Assessing the growth response of Vitis vinifera L. cv. Xynisteri, Maratheftiko, Shiraz and Sauvignon Blanc to different irrigation regimes.

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Assessing the growth response of *Vitis vinifera L*. cv. Xynisteri, Maratheftiko, Shiraz and Sauvignon Blanc to different irrigation regimes.

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Abstract

The world's changing climate is placing great pressure on the resources for sustainable viticulture. With this, it has become necessary to investigate grape varieties that are well adapted to hot climates. The aims of this study were to (1) assess the response of Xynisteri to different irrigation regimes, and (2) compare the performance of Xynisteri, Maratheftiko, Shiraz and Sauvignon Blanc grown in pots with different irrigation regimes. Trial one was established in a commercial Xynisteri vineyard in Cyprus under three different irrigation regimes - full, 50% and no irrigation in 2019. Trial two compared three irrigation regimes - full, 50% and 25% in a potted trial of Xynisteri and Sauvignon Blanc conducted in Cyprus in 2019. Trial three was a potted trial of Xynisteri, Sauvignon Blanc, Maratheftiko and Shiraz with the same three irrigation regimes conducted in Australia in 2020/21. Vine performance and physiology measurements were taken in both trials. Fruit composition analysis, yield (field trial only), shoot, trunk and root mass measurements were performed at the end of the season. Few differences between measures were found between irrigation regimes in the field trial. Fruit composition analysis revealed fructose to be lowest in the full irrigation group compared to deficit and no irrigation treatments. The potted trial in 2019 demonstrated that for all three irrigation regimes, Xynisteri had higher stem water potential, stomatal conductance and chlorophyll content than Sauvignon Blanc. Xynisteri produced greater end of season root, shoot and leaf mass than Sauvignon Blanc under all irrigation regimes. In 2020/21, Xynisteri had greater end of season root, shoot and leaf mass than Maratheftiko and Sauvignon Blanc with Shiraz the lowest. Few significant differences in stem water potential were observed in the early stages of the trial. However, toward the end of the trial and with reduced irrigation, Xynisteri and Maratheftiko had higher stem water potential than Shiraz and Sauvignon Blanc. Xynisteri had higher stomatal conductance and chlorophyll content than Maratheftiko and both

were higher than Sauvignon Blanc and Shiraz. These results indicate that Xynisteri in particular may possess better cultivar specific growth traits than Shiraz and Sauvignon Blanc when grown under the same environmental conditions and in turn may be a more appropriate choice in areas where water is limited.

Introduction

The threat of climate change to the global wine industry is well documented. As such, many wine regions of the world are expected to face significant impacts in the next 50 years encompassing increasing temperatures, reduced rainfall, earlier harvests and heat induced berry composition changes (Jones *et al.,* 2005, Schultz and Jones 2010, Camps and Ramos 2012, Webb 2011, Keller 2010, Webb *et al.,* 2013, Jarvis *et al*., 2019, Cook and Wolkovich 2016, Krieger *et al*., 2011, Jones *et al*., 2010, Diffenbaugh *et al.,* 2011, van Leeuwen *et al.,* 2013 & 2019, Hannah *et al*., 2013, Remenyi *et al.,* 2019). This threat has led to many countries investigating options to adapt to these challenges, with a particular focus on the drought and heat tolerant indigenous grape varieties of hot Mediterranean climates. Recently, in Australia many producers have been seeking varieties able to cope with water limited conditions from Greece, Portugal, Spain and Georgia. However, very little research has assessed these varieties under Australian conditions and there is a lack of knowledge on how they perform.

The island of Cyprus is another hot wine growing region (Adamides 2020) with a recent upsurge in interest and research into heat and drought tolerance and a return to cultivation of their indigenous varieties (Grigoriou *et al.,* 2020, Litskas *et al*., 2020, Vink *et al.,* 2021, Heyman *et al.,* 2021). Chrysargyris *et al*., (2018a) investigated the indigenous Cypriot red variety Maratheftiko by conducting a trial that compared tillage and no tillage with irrigated and non-irrigated treatments. The authors concluded that when comparing irrigation and no irrigation treatment groups in vineyards that did not undergo tillage, there was no change in yield. Also, the no tillage, no irrigation groups had an increase in berry chemistry measures; total soluble solids, total phenolics and total anthocyanins. Overall, the authors concluded that Maratheftiko is suited to cultivation in arid environments and suggested that Maratheftiko is able to tolerate arid conditions by decreasing stomatal conductance as an adaptive mechanism (Chrysargyris *et al*., 2018b).

In a vineyard and in a potted trial the performance of Xynisteri and Chardonnay were compared under different irrigation and tillage regimes (Chrysargyris *et al*., 2020; Tzortzakis *et al*., 2020). Xynisteri in a vineyard (clay soils) maintained yields and total soluble solid concentrations with no irrigation and low tillage levels, while in comparison, Chardonnay required irrigation and tillage to obtain high yields and adequate quality. Authors suggest that if irrigation is not available, Xynisteri is preferred over Chardonnay for cultivation. They also proposed that under drought conditions, a possible mechanism for Xynisteri to adapt to arid climates could include its ability to decrease stomatal conductance and photosynthetic rate along with increasing total phenols and antioxidant enzyme capacity in leaf tissue.

A similar approach has been used to evaluate indigenous Greek varieties. Koufos *et al.,* (2020) reviewed historical data in Greece and assessed 16 indigenous Greek and 13 international varieties cultivated across 14 different regions for harvest dates, potential alcohol and titratable acidity levels. They found that indigenous Greek varieties had greater heat requirements (Growing Degree Days) compared to international varieties; and that international varieties were skewed towards earlier ripening while Greek varieties were late ripening. Average harvest dates were the 30th of August and the 10th of September for the international and Greek varieties, respectively. The later ripening indigenous Greek varieties experienced fewer impacts (better growth, higher stem water potential, less potential alcohol increases and acidity decreases) due to temperature increases than the international varieties and therefore potentially better adapted to future warmer climates.

These studies highlight the possibility of indigenous varieties from the Eastern Mediterranean to be cultivated with reduced irrigation and be suitable for wine producers adapting to the challenges of climate change. Yet, little is known about their tolerance to reduced irrigation when compared to other more traditionally grown varieties and when grown in other environments.

Methods for scheduling irrigation times and rates can also be varied. The rate of evapotranspiration (ET) is most frequently used in vineyard/field trials to determine irrigation rates (Phogat *et al.*, 2020). Volumetric water content is commonly used in potted trials and has been used by Tzortzakis *et al*., (2020) to study Maratheftiko grown in small (8 litre) containers. Nambuthiri *et al.,* (2017) believe volumetric water content is a better method for determining irrigation rates in container grown crops than ET due to the need to determine specific crop coefficients for numerous cultivars. ET estimates also assume that the crop has access to unlimited water resources, which is often not the case in container grown crops (Incrocci *et al.,* 2014). Girona *et al*., (2006) have studied the use of midday leaf water potential for scheduling deficit irrigation in vineyards and concluded that the method can increase the precision of irrigation with highly repeatable results. To date, no studies have looked at the specific irrigation rates required for optimal growth of Xynisteri and Maratheftiko and their irrigation limits remain largely unknown.

Therefore, the aims of this study were to (1) assess the response of the indigenous Cypriot variety Xynisteri to different irrigation regimes, and (2) compare the performance of Xynisteri, Maratheftiko, Shiraz and Sauvignon Blanc grown in pots with different irrigation regimes in Cyprus and Australia.

Materials and Methods

1. Plant material, experimental design and treatments

The investigation involved two irrigation trials conducted in Lemesos, Cyprus during the 2019 season and one in the 2020-2021 growing season in Adelaide, Australia. Both potted trials were performed under field conditions rather than controlled environments. Trial one was established in a commercial Xynisteri vineyard, latitude 34°53N and elevation 840 metres. The vineyard was planted at a density of 3300 vines per hectare, with 1.5 metre vine spacing by 2 metre row spacing. All vines were own rooted, with no rootstocks used. Vineyard management practices included mid row cultivation in mid-April, mid-May and mid-June. Sulphur sprays were applied three times during the growing season and pesticide sprays twice. Three different irrigation regimes were utilised: full irrigation (44 litres per vine/0.14 ML per hectare), 50% (22 litres per vine/0.07 ML per hectare) and no irrigation. These regimes were randomly allocated to twelve vines, (four vines per treatment within a row and replicated three times) in a randomised block design, (Figure 1). Full irrigation was determined to be the usual rate at which the vineyard owner irrigated and represented the total irrigation per vine for the entire growing season. Irrigation was delivered by an in-line drip system with water meters attached to each row to measure volumes. Irrigation occurred once per week up until 2 weeks prior to the harvest date. Measurements were taken 7 days after the last irrigation episode and prior to the next. All the vines in the study were pruned to approximately 30 buds per vine.

Figure 1: Randomised block design of the vineyard. Orange line- n=4 vines full irrigation, Yellow line- n=4 vines deficit irrigation, Blue line n=4 vines no irrigation.

Trial two was a potted vine trial established from cuttings from two different Xynisteri vineyards located in two different regions in Cyprus (XK, Xynisteri Kathikas from the Kathikas region and XM, Xynisteri Mandria, from the Mandria region) and Sauvignon Blanc (SBC) sourced from a nearby vineyard. Recent work by Grigoriou *et al.,* (2020) has identified the possibility of different clones (biotypes) within the germplasm of Xynisteri from different regions. These potential clones have yet to be identified and their characteristic differences are largely unknown at present. However, suspected different clones (biotypes) potentially exist and may have different growth properties (Koundouras pers. Comm 2019), thus determining the selection of the two Xynisteri samples used in this study.

Trial three was a potted trial set up at the University of Adelaide, South Australia of Xynisteri Paphos (XP), Sauvignon Blanc (SBA), Maratheftiko Paphos (MP) and Shiraz (SZ) with the same three irrigation regimes as the 2019 trial. XP and MP cuttings were sourced from the Cyprus Department of Agriculture vineyard research facility in the Paphos region in 2018 and transported to Australia for quarantine and testing to ensure material was free of plant pests and/or pathogens before being released in 2019 for propagation. SZ and SBA cuttings were sourced from the University of Adelaide, Waite Campus vineyard.

For both potted trials, three irrigation regimes - full irrigation (100%), 50% and 25% were applied to ten treatment replicates (vines) in 2019 and seven treatment replicates in 2020-2021 (due to limited scion material). All cuttings consisted of 4 nodes and were approximately 20cm long. The basal end of the scion was coated in a rooting hormone gel, Clonex (Growth Technology Pty Ltd, O'Connor, Australia) prior to being planted in a growth medium. No rootstocks were used. The media used in both potted trials were readily available commercial potting mixes. In Cyprus, a decomposed peat and clay-based medium was used and in Adelaide a medium of decomposed bark, sand, coconut fibre and clay was used. The cuttings were then grown outside in field conditions in 55 litre pots for 18 months prior to testing to ensure root establishment; no rootstocks were used.

The full irrigation rate was determined by the Volumetric Water Content (VWC) capacity of the growing media in the pots. Water was added to the dry media in the pots until it began to exit from the drainage holes. This volume of water was recorded as the 100% VWC (Rhie and Kim 2017). Prior to the trial commencing, all pots received 8 litres three times per week, ceasing on day zero. All irrigation treatments were delivered once per week by hand using volumetric containers to ensure accurate volumes. Irrigation treatments commenced on day 7 of the trial. Full irrigation was 8 litres per vine, 50%- 4 litres per vine and 25%- 2 litres per vine. Pots were arranged in randomised block designs as per Figures 2 and 3.

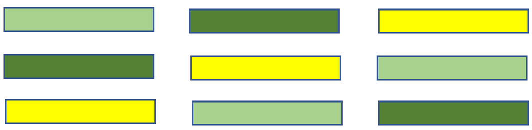


Figure 2: Randomised block design of pots in Cyprus 2019. Light Green- Xynisteri XK, Dark Green- Xynisteri XM, Yellow- Sauvignon Blanc. n=10 vines per block.

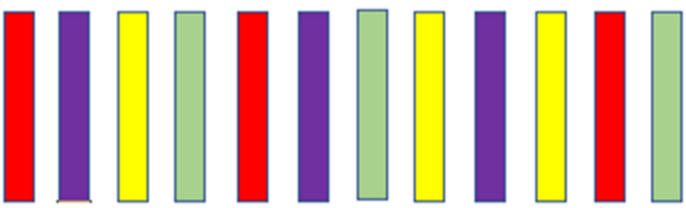


Figure 3: Randomised block design of pots in South Australia in 2020/21. Green- Xynisteri, Yellow- Sauvignon Blanc, Red- Maratheftiko, Purple- Shiraz. n=7 vines per block.

2. Measurements

2.1 Climate

A weather station (IC6250AU Davis Vantage Vue Weather Station, 4/33 The Concourse, Cowes, VIC 3922 Australia) was installed at each of the trial sites in Cyprus. General climate data for the region was supplied by the Cypriot Department of Meteorology (Republic of Cyprus Department of Meterology, 2019) and was collected from the nearest weather station at the Agriculture Research Institute in Saittas (Latitude 34°52’N, Longitude 32°55’E, at a distance of 16 km from the vineyard site). Climate data for the Adelaide potted trial was collected from a weather station (MEA Magpie Weather Stations, 1 Vine Street 5072, Magill SA, Australia) located adjacent to the testing site (Latitude 34°96’S, Longitude 138°63’E, 0.02km from the trial site).

2.2 Vine performance measures

Vine growth measurements at flowering (EL-21) were made, along with shoot, trunk and root mass measurements at the end of the season (EL-38) for both potted trials. Inflorescences were removed due to the age of the scion material and the limited time to produce quality fruit. Vine performance measurements, including shoot number, bunch number, bunches per shoot, shoot length, leaves per shoot, shoot diameter (at fourth internode), bunch length, bunch width and internode length (at fourth internode), were taken at flowering in the Cypriot vineyard trial to avoid any concerns associated with shoot tipping by the commercial vineyard, n=12 vines per treatment. All fruit from each sample vine was collected separately at harvest, bunches counted and weighed. All berries were removed from the rachis of every bunch (5-6 kg per vine, n=12 vines per treatment) and the fruit was homogenised in a blender.

Samples were allowed to settle overnight at 5°C, then a 100 ml sample of the supernatant juice underwent compositional analysis with FOSS Wine Scan FT120 (Nils Foss Allé 1, DK-3400 Hilleroed, Denmark).

2.3 Physiology measurements

Data was collected for all three trials as per Table 1. The first measurements were taken at day 0, with irrigation treatments applied at day 7 for the potted trials and day 29 for the vineyard trial.

Table 1: Trial data collection timetable for 2019, 2020/21

Site	Start date	End date	Total days	Measurements
Cyprus vineyard trial 2019	11th June (EL-21)	26th September (EL-38)	107	5
Cyprus potted trial 2019	19th July (EL-33)	24th September (EL-38)	67	6
Australian potted trial 2020/21	16th December (EL-33)	27th February (EL-38)	74	7

Potted trial testing start dates were based on 16 weeks after bud burst (EL-4) and 4 weeks after fruit set (EL-27).

A Skye SKPM1400 series Plant Moisture Vessel (Skye Instruments Ltd, Llandrindod Wells Powys, LD1 6DF, UK) was used to measure stem water potential as described by Meron *et al.* (1987). Midday stem water potential was measured between 12:00 and 14:00 on one fully expanded and undamaged leaf chosen from the mid-upper part of the canopy from every vine. Each leaf was selected from the midday sunlit side of the canopy, n=12 leaves per treatment were measured in the vineyard trial, n=10 in Cypriot potted trial and n=7 in Australian potted trial.

Leaves were covered with a Ziplock aluminium foil-coated plastic bag for 60 min before the measurement, in order to allow leaf water potential to equilibrate (Begg and Turner, 1970). After the equilibration period, the leaves were cut with a sharp blade and the stem water potential measured. A maximum of 60 seconds elapsed between cutting the leaves and recording the measurements. The same pressure chamber operator performed all the measurements with the goal of standardising the interpretation of the moment sap emerged from the petiole (Meron et al., 1987).

Leaf stomatal conductance was measured using a diffusion porometer (AP4, 2000 Delta-T Leaf Porometer Devices, Cambridge, UK). The porometer head was placed onto the required leaf and measurements were taken, which were recorded after three consecutive readings. Leaves were selected at the 4th node along the shoot and an average of three readings was recorded for each leaf), n=12 leaves per treatment were measured.

SPAD readings were taken as described by Marquard and Tipton (1987) using a SPAD 502 Meter 2900 (Minolta Japan), giving an approximation for chlorophyll content. Leaves were selected at the 4th node along the shoot and an average of three readings was recorded for each leaf, n=10 vines were sampled in 2019 and n=7 vines in 2020/21.

2.4 Stomatal density

Stomatal density was determined by selecting one leaf per vine from the varieties using a modified method described by Hilu and Randall (1984). Nail-polish imprints were made by applying nail-polish to the abaxial side of the leaf and allowing it to dry. Adhesive tape was placed over the area covered by nail polish and pressed down firmly. The adhesive tape was peeled from the leaf, mounted on a dry microscope slide, and viewed under a light microscope. Images were acquired on a Zeiss Axiophot Fluorescent Microscope equipped with a metric ocular 20× objective. Stomata number was counted in three different regions of each leaf and mean number per mm^2 calculated. The varieties sampled were Xynisteri, Maratheftiko, Shiraz, Sauvignon Blanc and mean values for each variety determined.

2.5 Statistical analysis

Measurements were analysed by using the statistical package XLSTAT (version 2019.4.2, Addinsoft SARL, Paris, France). Data were reported as mean and standard error of the mean. ANOVA was used to examine the differences between irrigation treatments at each sampling date, the differences among means were identified by Tukey Honest Significance Difference (HSD) post hoc tests. Two fixed factors (time and irrigation) and one interaction factor (time*irrigation) were analysed by repeated measure ANOVA Restricted Maximum Likelihood (REML) method. P values < 0.05 were considered significant.

Results and Discussion

1. Climate

The long-term average Mean July/January Temperature (MJT) for the Krasachoria wine growing region in Cyprus is 26.1°C and growing season rainfall 129mm (1st April- 30th September). The long-term average MJT for Adelaide is 22.6°C and growing season rainfall is 140mm (1st October-31st March).

The potted trial site in 2019 received 49mm of rain during the testing period (July-September average 33mm) and the 2020/21 trial site received 127mm (December-February average 42mm) during the testing period. The mean daily temperature for the 2019 testing period was 24°C and 21°C in 2020/21 (Figure 4). The temperature during the 2019 trial was consistent with the long-term averages, however, the total rainfall was 370 mm above the long-term average with large falls recorded in January, February, March, June, August and December (Figure 4).

In 2020/21 the weather was more varied compared to the long-term average data (Figure 4). The trial site experienced large variability in climate with higher maxima and lower minima than the 2019 trial and also higher rainfall. This was partly due to the La Nina phase of the El Niño–Southern Oscillation (ENSO) event experienced and is associated with a warming of the central and eastern tropical Pacific oceans that influences the climate of Eastern and Southern Australia by causing lower than average temperatures and increased rainfall (Bureau of Meteorology 2012). Typically, the Adelaide trial site experiences large climate variability, with long term records (1887-2021) indicating that January maximum temperatures can range from 17.1-46.6°C and minimum temperatures ranging from 8.8-33.2°C (Bureau of Meteorology 2021).

Liles and Verdon-Kidd (2020) investigated Growing Season Temperatures (GST) in south-east Australia and concluded that traditional growing season temperature thresholds may not be suitable for Australian wine regions. They state that a latitude adjustment is necessary to improve growing season models in Australia. While suggesting the use of a hot region classification being GST of 19-22°C and a very hot region being 22-24°C. The climate of the Krasachoria wine region of Cyprus would therefore be classified as very hot and in a typical year so would Adelaide, South Australia. However, in the 2020/21 season Adelaide was only classified as hot.

2. Vine growth and physiology measurements

2.1 Cyprus Xynisteri vineyard trial

No significant differences between physiological measures and growth data at fruit set and harvest were found when comparing the three irrigation regimes in the commercial Xynisteri vineyard (Table 2 and Figure 5a, b, c). While the results for stomatal conductance, chlorophyll content and in particular water potential were not significantly different between the irrigation groups, they were however similar to those reported by Copper *et al.,* (2020). It has been demonstrated that water potential measurements respond not only to water shortage but also to other factors including cultivar, environment, soil type and the relationships between canopy and root system (García-Tejera *et al.,* 2021). It is therefore possible that Xynisteri has some unique cultivar properties that enable it to maintain water potential under different water status conditions. This possibility requires further research for confirmation.

Table 2: Vine performance measures at fruit set and harvest for Xynisteri, field trial, Kato Mylos, Cyprus, 2019 growing season

	Shoot number	Shoot length (cm)	Leaf number	Shoot diameter (cm)	Internode length (cm)	Bunch length flower (cm)	Bunch width flower (cm)	Bunch number	Average bunch weight (gm)	Yield per vine (kg)
Nil	28.2	163	46	1.0	9.1	17.9	8.9	25.9	209	5.4
50%	25.3	146	43	0.96	9.5	15.9	8.5	25.9	251	6.5
Full	27.3	141	42	0.96	9.5	17.6	9.4	24.1	257	6.2
p<0.05	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Full- 44 litres, 50%- 22 litres, Nil- no irrigation, ns- not statistically significant different. Shoot, bunch number, bunch length and bunch width are means of n=12 vines.

Must composition analysis revealed fructose to be lowest in the full irrigation group compared to deficit and non-irrigated treatments (Table 3). Fructose production is favoured in warmer conditions and can be an indication of over ripeness and higher potential alcohol (Amerine and Thoukis 1958 cited in Trad *et al.* 2021). The full irrigation regime may have had a role in reducing the amount of fructose produced. Similar reductions in Total Soluble Solids (TSS) with full irrigation have been demonstrated with the Cypriot variety Maratheftiko (Chrysargyris *et al*., 2018a) and the Greek varieties Agiorgitiko and Xinomavro (Theodorou *et al*., 2019). In 2019, the vineyard region received 194mm of rain in the growing season (April-September). However, 106mm of the rain occurred in early June and 34mm occurred during two episodes in August, which may have influenced the results, especially when considering the long-term average growing season rainfall is 129mm.

After 52 days of the irrigation regimes, the stem water potential for all three irrigation regimes was approximately -1.2 MPa which is regarded as moderately stressed (Girona *et al.,* 2006). Therefore, it is difficult to conclude whether the testing period rainfall had an impact on the results or not.

Table 3: Must analysis of fruit from three irrigation regimes for Xynisteri field trial, Kato Mylos, Cyprus, 2019 growing season

Treatment	ETH (g/l)	pH	TA (g/l)	VA (g/l)	Malic Acid (g/l)	Fruct (g/l)	Gluc (g/l)	Red Sug (g/l)	FolinC (mg/l)
Nil	0.30	3.90	1.71	0.31	1.53	109 a	114	206	104
50%	0.25	3.96	1.66	0.29	1.57	109 a	112	204	108
Full	0.31	3.85	1.85	0.27	1.60	103 b	107	194	103
Pr > F	ns	ns	ns	ns	ns	0.04*	ns	ns	ns

FolinC (Folin–Ciocâlteu)-Gallic Acid Equivalence phenolic index. ETH-ethanol, TA-titratable acidity, VA- volatile acidity, Fruct- fructose, Gluc- glucose, RedSug- reducing sugars. Full- 44 litres, 50%- 22 litres, Nil- no irrigation. ns- not statistically different. Each data point is a mean of n=12 samples. Means were separated by ANOVA using Tukey's test. * indicate significance at $p < 0.05$, ns = not significant.

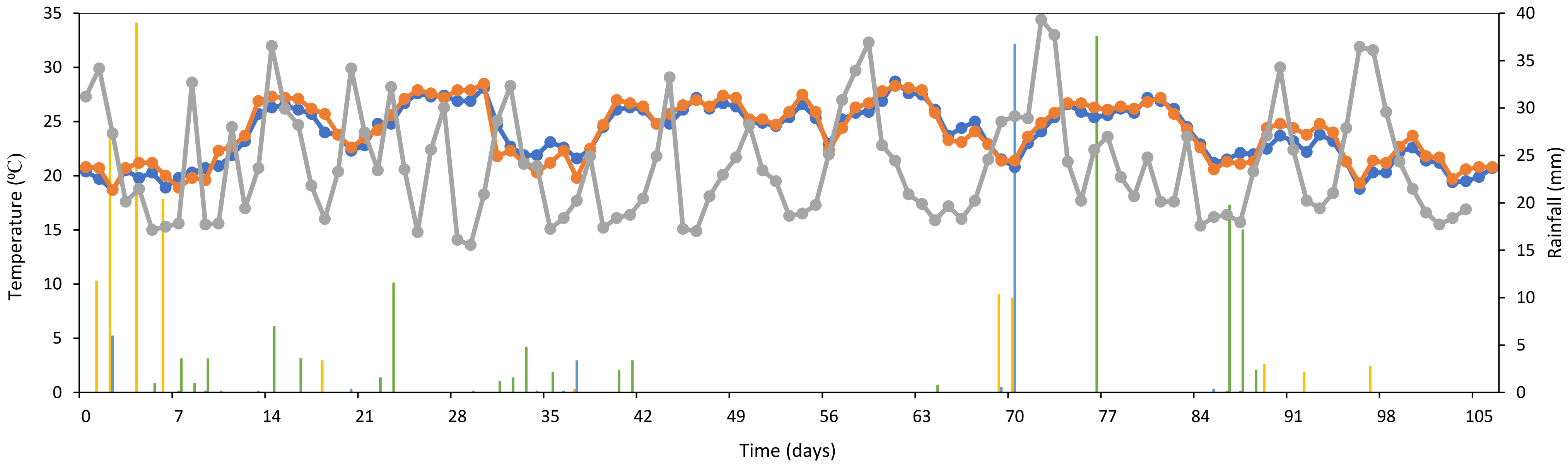


Figure 4: Climate and rainfall data for Cyprus Xynisteri vineyard site (Kato Mylos), Cyprus potted trial (Omodhos) and Adelaide Waite Campus, Australia for the testing periods 2019 and 2020/21.

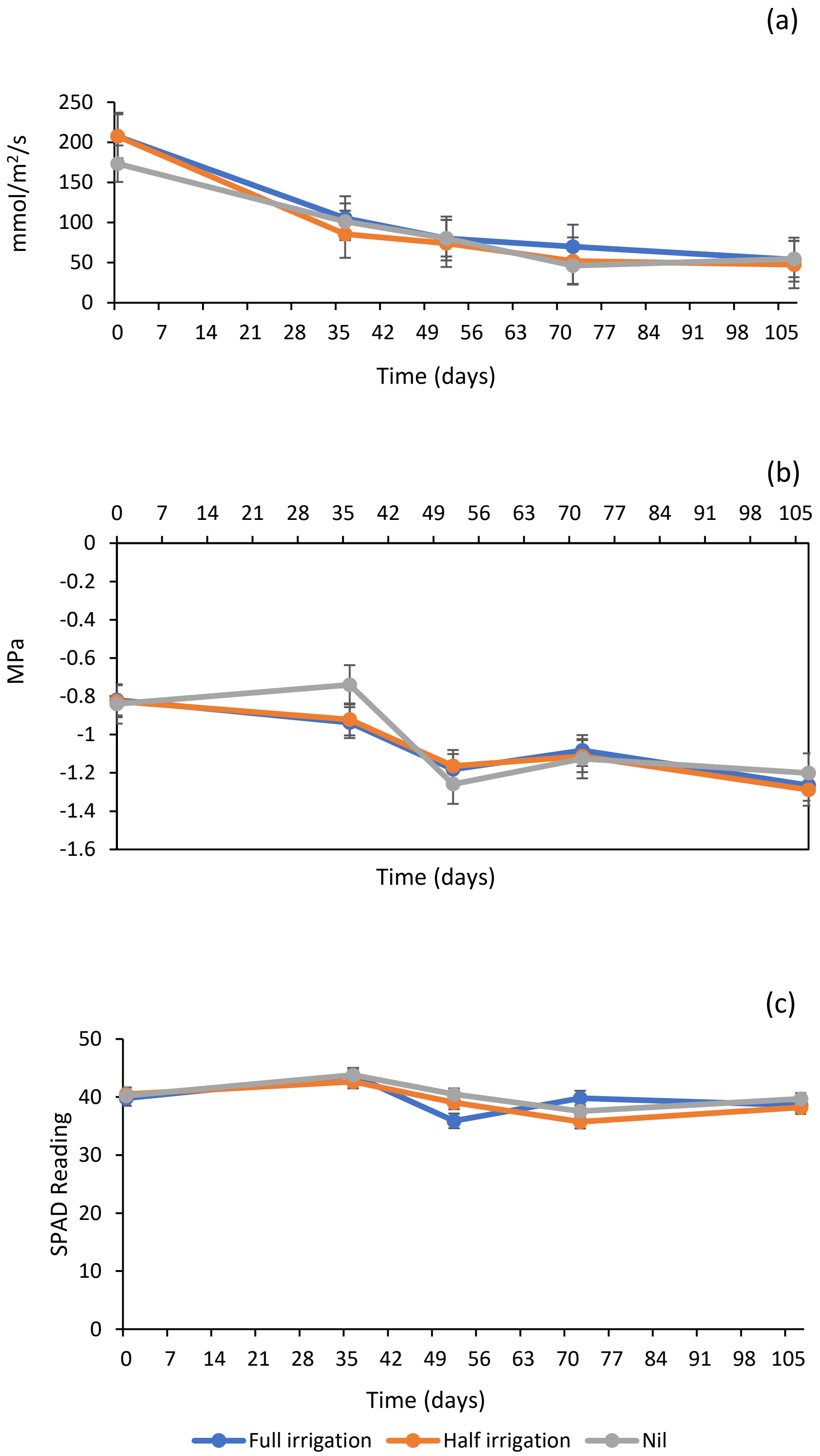


Figure 5: Vine leaf physiological measurements for 107-day test period in commercial Cypriot Xynisteri vineyard 2019. (a) stomatal conductance (mmol/m^2/s), (b) stem water potential (MPa), (c) SPAD reading- indicative chlorophyll content. Full irrigation- 44 litres/vine, Half irrigation- 22 litres/vine, Nil- no irrigation. Each data point is a mean of n=12 vines. Bars indicate the standard error. Means were separated by ANOVA using Tukey's test.

2.2 Potted vine trials

In 2019, vine growth measurements were taken at flowering (Table 4). XM and XK had longer shoots and internode length than SBC as well as a greater shoot diameter. XK also had longer shoots than XM. Shoot length is important in terms of canopy capacity, Smart (1985) described vineyards that produce long shoots, large leaves and extensive lateral growth as having high vigour. This high vigour growth can have an impact on the canopy density and the exposure of fruit to sunlight and the resultant wine composition.

In 2020/21, the potted vine trial consisted of XP, MP, SBA and SZ. Growth measurements at flowering showed XP, MP and SZ had longer shoots than SBA. SZ had the most leaves per shoot and MP the least. XP and MP had the largest shoot diameter and MP had the longest internode length with SBA the shortest (Table 3). These findings are consistent with the field trial data from Copper *et al.,* (2020) where Xynisteri had the longest shoots and the largest shoot diameter and Maratheftiko had the least leaves per shoot and longest internode length.

When comparing shoot length and internode length for XM, XK, XP, SBC and SBA between the two seasons, we can see that in the warmer 2019 season vines had longer shoots than those from the cooler 2020/21 season. This is consistent with results seen by Galat-Giorgi *et al.,* (2020) who noted that Malbec shoots were longer with increased temperature. Likewise with internode length and leaf number per shoot, increased temperatures are associated with shorter internode lengths and a greater number of nodes and leaves per shoot as demonstrated by Allen *et al.,* (2017) with soybean crops. A warming climate can have significant effects on grapevines and other crops. Keller and Tarara (2010) studying Cabernet Sauvignon, report that warmer spring temperatures at bud burst can lead to large differences in shoot growth, shoot architecture and leaf development. These changes can be maintained or amplified during the growing season. They demonstrated that early season temperatures have a persistent effect on the shoot growth rate regardless of the growing season temperature.

Table 4: Vine growth assessments at flowering for all varieties in potted trials in season 2019 and 2020/21.

Treatment	Shoot length (cm)	Leaves per shoot	Shoot diameter (cm)	Internode length (cm)
2019				
XM	196 b	87 a	0.97 a	7.2 a
XK	2358 a	105 a	1.03 a	7.5 a
SBC	118 c	98 a	0.45 b	5.9 b
Pr>F	<0.0001	0.079	<0.0001	<0.0001
2020/21				
XP	152 a	44 ab	1.03 a	10.4 b
MP	166 a	36 b	1.09 a	12.6 a
SZ	171 a	47 a	0.75 b	11.9 ab
SBA	101 b	42 ab	0.74 b	8.01 c
Pr>F	<0.0001	0.004	<0.0001	<0.0001

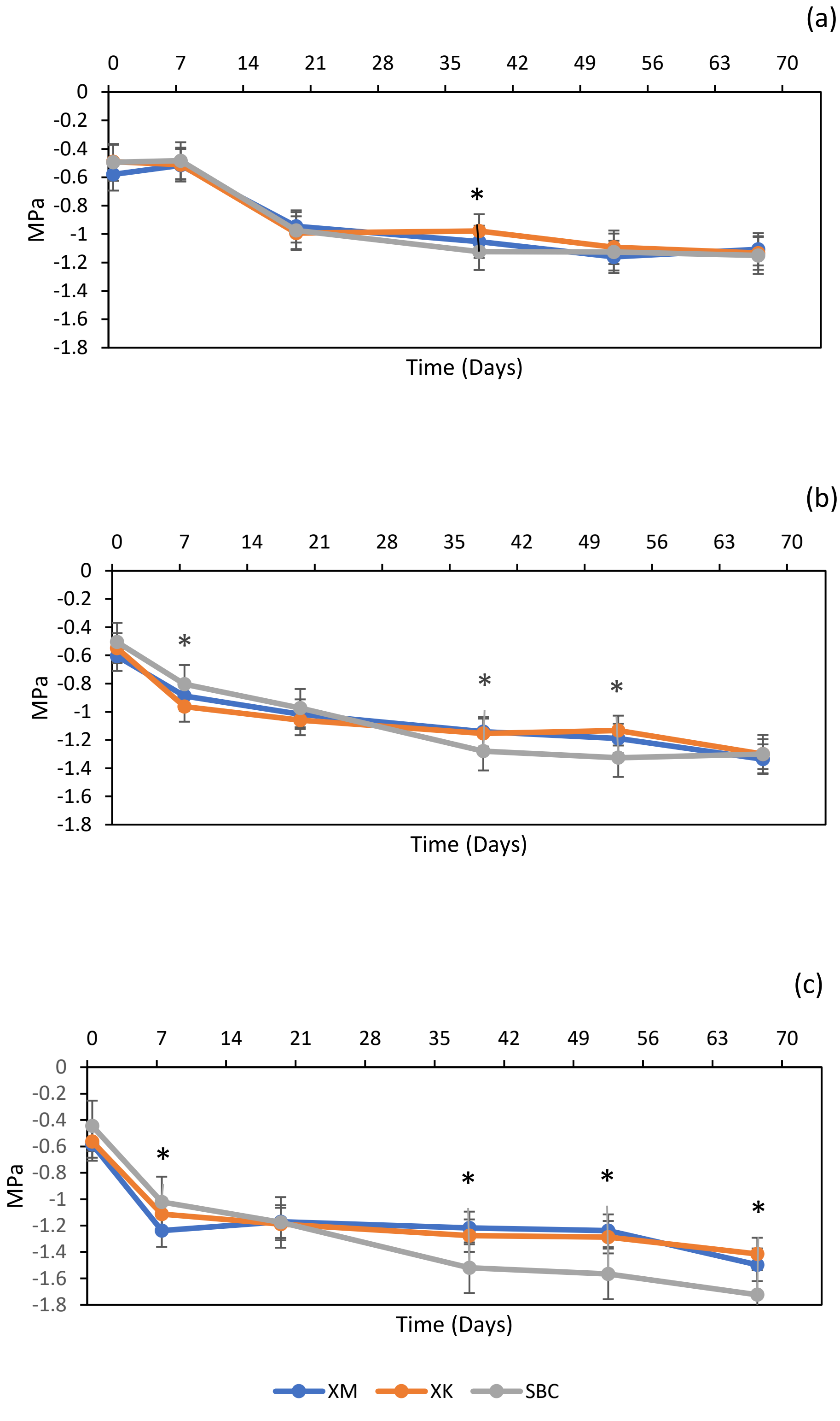
XM- Xynisteri Mandria, XK- Xynisteri Kathikas, XP- Xynisteri Paphos, MP- Maratheftiko Paphos, SBC- Sauvignon Blanc Cyprus, SBA- Sauvignon Blanc Adelaide, SZ- Shiraz. Different letters next to the measures indicate significant differences ($p<0.05$), measures with the same letters are not statistically significantly different. Measures for shoot length, Shoot diameter and internode length are means of $n=10$ vines in 2019 and $n=7$ vines in 2020/21 with 2 shoots per vine.

In 2019, under full irrigation XM and XK had higher stem water potential than SBC on day 38 only (Figure 6a), with XM and SBC at moderate levels of stress (between -1.1 and -1.2 MPa).
Under 50% irrigation, on day 7 SBC had higher stem water potential than XM and XK. XM and XK were higher than SBC at days 38 and 52. Additionally, at day 52 XK was higher than XM (Figure 6b). XM and XK were under moderate stress levels at day 19, followed by SBC at day 38.
Under 25% irrigation, at day 7 SBC had higher stem water potential than XK and XM. XM and XK had higher stem water potential than SBC at days 38, 52 and 67 (Figure 6c). All varieties were under moderate stress by day 7 with SBC under severe stress (-1.5 MPa) by day 38. Repeated ANOVA indicated that stem water potential was significantly affected by time, irrigation rate and their interactions (Table 5), that is, stem water potential decreased significantly with time and for all irrigation levels, with the largest decrease occurring for SBA under 25% irrigation.

In 2020/21 under full irrigation MP, XP and SZ stem water potentials were all higher than SBA on day 73 only (Figure 6d), with all except for MP under moderate stress.
At day 35 SZ had the highest stem water potential, followed by MP, SBA, and XP the lowest. Under 50% irrigation, on day 39 MP and SZ stem water potentials were highest followed by SBA and XP. On day 73 XP had the highest stem water potential followed by SZ, MP and SBA (Figure 6e).

XP was under moderate stress from day 35 onwards, SBA from day 39 onwards and MP and SZ from day 64 onwards.
Under 25% irrigation SBA stem water potential was highest on day 7 followed by SZ, MP with XP the lowest. On day 35 SZ was the highest followed by SBA, XP and MP the lowest (Figure 6f). All varieties were under moderate stress by day 21 with all under severe stress (-1.5 MPa) by day 73. Repeated ANOVA indicated that stem water potential was significantly affected by time, irrigation rate and their interactions (Table 5). Under full and 50% irrigation SBA stem water potential decreased the most at the end of the trial, while under 25% irrigation XP and XM stem water potentials decreased earlier and mid-way through the trial when compared with SZ and SBA.

While the results for 2020/21 were not conclusive, findings for 2019 were similar to those reported by Copper *et al.,* (2020) who demonstrated that Xynisteri had higher stem water potential than Maratheftiko and Shiraz, while Sauvignon Blanc had the lowest stem water potential. Water potential has been widely used as an indicator of plant water status for irrigation management purposes (García-Tejera *et al.,* 2021). There is however some conjecture about the levels of stem water potential that are considered as moderate and severely stressed. Alatzas *et al*., (2021) consider values lower than -1.1 MPa as severe stress and Girona *et al*., (2006) consider -1.2 MPa moderate stress and -1.5 MPa severe stress. This could also be cultivar dependant, with modifications of the ratio of root to leaf area inducing changes in the relationship between water potential, transpiration and soil water content (Bauerle *et al*., 2008). For example, Bauerle *et al*., (2008) studied high and low vigour rootstocks and concluded that some high vigour rootstocks may be more plastic and have evolved to grow roots in the deeper, moister soil regions later in the growing season. This could help to explain the root mass results seen, particularly for Xynisteri in 2020/21. The cultivar and root structure differences for Xynisteri and Maratheftiko may be the reason why the water potential decreases were not significant until the end of the testing period after 74 days of water stress. That is, they have evolved to develop deeper roots later in the growing season, when soil water content has decreased. Future longitudinal studies of root development of Maratheftiko and in particular Xynisteri compared to other varieties over the growing period could confirm this.



(a)
MPa
Time (Days)
(b)
MPa
Time (Days)
(c)
MPa
Time (Days)
XM
XK
SBC

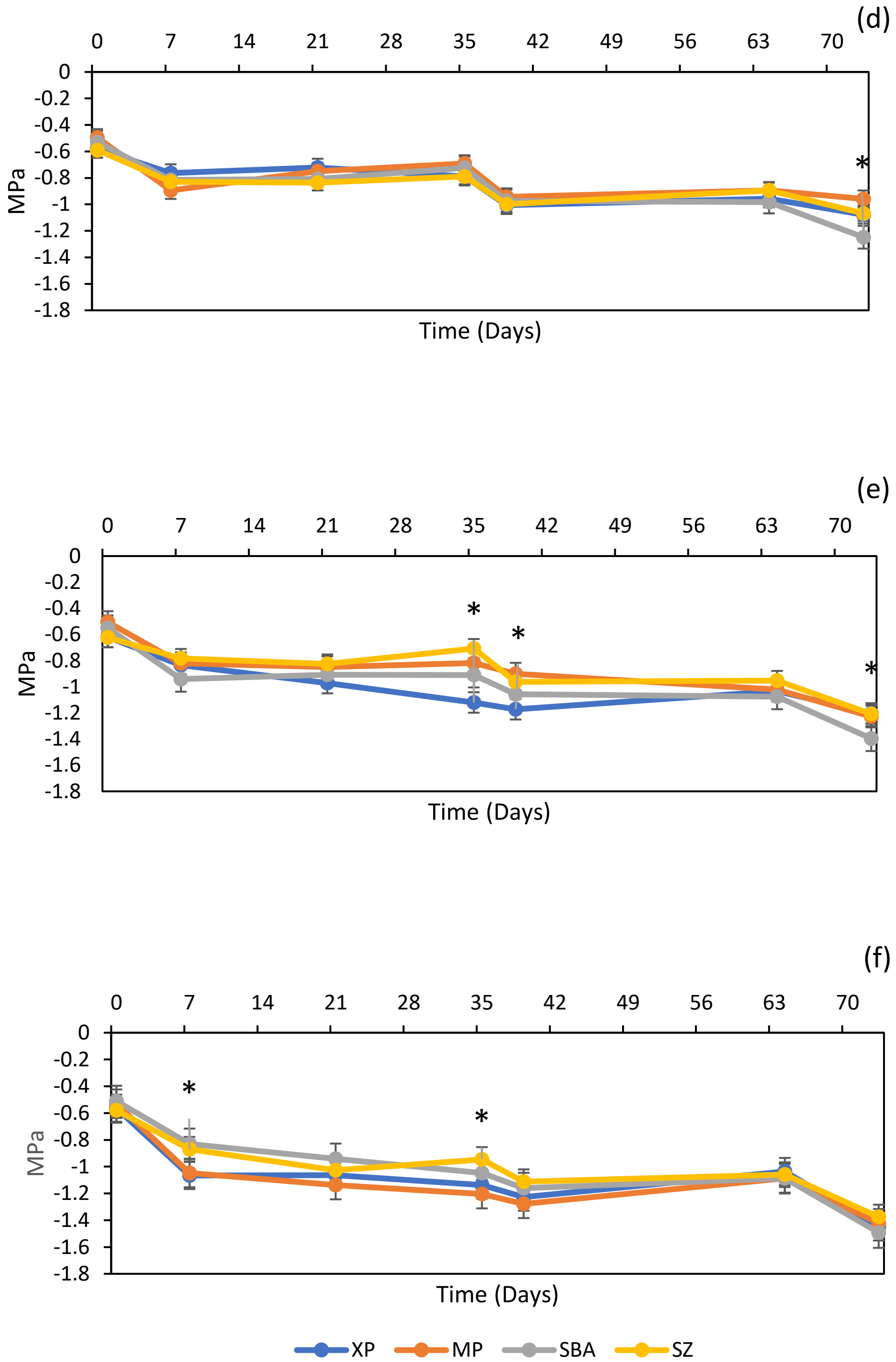


Figure 6: Stem water potential (MPa) measures for potted trials for seasons 2019 and 2020/21.

XM- Xynisteri Mandria, XK- Xynisteri Kathikas, XP- Xynisteri Paphos, MP- Maratheftiko Paphos, SBC- Sauvignon Blanc Cyprus, SBA- Sauvignon Blanc Adelaide, SZ- Shiraz.

(a) Full irrigation 2019, (b) 50% irrigation 2019, (c) 25% irrigation 2019, (d) Full irrigation 2020/21, (e) 50% irrigation 2020/21, (f) 25% irrigation 2020/21. Each data point are means of n=10 vines in 2019 and n=7 vines in 2020/21. Bars indicate the standard error. Means were separated by ANOVA using Tukey's test. * indicate significance at $p < 0.05$.

Table 5: Repeated measures ANOVA applied to stem water potential, stomatal conductance and SPAD reading in relation to time, irrigation (treatment) and their interactions.

Factor	F value	p-value
2019		
Stem water potential		
Time	104.376	< 0.0001*
Irrigation	51.823	0.0002*
Time*Irrigation	4.484	0.001*
Stomatal conductance		
Time	118.548	< 0.0001*
Irrigation	27.634	0.001*
Time*Irrigation	5.131	0.0002*
SPAD reading		
Time	1.286	0.296
Irrigation	0.234	0.799
Time*Irrigation	1.976	0.073
2020/21		
Stem water potential		
Time	119.446	< 0.0001*
Irrigation	15.992	0.001*
Time*Irrigation	3.731	0.0004*
Stomatal conductance		
Time	90.098	< 0.0001*
Irrigation	2.065	0.183
Time*Irrigation	1.367	0.210
SPAD reading		
Time	16.054	< 0.0001*
Irrigation	0.133	0.877
Time*Irrigation	0.127	1.000

* indicate significance at $p < 0.05$

In 2019 under full irrigation, XM and XK had higher stomatal conductance than SBC on day 38 (Figure 7a), XM was also higher than XK. Cifre *et al.*, (2005) consider 50-150 mmol/m^2/s the threshold for severe water stress, using this classification XK and SBC were stressed from day 39 and XM from day 38.

Under 50% irrigation XM and XK had higher stomatal conductance than SBC at days 19, 38 and XK was higher than XM (Figure 7b). All varieties were classed as stressed after day 7.

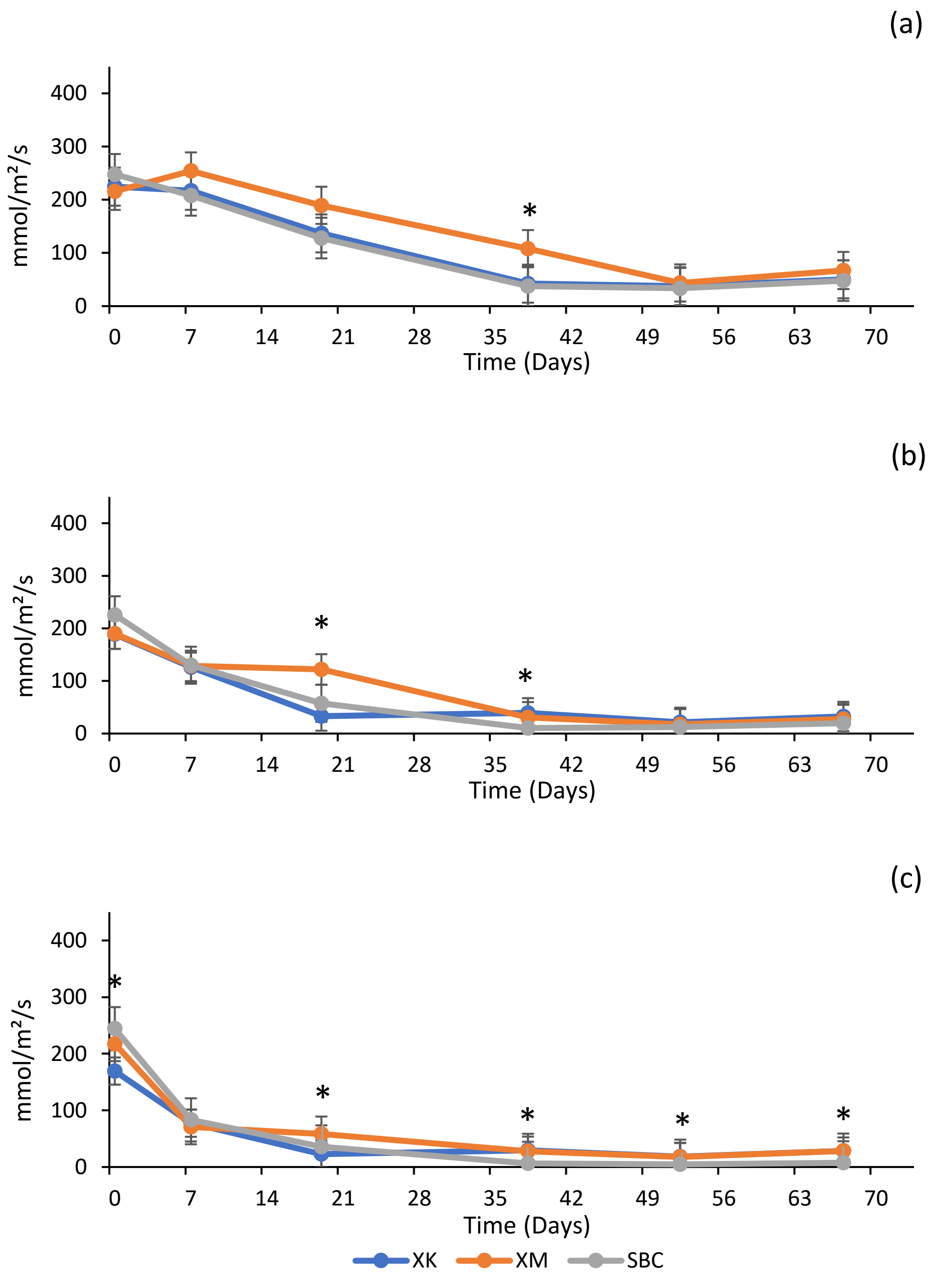
Under 25% irrigation XM and XK were higher at days 0, 19, 38, 52 and 67 than SBC. Additionally, at day 19 XM was higher that XK and at day 52 XK was higher than XM (Figure 7c). All varieties were classed as stressed after day 7.

Repeated measures ANOVA indicated that stomatal conductance was significantly affected by time, irrigation rate and their interactions (Table 5), that is, there was a decrease in stomatal conductance for all three varieties, with the largest decrease occurring for SBC under 25% irrigation rates.

In 2020/21 under full irrigation stomatal conductance for XP was the highest on every occasion. SZ was the lowest on days 0 and 7, while SBA had the lowest stomatal conductance on days 21, 35, 39, 64 and 73 (Figure 7d). SZ was considered stressed at day 7, SBA at day 35, XP and MP never fell below 150 mmol/m^2/s for the entire testing period.

Under 50% irrigation XP was the highest on days 0, 7, 21, 64 and 73, While MP was the highest on days 35 and 39. The lowest stomatal conductance was SZ on days 0, 7, 21 and 35 with SBA the lowest on days 39, 64 and 73 (Figure 7e). SZ was considered stressed at day 7, SBA at day 21, XP at day 35 and MP at day 39.

Under 25% irrigation XP had the highest stomatal conductance on days 0, 7, 21, 64 and 73. SZ had the lowest on days 0, 7 and 21 while SBA was the lowest on days 64 and 73 (Figure 7f). SZ was considered stressed at day 7, SBA at day 21, MP at day 21 and XP at day 35. Repeated ANOVA indicated that stomatal conductance was significantly affected by time only (Table 5), that is, it decreased over time at a much greater rate for SBA and SZ than for MP and XP.



(a)
400
300
200
100
0
mmol/m²/s
*
0
7
14
21
28
35
42
49
56
63
70
Time (Days)
(b)
400
300
200
100
0
mmol/m²/s
*
*
0
7
14
21
28
35
42
49
56
63
70
Time (Days)
(c)
400
300
200
100
0
mmol/m²/s
*
*
*
*
*
0
7
14
21
28
35
42
49
56
63
70
Time (Days)
XK
XM
SBC

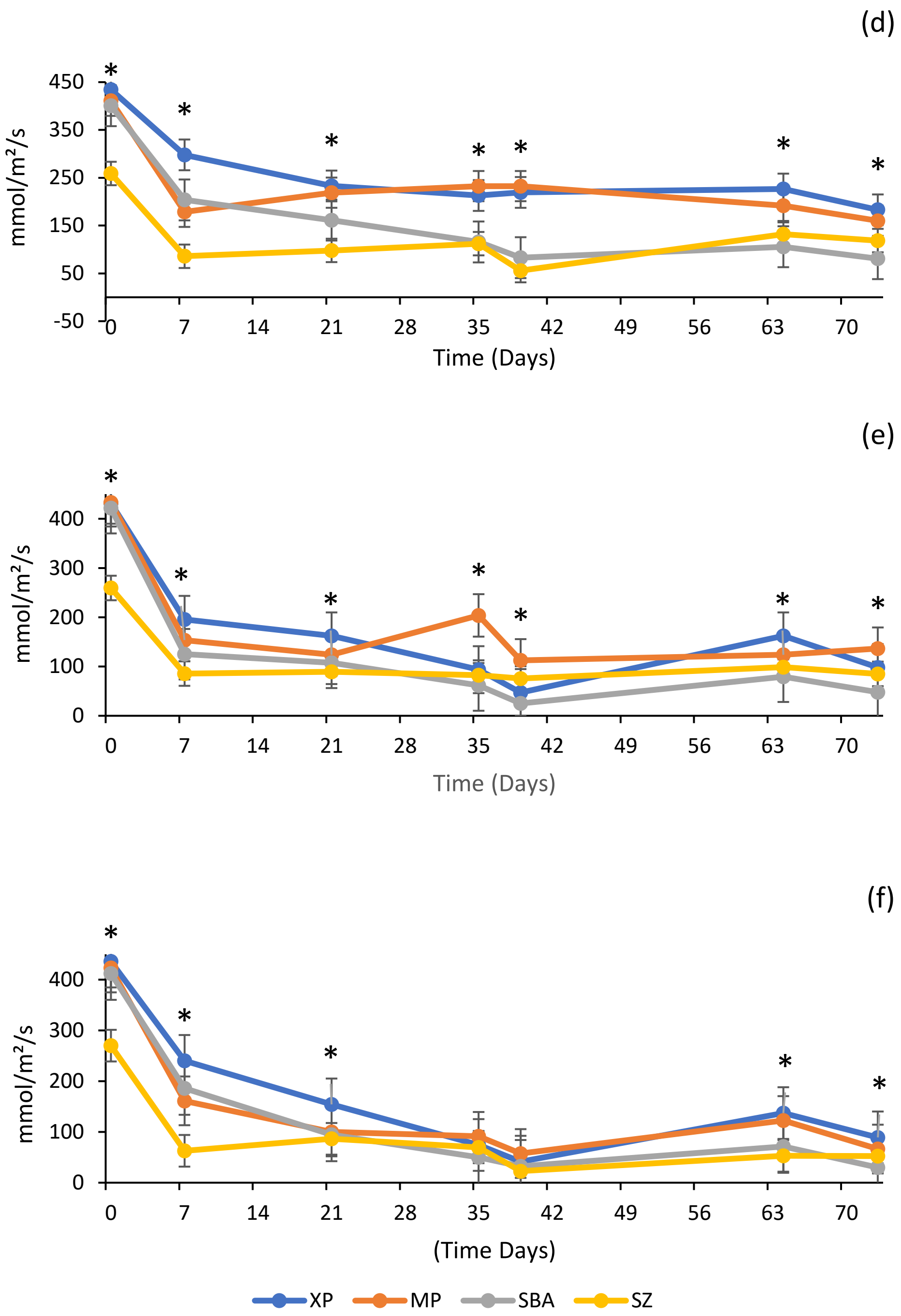


Figure 7: Stomatal Conductance (mmol/m²/s) measures for potted trial for seasons 2019 and 2020/21.

XM- Xynisteri Mandria, XK- Xynisteri Kathikas, XP- Xynisteri Paphos, MP- Maratheftiko Paphos, SBC- Sauvignon Blanc Cyprus, SBA- Sauvignon Blanc Adelaide, SZ- Shiraz.

(a) Full irrigation 2019, (b) 50% irrigation 2019, (c) 25% irrigation 2019, (d) Full irrigation 2020/21, (e) 50% irrigation 2020/21, (f) 25% irrigation 2020/21. Each data point are means of n=10 vines in 2019 and n=7 vines in 2020/21. Bars indicate the standard error. Means were separated by ANOVA using Tukey's test. * indicate significance at

$p < 0.05$.

From this data we can see that XP had higher stomatal conductance and SZ had the lowest in the early stages of testing, with SBA being the lowest in the later developmental stages. This is similar to the results seen by Copper *et al*., (2020) who found that Xynisteri and Maratheftiko had greater stomatal conductance than Shiraz and Sauvignon Blanc in a vineyard trial. All the varieties in this study showed a reduction in stomatal conductance over time but at differing rates.
Tzortzakis *et al*., (2020) studying Xynisteri and Chardonnay showed similar results for Xynisteri but saw that stomatal conductance for Chardonnay was relatively constant throughout their testing period. They concluded that this was a possible mechanism in which Xynisteri responds to drought stress by improved stomata conductance regulation.
The literature, however, is not so clear and in recent times, stomatal regulation has been a topic of much research and conjecture. The classification for drought tolerance in grapevines often utilises the binary terms isohydric and anisohydric (Gerzon *et al.,* 2015). Isohydric vines are said to be able to maintain constant low water potentials through rapid stomatal closure, while anisohydric vines only close stomata at very low water potentials. Chaves *et al*., (2010) state that the distinction between isohydric and anisohydric plants is not clear, and that they may be able to switch between strategies depending on drought severity and environmental conditions.
Levin *et al*., (2020) however, reject the premise of isohydric and anisohydric behaviour entirely. They studied 17 different cultivars in a field experiment under three irrigation regimes. They measured pre-dawn and midday leaf water potential as well as midday stomatal conductance. They concluded that stomatal behaviour is an across-cultivar continuum and call into question the isohydric and anisohydric classification system. They state that in general, cultivars respond similarly to one another at high and low water status, but stomatal behaviour differs at moderate water status. They believe that *V. vinifera* cultivars possess both isohydric and anisohydric stomatal behaviour that is dependent on the intensity of water deficits. Hochberg *et al*., (2018) agree and state that the use of the iso/anisohydric terminology should be abandoned for two reasons: (i) the different definitions are not necessarily in agreement with one another, creating confusion as to the actual meaning of the terms; and (ii) the environmental effects are at least as significant as the genotypic effect, and thus a cultivar's hydraulic behaviour cannot be predicted without accounting for the environment (Hochberg *et al.,* 2018 and Villalobos-González *et al.*, 2019, Dayer *et al*., 2020). This may be the reason for the results that were seen in this study. That is, all varieties had a decrease in stomatal conductance over the testing period, with SBC, SBA and SZ showing the largest decreases with all irrigation regimes.

SPAD readings in both seasons ranged between 15 and 40, which is consistent with results seen by Steele *et al.,* (2008) who state that SPAD readings are adequately sensitive at around 35 (chlorophyll content approximately 300 mg/m^2). Their research demonstrated that grapevine leaves can have SPAD values of between 7 and 44 which equates to a chlorophyll content of between 63 to 576 mg/mm^2. Ling *et al*., (2011) report that SPAD readings are proportional to the amount of chlorophyll present in the leaf and that converted SPAD values differ from photometric measurements of solvent-extracted chlorophyll by just 6%, as well as being a non-destructive method suitable for preserving the leaves of plants being studied.

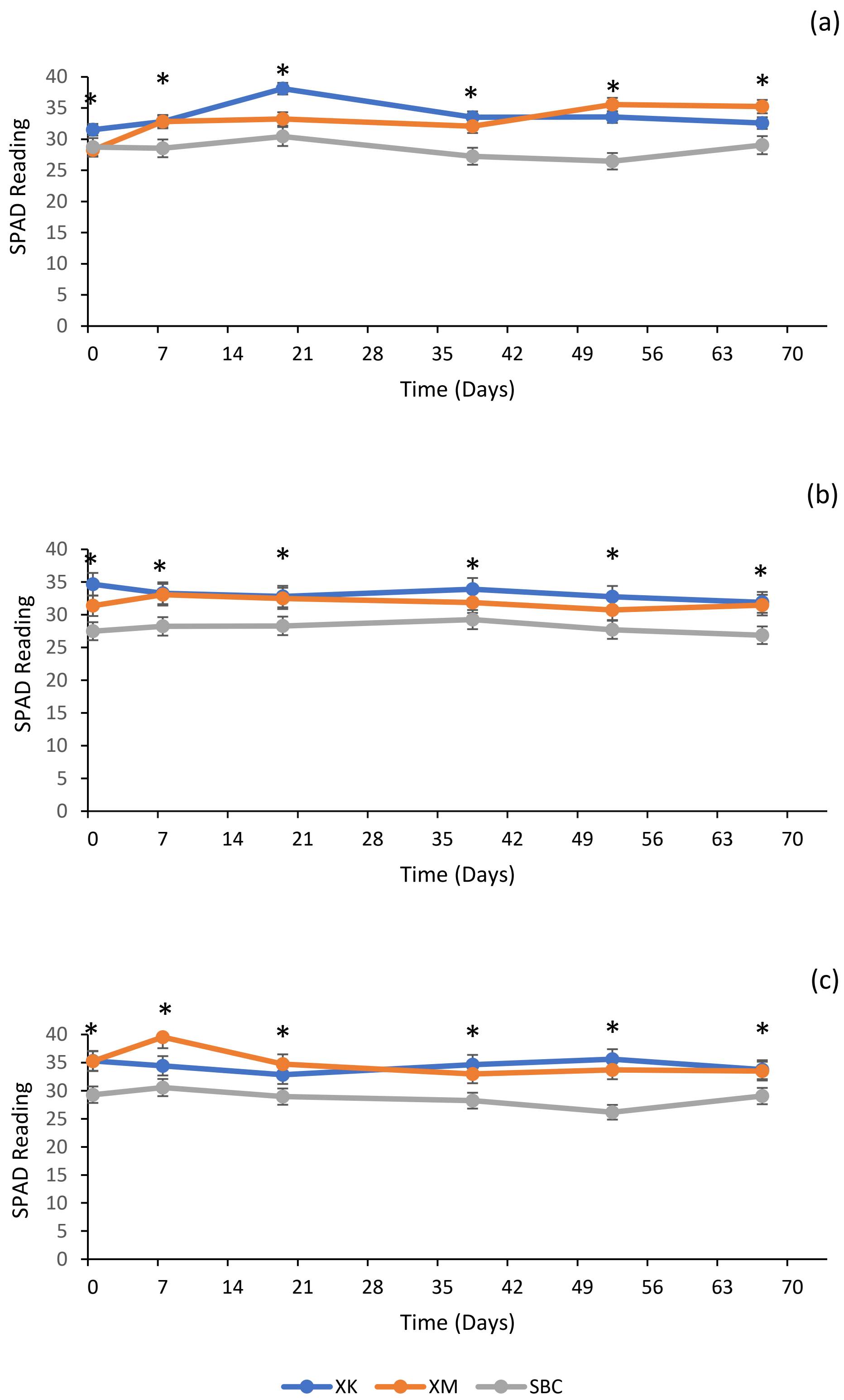
Both XM and XK had higher SPAD readings/chlorophyll content when compared to SBC throughout the testing period in 2019. Chlorophyll content for all three varieties remained constant throughout the testing period (Figure 8a, b, c). Repeated ANOVA indicated that there were no interactions for time and irrigation rates in SPAD readings (Table 5).

In 2020/21 SPAD readings/chlorophyll content for XP and MP were the highest for all three irrigation regimes at every testing period. Conversely SBA and SZ were the lowest for all three irrigation regimes and testing period. Overall, all the four varieties increased their chlorophyll content over the testing period, XP and MP in particular increased their chlorophyll content with 50% and 25% irrigation (Figure 8d, e, f). Repeated ANOVA indicated that SPAD readings were significantly affected by time only (Table 5), that is XP and MP had higher chlorophyll content than SZ and SBA under all irrigation regimes over the course of the trial

This again concurs with results seen by Copper *et al.,* (2020) where Xynisteri and Maratheftiko had higher chlorophyll content than Shiraz and Sauvignon Blanc. They however identified that in a vineyard, Maratheftiko had higher chlorophyll content than Xynisteri, which was not the case with the potted trials.

Chrysargyris *et al*., (2020) compared Xynisteri with Chardonnay in a vineyard and found that the level of chlorophyll amongst irrigation and no irrigation groups varied. In irrigated vines, chlorophyll decreased at flowering and increased at veraison with no irrigation. Xynisteri chlorophyll levels were unchanged between treatment groups at flowering, veraison and harvest, but overall levels showed a decreasing trend throughout the testing period. Tzortzakis *et al*., (2020) studying Xynisteri and Chardonnay in pots showed Chardonnay chlorophyll content levels decreased after eight days of drought stress and Xynisteri showed similar affects after 20 days. With heat stress conditions, both Xynisteri and Chardonnay showed reduced levels after 20 days.

Chrysargyris *et al*., (2018a) studying Maratheftiko in a vineyard trial with varying irrigation found that chlorophyll content was constant throughout the testing period for all irrigation groups except for the irrigated group at harvest, where a decrease in chlorophyll content was seen. Similar results were demonstrated by Chrysargyris *et al*., (2018b) studying Maratheftiko in pots under heat and drought stress conditions. Chlorophyll content was maintained after 20 days of light drought stress compared to full irrigation. However, moderate drought stress caused a decrease. Heat stress caused a decrease in chlorophyll content after 20 days, but overall drought stress had a larger impact than heat stress. These results indicate that both Xynisteri and Maratheftiko overall are able to maintain or in some cases increase their chlorophyll content across a growing season and are able to do this more efficiently than Chardonnay, Sauvignon Blanc and Shiraz. Liu *et al.,* (2019) investigating chlorophyll content as a predictor of above ground biomass in rice crops, demonstrated that higher chlorophyll content in leaves correlated with an increase in above ground biomass. This may be the reason XM, XK, XP and MP developed higher biomass than SBC, SBA and SZ. Chlorophyll content and the nitrogen status of Shiraz grapevines (measured by SPAD) was studied by Metya *et al*., (2014) grown in pots. They concluded that nitrogen supply altered the whole plant biomass and its distribution between annual and perennial parts of the plants. Nitrogen deficiency slows growth and causes a higher biomass allocation to perennial parts of the plant (particularly the trunk), this however is cultivar dependant, Merlot grown under reduced nitrogen levels demonstrated enhanced root growth at the expense of aboveground growth. This adds to the paradox of the Xynisteri results, with it demonstrating high chlorophyll content (leaf nitrogen), large above ground biomass and large root biomass.


(a)
(b)
(c)
SPAD Reading
Time (Days)
0
7
14
21
28
35
42
49
56
63
70
40
35
30
25
20
15
10
5
0
XK
XM
SBC

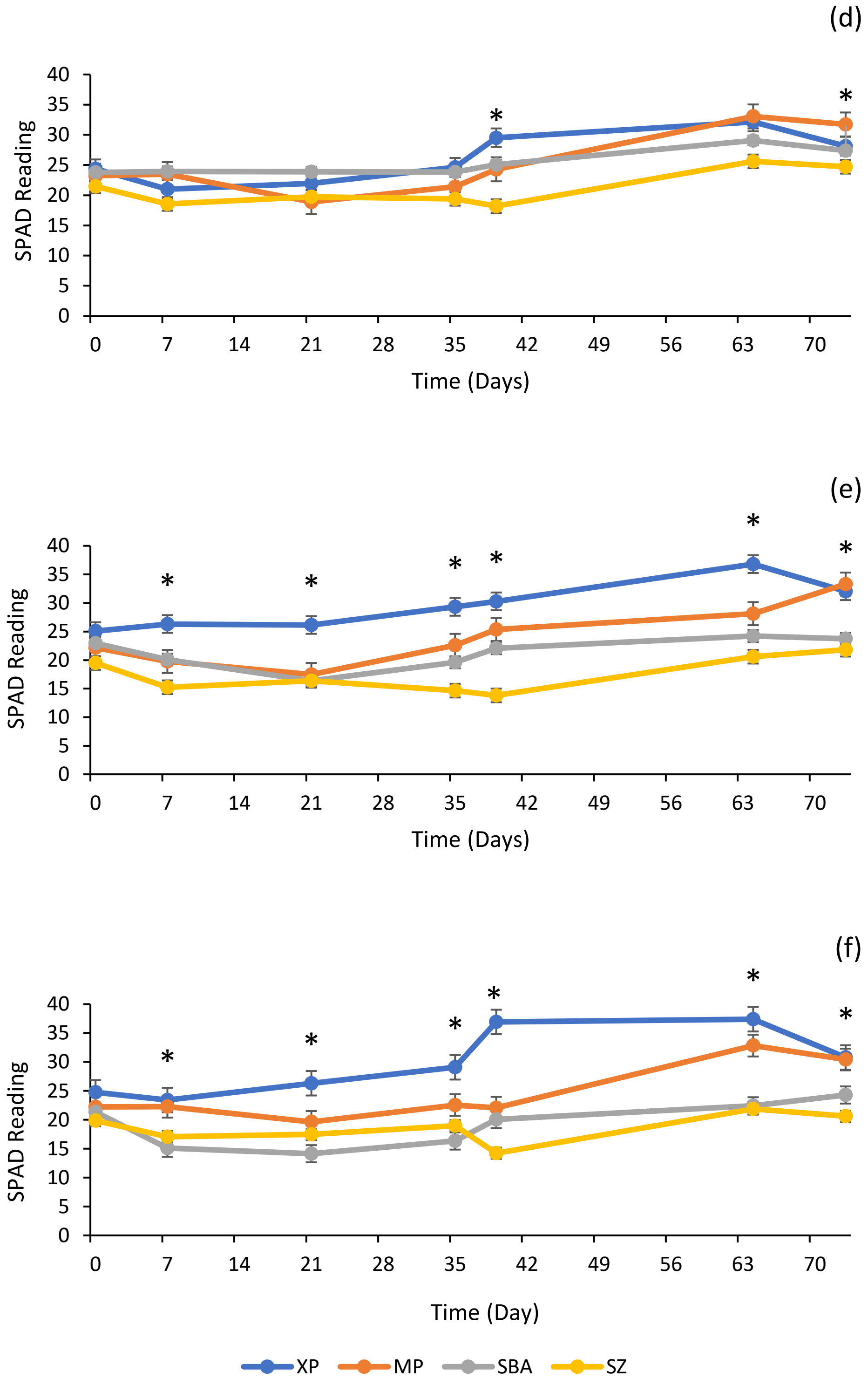


Figure 8: SPAD reading (indicative chlorophyll content) measures in potted trials for seasons 2019 and 2020/21.

XM- Xynisteri Mandria, XK- Xynisteri Kathikas, XP- Xynisteri Paphos, MP- Maratheftiko Paphos, SBC- Sauvignon Blanc Cyprus, SBA- Sauvignon Blanc Adelaide, SZ- Shiraz.

(a) Full irrigation 2019, (b) 50% irrigation 2019, (c) 25% irrigation 2019, (d) Full irrigation 2020/21, (e) 50% irrigation 2020/21, (f) 25% irrigation 2020/21. Each data point are means of n=10 in 2019 and n=7 in 2020/21, Bars indicate the standard error. Means were separated by ANOVA using Tukey's test. * indicate significance at $p < 0.05$

In 2019 XK and XM produced greater end of season root, trunk and shoot mass than SBC under all irrigation regimes and XK had greater root, trunk and shoot mass than XM with full irrigation (Table 6). All mass values were fresh weights taken one day after the final testing day. In 2020/21 XP had the highest root, shoot and leaf mass followed by MP, SBA and SZ. SZ had the lowest root, shoot and leaf mass at all irrigation levels except in the case of shoot mass with 25% irrigation where it was not statistically different to that of MP and SBA had the lowest mass (Table 6). When a 2-way ANOVA was applied to the data to assess for interactions between variety and irrigation, only the shoot length of Xynisteri when receiving full irrigation in 2019 was significantly affected.

In both seasons, root mass for Xynisteri was greater than the shoot and leaf mass (above ground biomass), while Maratheftiko, Shiraz and Sauvignon Blanc had similar root and above ground biomass ratios. However, in the cooler 2020/21 season root and shoot/leaf masses were higher than the warmer 2019 season. Jumrani *et al.,* (2017) studying the effect of increased temperatures on soybean crops found that leaf weight and thickness decreased and the rate of photosynthesis and stomatal conductance also decreased with increased temperatures. Conversely, they observed that stomatal density increased significantly with increased temperatures. These changes were also seen in the cooler 2020/2021 season when compared to 2019, with the cooler season producing a decreased stomatal density (Table 6). Ferlito *et al.,* (2020) studying Nerello Mascalese and Nero d'Avola on own roots and on drought tolerant rootstocks in a vineyard trial showed similar ratios with the drought tolerant rootstocks 140 Ruggeri and 1103 Paulsen having higher root masses than above ground masses when compared to corresponding own rooted vines. Yildirm *et al.,* (2018) investigating Sultana grape vines grafted to three rootstocks, found that the drought tolerant rootstock 110R increased its root mass under drought and well-watered conditions at a greater rate than the rootstocks 5BB and 41B. They concluded that 110R was able to do this via drought dependent sugar and protein induction genes located in the roots. Gambetta *et al.,* (2020) describe root volume as one of the most basic and enigmatic physiological traits of grapevines and that root volume has the potential to be used to determine the soil water reservoir that is available to the vine. Alsina *et al.,* (2011) also found that drought-adapted rootstocks tend to have deeper roots. This may be one mechanism by which Xynisteri is also able to increase root mass in drought and well-watered conditions. To a lesser extent Maratheftiko also had a larger root mass, but this was only statistically significant under 25% irrigation conditions. Prinsi *et al.,* (2018) agree that roots may play a role in the grapevine's response to drought stress. They studied M4 rootstocks and concluded that carbon metabolism, mitochondrial function and other as yet unidentified mechanisms may be involved in the root mass, drought tolerance effect in grapevines.

Chrysargyris *et al*., (2020) in their study comparing Xynisteri to Chardonnay found that non-irrigated Xynisteri had an increased level of the hormone abscisic acid (ABA). ABA is thought to play a role in the behaviour of stomata and reflects an increased capacity to react to water stress by altering stomatal conductance (Dayer *et al*., 2020, Prinsi *et al*., 2021). Sharp and LeNoble (2002), state that ABA accumulation during water stress may often function to help maintain root as well as shoot growth, rather than to inhibit growth as is commonly believed. In recent times, the role of ABA and the expression of genes involved in its activity have been the subject of much research for possible drought resistance (Hopper *et al.* 2016, Wang *et al.,* 2019, Liu *et al.,* 2019b), however, no definitive mechanisms have been concluded to date. Li *et al.,* (2020) state that root architecture is very important for *V. vinifera* and that ABA plays an important role in increased root growth, root hair growth and enhanced drought resistance.

The role of Xynisteri and Maratheftiko roots could be an important factor for vineyards in Australia were 80-95% of vineyards are planted without the use of rootstocks. Only one region (Riverland) has 45% of vineyards planted with vines using rootstocks (Vinehealth Australia 2021). Further research into the role of these root structures is therefore warranted.

Table 6: Fresh root, shoot and leaf mass (EL 38 Harvest) measures for potted trials for seasons 2019 and 2020/21.

Mass (gm)	**Root**			**Shoot**			**Leaf**		
IR	Full	50%	25%	Full	50%	25%	Full	50%	25%
Cyprus 2019									
XM	693b	582ab	387ab	264b	204a	112b	243b	184a	102ab
XK	939a	643a	486a	377a	234a	180a	359a	208a	156a
SBC	493c	352b	182b	109c	93b	63c	129c	93b	48b
Pr>F	<0.0001	0.01	<0.0001	<0.0001	0.0001	0.0002	<0.0001	0.0017	0.0001
Adelaide 20/21									
XP	1233 a	1135 a	892 a	458a	411a	342a	357a	291a	259a
MP	620 b	567 b	539b	425ab	366ab	296ab	252ab	240ab	201ab
SZ	592 b	445 b	320c	299b	286b	238ab	215b	154c	137b
SBA	610 b	494 b	443b	307b	274b	206b	236b	205bc	140b
Pr>F	0.0004	<0.0001	<0.0001	0.009	0.035	0.029	0.011	0.0003	0.001

IR- Irrigation regime, Full= 8 litres per pot per week, 50%= 4 litres per pot per week, 25%= 2 litres per pot per week. XM- Xynisteri Mandria, XK- Xynisteri Kathikas, XP- Xynisteri Paphos, MP- Maratheftiko Paphos, SBC- Sauvignon Blanc Cyprus, SBA- Sauvignon Blanc Adelaide, SZ- Shiraz. Each data point are means of n=10 in 2019 and n=7 in 2020/21. Bars indicate the standard error. Means were separated by ANOVA using Tukey's test. *indicate significance at $p < 0.05$, different letters next to the measures indicate significant differences.

2.3 Stomatal density

In 2019, XK and XM had greater stomatal density than SBC. The Xynisteri stomatal density were the highest (similar to the findings of Copper *et al.,* 2020), followed by MP, SZ and SBA (Table 6) in 2020/21. There was however some difference between the two seasons. As discussed previously, the 2019 testing period had a mean temperature of 24°C and the 2020/21 growing season had a mean temperature of 21°C. Rogiers *et al.,* (2011) have reported that stomatal density is correlated to temperature, they report that the stomatal density can be as much as 1.4 times greater in warm temperatures when compared to cooler temperatures. This could help to explain the differences seen between 2019 and 2020/21 for Xynisteri with the warmer season producing higher stomatal densities. High stomatal density has been associated with drought tolerance, with Boso *et al.,* (2011) suggesting that the high stomatal density of Albarinho may be responsible for its greater drought tolerance as it has an increased photosynthetic capacity. However, leaves for stomatal density were only collected at flowering, future studies to determine the impact of water status on these varieties could involve collecting leaves at different time points during an irrigation trial.

Table 7: Stomatal Density measures for potted trials for seasons 2019 and 2020/21.

Season	Variety	Stomatal Density
2019	XCV	238.6a
	XK	227.5a
	XM	233.2a
	SBC	139.8b
	Pr>F	<0.0001
2020/21	XP	206.1a
	MP	189.0b
	SZ	170.5c
	SBA	151.4d
	Pr>F	<0.0001

XCV- Xynisteri Cyprus Vineyard. XK, XM, XP- Xynisteri, MP- Maratheftiko, SBA, SBC- Sauvignon Blanc. Stomatal density- number of stomata per mm^2. Each data point are means of n=10 in 2019 and n=7 in 2020/21. Bars indicate the standard error. Means were separated by ANOVA using Tukey's test. *indicate significance at $p < 0.05$, different letters next to the measures indicate significant differences.

Conclusion

This study, along with recent studies described above highlight the potential of the indigenous Cypriot varieties to tolerate reduced irrigation levels. Xynisteri and to a lesser extent Maratheftiko, were shown to have more vigorous growth than the commonly cultivated varieties of Sauvignon Blanc, Shiraz and Chardonnay in lower irrigation regimes. The study also demonstrated that while Xynisteri and Maratheftiko may be classified as stressed using conventional stem water potential and stomatal conductance parameters, they are able to continue to increase their biomass at greater rates than Shiraz and Sauvignon Blanc. In regions where irrigation is used to supplement rainfall, cultivation of these varieties could result in a reduction of the irrigation required and warrants further investigation with vineyard trials.

Overall, the biomass of Xynisteri above and below ground was far greater than all the other varieties investigated and under all irrigation regimes. Maratheftiko had greater leaf and shoot mass than Shiraz and Sauvignon Blanc for all irrigation regimes, but only had greater root mass under 25% irrigation. The role of the extensive root structure of Xynisteri is one area that requires further investigation. Although to date root biomass data for Xynisteri only exists for potted vines, field grown vines could better explain the role of the roots in drought tolerance in the future. To determine whether scion or root structure is more important to Xynisteri, trials with scion material grafted to differing rootstocks could be studied to assess the performance and assist in guiding future research. Future studies involving leaf anatomy and stomatal density of Maratheftiko could assist in determining the mechanism of its drought resilient properties.

A limitation to the potted trial was that no soil moisture sensors were utilised to determine the frequency of irrigation. Future vineyard trials could include soil moisture sensors to better guide the frequency and volume of irrigation under field conditions. Vineyard trials are currently being established in Australia, however this is a prolonged process due to the limited availability of scion material related to Australian government plant importation and quarantine laws.

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