification of embolism in grapevine petioles and sheds new light on their hydraulic strategy, *Tree Physiology*, *36*(6), 748–755, https://doi.org/10.1093/treephys/tpv145

Hochberg, U., Bonel, A. G., David-Schwartz, R., Degu, A., Fait, A., Cochard, H., Peterlunger, E. & Herrera, J. C. (2017). Grapevine acclimation to water deficit: the adjustment of stomatal and hydraulic conductance differs from petiole embolism vulnerability. Planta 245, 1091–1104. https://doi.org/10.1007/s00425-017-2662-3

Hofmann, U., Köpfer, P., Spieß, H. & Werner, A. (2014). Biologischer Weinbau. Verlag Eugen Ulmer Stuttgart.

Lavoie-Lamoureux, A., Sacco, D., Risse, P.-A. & Lovisolo, C. (2017). Factors influencing stomatal conductance in response to waater availability in grapevine: a meta-analysis. *Physiologia Plantarum, 159*(4), 468-482. https://doi.org/10.1111/ppl.12530

Levin, A. D. (2019). Re-evaluating pressure chamber methods of water status determination in field-grown grapevine (*Vitis spp.)*. *Agricultural Water Management*, *221*, 422-429. https://doi.org/10.1016/j.agwat.2019.03.026

Levin, A. D., Williams, L. E., & Matthews, M. A. (2019). A continuum of stomatal responses to water deficits among 17 wine grape cultivars (*Vitis vinifera*). *Functional Plant Biology, 47*, 11-25. https://doi.org/10.1071/FP19073

Medrano, H., Escalona, J. M., Bota, J., Gulías, J., & Flexas, J. (2002). Regulation of Photosynthesis of C_3 Plants in Response to Progressive Drought: Stomatal Conductance as a Reference Parameter, Annals of Botany, *89*(7), 895–905. https://doi.org/10.1093/aob/mcf079

Medrano, H., Tomás, M., Martorell, S., Escalona, J. M., Pou, A., Fuentes, S., Flexas, J., & Bota, J. (2015). Improving water use efficiency of vineyards in semi-arid regions. A review. *Agronomy for Sustainable Development, 35*, 499–517. https://doi.org/10.1007/s13593-014-0280-z

Medrano, H., Tortosa, I., Montes, E., Pou, A., Balda, P., Bota, J., Escalona, J. M. (2018). Chapter 16 - Genetic Improvement of Grapevine (*Vitis vinifera* L.) Water Use Efficiency: Variability Among Varieties and Clones. In Tejero, I. F. G. & Zuazo, V. H. D. (Editors). Water Scarcity and Sustainable Agriculture in Semiarid Environment, *Academic Press*, 377-401. https://doi.org/10.1016/B978-0-12-813164-0.00016-8

Merdinoglu, D., Schneider, C., Prado, E., Wiedemann-Merdinoglu, S., & Mestre, P. (2018). Breeding for durable resistance to downy and powdery mildew in grapevine. *OENO One*, *52*(3), 203–209. https://doi.org/10.20870/oeno-one.2018.52.3.2116

Montaigne, E., Coelho, A., & Khefifi, L. (2016). Economic issues and perspectives on innovation in new resistant grapevine varieties in France. *Wine Economics and Policy*, *5*(2), 73-77. https://doi.org/10.1016/j.wep.2016.11.002

Moriondo, M., Jones, G.V., Bois, B., Dibari, C., Ferrise, R., Trombi, G., & Bindi, M. (2013). Projected shifts of wine regions in response to climate change. *Climatic Change*, *119*, 825–839. https://doi.org/10.1007/s10584-013-0739-y

Pedneault, K., & Provost, C. (2016). Fungus resistant grape varieties as a suitable alternative for organic wine production: Benefits, limits, and challenges, *Scientia Horticulturae*, *208*, 57-77. https://dx.doi.org/10.1016/j.scienta.2016.03.016

Pertot, I., Caffi, T., Rossi, V., Mugnai, L., Hoffmann, Grando, M. S., Gary, C., Lafond, D., Duso, C., Thiery, D., Mazzoni, V., & Anfora, G. (2017). A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. *Crop Protection*, *97*, 70-84, https://doi.org/10.1016/j.cropro.2016.11.025

Reynolds, A. G. (Editor) (2015). *Grapevine Breeding Programs for the Wine Industry*. Sawston, Cambridge, United Kingdome: Woodhead Publishing. https://doi.org/10.1016/B978-1-78242-075-0.00013-2

Schultz, H. R., & Stoll, M. (2009). Some critical issues in environmental physiology of grapevines: Future challenges and current limitations. *Australian Journal of Grape Wine Research*, *16*, 4–24. https://doi.org/10.1111/j.1755-0238.2009.00074.x

Sorek, Y., Greenstein, S., Netzer, Y., Shtein, I., Jansen, S., & Hochberg, U. (2021). An increase in xylem embolism resistance of grapevine leaves during the growing season is coordinated with stomatal regulation, turgor loss point and intervessel pit membranes. *New Phytologist*, *229*, 1955-1969. https://doi.org/10.1111/nph.17025

Tomás, M., Medrano, H., Escalona, J. M., Martorell, S., Pou, A., Ribas-Carbó, M., & Flexas, J. (2014). Genetic variability in water use efficiency in grapevines. *Environmental and Experimental Botany*, *103*, 148–157. https://doi.org/10.1016/j.envexpbot.2013.09.003

Tombesi, S., Nardini, A., Farinelli, D., & Palliotti, A. (2014). Relationships between stomatal behavior, xylem vulnerability to cavitation and leaf water relations in two cultivars of *Vitis vinifera*. *Physiol Plantarum*, *152*, 453-464. https://doi.org/10.1111/ppl.12180

Töpfer, R., & Trapp, O. (2022). A cool climate perspective on grapevine breeding: climate change and sustainability are driving forces for changing varieties in a traditional market. *Theoretical and Applied Genetics* (2022). https://doi.org/10.1007/s00122-022-04077-0

Van Leeuwen, C., Destrac-Irvine, A., Dubernet, M., Duchêne, E., Gowdy, M., Marguerit, E., ... & Ollat, N. (2019). An update on the impact of climate change in viticulture and potential adaptations. *Agronomy*, *9*(9), 514. https://doi.org/10.3390/agronomy9090514

Wickham, H. (2016). *Data Analysis*. In: ggplot2. Use R!. Springer, Cham. https://doi.org/10.1007/978-3-319-24277-4_9.