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Vine performance benchmarking of indigenous Cypriot grape varieties Xynisteri and Maratheftiko

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ABSTRACT

Aim: The aims of this study were to (1) formulate a baseline understanding of the performance of the indigenous Cypriot white grape Xynisteri and the red grape Maratheftiko (*Vitis vinifera* L.), and (2) compare these varieties to Shiraz and Sauvignon blanc grown in a Cypriot vineyard.

Materials and results: The investigation involved multiple dry grown vineyards from the Krasochoria region of Lemesos, Cyprus, during the 2017, 2018 and 2019 vintages. Vine performance measurements, including midday stem water potential, stomatal conductance, chlorophyll content, stomata density, vine phenology and vegetative and reproductive measurements, were taken at flowering, veraison and pre-harvest. Xynisteri had the greatest stomatal density, more shoots, more leaves, heavier bunches, greater yield, higher leaf water potential at harvest, and a stomatal conductance equal to Maratheftiko, but greater than that of both Shiraz and Sauvignon blanc. Maratheftiko had the longest shoots, largest shoot diameter and the greatest chlorophyll content out of all four varieties.

Conclusions: This study identified the ability of the indigenous Cypriot grape varieties, Xynisteri and Maratheftiko, to better tolerate hot and dry conditions when compared to more commonly cultivated varieties grown in the same environmental conditions.

Significance and impact of the study: The changing climate of wine growing regions worldwide is placing great pressure on the resources for sustainable viticulture. Many vineyards in hot climate zones base their businesses on European grape varieties traditionally grown in regions with abundant water resources. It is therefore necessary for the global wine industry to investigate grape varieties that are indigenous to hot climates. The eastern Mediterranean island of Cyprus is one such place, with more than 10 indigenous grape varieties that grow well in a hot climate without irrigation. Consumer studies have demonstrated that wines made from these Cypriot varieties are equally, if not more, acceptable than wines made from more traditional European grapes; therefore, the potential for their use in other hot wine growing regions is promising.

KEYWORDS

climate change, vine performance, adaptation, stomata density, water potential, chlorophyll content

INTRODUCTION

The world's wine growing regions are experiencing rapid climate change. Jones *et al.* (2005) reported on climate data from 1950-1999 and found that wine growing regions in Europe and the United States had experienced significant increases in growing season temperatures. This continues to be the case, with Schultz and Jones (2010) reporting that temperatures in French, German and Swiss wine growing regions are continuing to rise. Camps and Ramos (2012) state that in the Penedes wine region of Northern Spain, maximum temperatures have increased and rainfall has decreased in the period 1999–2009. Likewise, Australia is also experiencing the effects of climate change with 2019 reported as the hottest and driest year since records began in 1910. The area-averaged mean temperature for 2019 was 1.52 °C above the 1961–1990 average, while mean maximum temperatures were 2.09 °C above average and mean minimum temperatures were 0.95 °C above average. It was also the driest year on record, with a nationally averaged rainfall of 277.6 mm, which is 40 % below the 1961-1990 average (Australian Bureau of Meterology, 2020). This far exceeds the previously reported increase in average temperatures, which has been approximately 1 °C since the middle of the 20th Century (Webb, 2011).

The changes in climate have made viticulture more challenging in many regions of the world. For example, Keller (2010) and Webb *et al.* (2013) report of advancement in phenological development, particularly in hot years, which results in earlier harvest dates at higher temperatures and higher grape sugar concentration. This is partly due to warming climates, but it is also due to drought conditions and reduced water availability. Jarvis *et al.* (2017) state that wine grape maturity is occurring earlier due to the warming climate. This creates the effect known as 'vintage/harvest compression', whereby different varieties ripen at the same time, placing great pressure on winery resources and logistics. Cook and Wolkovich (2016) report that harvest dates are occurring earlier in France and Switzerland, and Krieger *et al.* (2011) state that increases in winter temperatures since the 1980's has caused harvest dates in Burgundy to be earlier. Jones and Goodrich (2008) and Diffenbaugh *et al.* (2011) concurred when describing very similar warming climate scenarios in the Napa Valley and other wine growing regions of the United States of America. Hotter and drier growing conditions in Spain have also resulted in reduced yields (Camps and Ramos, 2012).

Ongoing climate change and a further reduction in average rainfall is expected over the coming decades (Johnson *et al.*, 2018). For example, it is predicted that by 2030 most coastal regions in Australia will experience an increase in average temperatures of 0.7-0.9 °C and 1-1.2 °C inland. Annual rainfall is also predicted to decrease by 2.5 to 5 % in most regions of Australia. Studies by van Leeuwen *et al.* (2013) and van Leeuwen *et al.* (2019) conclude that wine growing regions in France and Germany will not dramatically decrease over the next 30 years. However, Hannah *et al.* (2013) and Remenyi *et al.* (2019) disagree and demonstrate that in marginal wine growing regions, such as Australia, New Zealand, North and South America and South Africa, the suitability for growing grapes will decline more rapidly. It is therefore imperative for wine producers in warm to hot growing regions around the world to develop strategies to mitigate and adapt to these changes in climate.

The eastern Mediterranean island of Cyprus is reported to have a more than 5,500-year history of wine production (Chrysargyris *et al.*, 2018b). The region is also gradually and steadily becoming hotter and drier due to climate change (Evans, 2008; Lelieveld *et al.*, 2016). There are more than 10 indigenous grape varieties from Cyprus and many of them are very well adapted to drought, having been hand selected for their resistance to heat and drought by farmers for millennia (Fraga *et al.*, 2016; Patakas *et al.*, 2005). These Cypriot varieties therefore require less water and fertiliser inputs when compared to other non-indigenous varieties and show great promise for adaptation to climate change (Litskas *et al.*, 2017).

The Cypriot climate scenario is very similar to other warm/hot climate grape growing regions and as such, these indigenous Cypriot varieties may form a suitable strategy to assist in mitigating the climate change effects. Consumers in Australia have demonstrated that they like the wines made from these varieties and rate them similarly to Australian wines made from French varieties, further supporting the case for their potential use in other warm/hot regions (Copper *et al.*, 2019).

To date, very little has been published on the performance of indigenous Cypriot varieties. Chrysargyris *et al.* (2018a) and

Chrysargyris *et al.* (2018b) have investigated the short-term effects of light and moderate drought and heat stress on the physiological and biochemical stress markers in Maratheftiko. Chrysargyris *et al.* (2018b) have evaluated the effect of tillage and irrigation on yield and quality characteristics of Maratheftiko. More recently, Constantinou *et al.* (2019) evaluated the effect of leaf removal at veraison on the metabolites of fresh and dehydrated grapes of Mavro and Xynisteri. However, to the best of our knowledge, very little information has been reported in the literature on fundamental performance metrics for these varieties.

The aims of this study were to (1) formulate a baseline understanding of the performance of the indigenous Cypriot white grape Xynisteri and the red grape Maratheftiko and (2) compare these varieties to Shiraz and Sauvignon blanc grown in a Cypriot vineyard.

MATERIALS AND METHODS

1. Experimental design and material

The investigation involved multiple dry grown vineyards from the Lemesos wine region in Cyprus in 2017, 2018 and 2019. In season 2017 and 2018, the study was carried out on both trellised and non-trellised vineyards in close proximity to each other, while in 2019 it focused on trellised vines from the same vineyard (Table 1). Bush vines (goblet style) were planted at 2.1 m by 2.1 m spacing, while trellised vines were planted at

1.5 m vine spacing and 2 m row spacing. Trellising was a two wire, Vertical Shoot Positioning (VSP) training system with fruiting wires set at 1.5 m. Twelve vines were sampled from each vineyard (4 adjacent vines in 3 rows and randomly selected). The clones of the French varieties were not known and to date, no clones of the Cypriot varieties have been identified. All vines were on their own roots and not grafted onto any rootstock. All vines were spur pruned to two buds per spur. All vineyards were of similar clay, sandy loam soil type with sandstone rocks of various sizes in the soil profile.

2. Measurements

2.1. Climate

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 between 11 and16 km from the vineyard sites). Mean January Temperature (MJT) and Growing Degree Days (GDD) were calculated for the three seasons studied, as well as the long-term average (1955-2017) (Table 2).

Rainfall was highly variable over the three seasons. In 2017, total rainfall was 481 mm compared with the long-term average of 735 mm. In 2018, the total rainfall was 941 mm, with large falls recorded in January, May, June and December of that year.

TABLE 1. Details of vineyards used in this study

| Season	Code	Variety	Planted	Area (Ha)	Elevation (m)	Training	Latitude	Cultivate	Fertiliser	Sulphur application	Tip Pruning
2017	EX	Xynisteri	1970	0.4	840	Bush	34°86'N	*nil*	*nil*	May & June	June
2018								February	*nil*	May & July	June
2017	ZX	Xynisteri	1989	0.45	950	Bush	34°86'N	*nil*	*nil*	May & June	June
2018								February	*nil*	May & July	June
2017	MB	Maretheftiko	2007	1.27	740	Bush	34°78'N	*nil*	*nil*	May & June	June
2018								February	*nil*	May & July	June
2017	MT	Maratheftiko	2010	0.49	740	Trellis	34°78'N	*nil*	*nil*	May & June	June
2018								February	*nil*	May & July	June
2019	VX	Xynisteri	2013	0.25	795	Trellis	34°50'N	April	*nil*	May & June	May
2019	VM	Maratheftiko	2006	0.16	710	Trellis	34°49'N	April	*nil*	May & June	May
2019	VSB	Sauvignon blanc	2006	0.16	710	Trellis	34°49'N	April	May	May & June	May
2019	VShz	Shiraz	2008	0.6	690	Trellis	34°49'N	April	*nil*	May & June	May
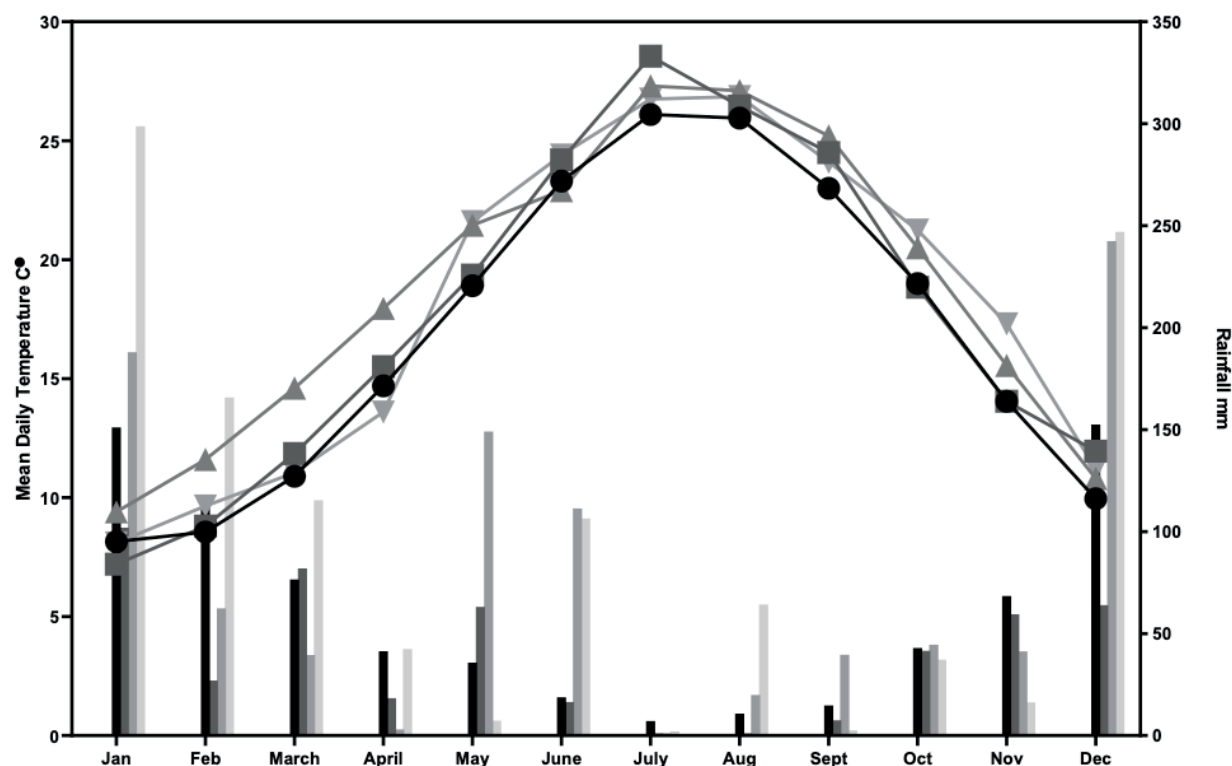
FIGURE 1. Mean daily temperature and rainfall per month for the three seasons studied compared to the long-term average.

Rainfall for each year is indicated by vertical lines; black line long term average, dark grey line 2017, medium grey line 2018, light grey line 2019. Temperature is indicated with a symbol and a horizontal line connecting symbols: black circle (●) long term average, dark grey square (■) 2017, medium grey upward pointing triangle (▲) 2018, light grey downward pointing triangle (▼) 2019.

TABLE 2. MJT and GDD for three seasons

Year	MJT (°C)	GDD	Total rain (mm)
Long-term average	26.1	2535	735
2017	28.5	2441	481
2018	27.3	2649	941
2019	26.8	2409	1105

In 2019, the total rainfall was 370 mm above the long-term average with large falls recorded in January, February, March, June, August and December (Figure 1).

2.2. Vine performance measurements

Vine performance measurements, such as shoot number, bunch number, bunches per shoot, shoot length, leaves per shoot, shoot diameter (at fourth internode), bunch length, bunch width, bunch weight and internode length (at fourth internode), were taken at flowering for all three seasons to avoid any concerns associated with tip pruning by the commercial vineyards. All the vines in the

study were pruned to approximately 30 buds per vine. Fruit weight per vine was recorded in 2017 and 2018 for the four vineyards; however, in 2019 only preharvest volume was available. Internode lengths were not available for the 2017 season.

2.3. Physiology measurements

In 2017 and 2018, physiology measurements were taken at flowering (EL-21), veraison (EL-35) and harvest (EL-38) (Coombe and Dry 1988). While in 2019, measurements were taken at flowering (EL-21), pea-sized berry formation (EL-30), veraison (El-35), mid-veraison (EL-36) and at harvest (El-38). In 2019, Sauvignon blanc only

received three periods of analysis and Shiraz only four, due to their earlier harvest date compared to Xynisteri and Maratheftiko.

A Skye SKPM1400 series Plant Moisture Vessel (Skye Instruments Ltd, Llandrindod Wells Powys, LD1 6DF, UK) was used to measure leaf water potential as described by Meron *et al.* (1987). Midday leaf 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. The leaf was collected from the midday sunlit side of the canopy.

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 was measured. A maximum of 60 seconds elapsed between cutting the leaves and 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 (De Bei *et al.*, 2011).

Leaf stomatal conductance (g) 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

Chlorophyll content was measured as described by Marquard and Tipton (1987) using a SPAD 502 Meter 2900 (Minolta Japan). Chlorophyll concentration per area was determined utilising radiation in the red and near-infrared wavelengths to derive a numerical value of chlorophyll in the leaf (Gonçalves *et al.*, 2009). Leaves were selected at the 4th node along the shoot and an average of three readings was recorded for each leaf.

2.4. Stomatal density

Stomatal density was determined by selecting leaves from seven 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 seven varieties sampled were Xynisteri, Maratheftiko, Shiraz, Sauvignon blanc, Semillon, Cabernet-Sauvignon and Chardonnay. The varieties chosen for this assessment were to be used for benchmarking Xynisteri and Maratheftiko when all grown in the same environment. Samples were taken from different vineyards over the 3-year period and mean values for each variety determined.

2.5. Statistical analysis

Data sorting and preparation was conducted with Microsoft Excel 2010 and analysed by one-way ANOVA using the statistical package XLSTAT (version 2019.4.2, Addinsoft SARL, Paris, France). Figures were prepared using GraphPad Prism 8.0.0 (224) for Windows (GraphPad Software, La Jolla California USA).

RESULTS AND DISCUSSION

1. Climate

The climate varied greatly over the 3-year study period. All three seasons were hotter than the long-term average supplied by the Cypriot Department of Meteorology (Table 2). In June and August in particular, rainfall had an impact on the growing season results (Figure 1).

Grape growing regions have traditionally been classified by various methods, such as Mean January/July Temperatures (MJT), Growing Season Temperature (GST), Growing Degree Days (GDD), Huglin Index (HI), Spring Index (SI), and Biologically Effective Degree Days (BEDD) (Coombe and Dry, 1988; Hall and Jones, 2010; Jarvis *et al.*, 2017; Cameron *et al.*, 2020). For this study MJT and GDD were used (Table 2), and when these classifications are applied to Cypriot vineyards, we can see that the area analysed for this study is very hot according to Coombe and Dry (1988). Webb *et al.* (2008) state that the optimum MJT, utilising the quality parameters (glycosyl-glucose, colour and price) for growing Cabernet-Sauvignon is 18.5 °C, Shiraz 19.1 °C and Ruby Cabernet 21.5 °C. The Cypriot varieties, however, are able to grow and produce medium to high yields, well outside of these optimum MJT values described in other studies. GDD also reflects this, with all three seasons and the long-term average GDD greater than 2,400 and approaching 2,700, which Hall and Jones (2010) consider the upper limit

for producing quality wine grapes in the western United States wine regions.

Wolkovich *et al.* (2017) reported on French varieties grown in Bordeaux and consider optimal ripening to occur between Day of Year (DOY) 200 and 245, with most varieties ripening around DOY 225. This can lead to the phenomenon of vintage/harvest compression that is currently occurring in many wine regions with warming climates (Jarvis *et al.*, 2018). The results from this study also highlight this. Harvest DOY (Table 3) for Xynisteri and Maratheftiko was between 270 and 280 for all three seasons. In 2019, Sauvignon blanc was harvested at DOY 222 and Shiraz at DOY 240. If wine producers in warm-hot wine regions were to adopt Xynisteri and Maratheftiko in place of some French varieties, this would greatly assist in reducing the logistical pressures associated with vintage/harvest compression. Later ripening of Xynisteri and Maratheftiko would also avoid high daytime and night-time temperatures during the later stages of ripening, considered as unfavourable for the expression of varietal characteristics due to the repression of key enzymes related to aroma synthesis (Rienth *et al.*, 2014).

The ability to ripen later, along with the capacity to grow in non-irrigated vineyards, means that it is possible to postulate that the indigenous Cypriot varieties are potentially well-adapted to coping with their current hot climates. This requires further investigation in future studies.

2. Vine performance measures

Vine performance measures for all varieties across the three seasons are summarised in Table 4. Xynisteri had the highest shoot number and yield per hectare, as well as the largest bunch volume or bunch weights, compared to Maratheftiko and the other varieties for all three seasons of this study. In 2019, the number of Xynisteri leaves per shoot was higher than for other varieties. Fruitfulness (bunch number per shoot; Dry *et al.*, 2010) was generally lower in Xynisteri than Maratheftiko and both Shiraz and Sauvignon blanc in 2019. In 2019, Maratheftiko had a larger bunch volume and overall yield than Shiraz and Sauvignon blanc.

Shoot number can be very difficult to compare as it depends on the pruning method applied. However, the vines in the study compared favourably with the literature, with shoot numbers per vine ranging from 10.4 (MT 2017) to 30.5 (VX 2019) see Table 4. This may suggest that Xynisteri has better bud viability than Maratheftiko, thus producing more shoots. While Maratheftiko had less shoots, they tended to be longer and with greater diameter, suggesting that the two varieties partition their reserves differently. This concurs with Miller *et al.* (1996) researching Chambourcin, who found that vines with more shoots had greater leaf area, shoot length and leaf number per vine,

TABLE 3. Key phenological development dates for the varieties and seasons studied.

Season	Code	Variety	Budburst	Flowering	Fruit-set	Veraison	Harvest
2017	EX	Xynisteri	25 March	5 June	16 June	10 August	21 September
2018			22 March	9 June	19 June	9 August	28 September
2017	ZX	Xynisteri	28 March	8 June	18 June	10 August	22 September
2018			25 March	12 June	20 June	9 August	29 September
2017	MB	Maratheftiko	15 March	3 June	17 June	10 August	19 September
2018			12 March	1 June	24 June	8 August	10 September
2017	MT	Maratheftiko	15 March	3 June	17 June	10 August	19 September
2018			12 March	1 June	24 June	8 August	10 September
2019	VX	Xynisteri	27 March	6 June	18 June	8 August	27 September
2019	VM	Maratheftiko	13 March	1 June	11 June	8 August	20 September
2019	VSB	Sauvignon blanc	6 April	15 June	20 June	5 July	11 August
2019	VShz	Shiraz	3 April	12 June	24 June	1 August	24 August

TABLE 4. Vine performance measures recorded during 2017, 2018 and 2019 seasons.

	Shoot No.	Bunch	Bunch	Shoot Length	Leaves per shoot	Shoot diam	Bunch length	Bunch width	Internode length	Yield per vine (kg)	Average bunch weight at harvest (g)	Bunch length at harvest (cm)	Bunch width at harvest (cm)	Bunch volume at harvest (cm³)
2017														
ZX	23.5a	30.0a	1.3b	116.2b	17.5a	1.0a	19.2a	8.7a	na	5.0a	166.6a	na	na	na
EX	20.4a	19.4b	1.0c	122.2b	20.2a	0.9bc	18.4a	6.7ab	na	2.5b	128.8b	na	na	na
MB	11.12b	21.6ab	1.9a	164.1a	18.0a	1.4a	15.4b	8.6a	na	1.7c	78.7c	na	na	na
MT	10.7b	18.7b	1.8a	124.8b	17.3a	1.2ab	12.6b	5.8b	na	1.6c	85.6c	na	na	na
Pr > F	< 0.0001	0.003	< 0.0001	0.003	0.13	< 0.0001	< 0.0001	0.002		< 0.0001	< 0.0001			
2018														
ZX	19.3a	18.8ab	1.1c	124.4a	17.3a	0.6bc	15.5b	6.3b	7.3a	4.6a	244.7a	na	na	na
EX	15.5ab	17.2ab	1.4b	115.6a	18.1a	0.5c	19.8a	9.0a	8.1a	2.9b	168.6b	na	na	na
MB	10.4c	14.6b	1.7a	143.4a	16.1a	1.0a	13.3b	7.0b	9.5a	1.8c	123.3c	na	na	na
MT	12.3bc	20.8a	1.0c	140.8a	17.1a	0.7b	12.8b	6.3b	8.9a	2.5b	120.2b	na	na	na
Pr > F	< 0.0001	0.04	< 0.0001	0.47	0.59	< 0.0001	< 0.0001	< 0.0001	0.12	< 0.0001	< 0.0001			
2019														
VX	30.5a	24.8a	0.8b	162.9ab	61.4a	0.9a	17.7a	9.7a	9.2b	na	na	20.9a	12.5a	871.3a
VM	20.6c	22.8a	1.1a	184.5a	25.1c	0.9a	13.8b	10.1a	13.5a	na	na	19.0a	11.4b	648.5b
VShz	24.3b	23.8a	1.0a	158.5bc	34.0c	0.5b	17.2a	5.8b	8.1b	na	na	19.9a	7.5c	295.3c
VSB	22.1bc	23.6a	1.1a	141.7c	46.5b	0.8a	10.5c	5.7b	9.1b	na	na	13.4b	7.6c	208.1c
Pr > F	< 0.0001	0.67	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001			< 0.0001	< 0.0001	< 0.0001

ZX, EX, VX-Xynisteri, MB, MT, VM- Maratheftiko, VShz-Shiraz, VSB-Sauvignon blanc
Different letters next to the measures indicate significant differences (p < 0.05), measures with the same letters are not statistically significant different.

but vines with fewer shoots had longer shoots, larger leaves, and greater leaf area and leaf number per shoot. When researching Shiraz/Syrah and Cabernet-Sauvignon stem starch reserves, Rustioni *et al.* (2019) demonstrated that water stress reduced the stem starch storage in Syrah, but Cabernet-Sauvignon was not affected. It is therefore possible that Xynisteri and Maratheftiko are more similar to Cabernet-Sauvignon than Shiraz in how they respond to drought conditions; that is, their mechanism for carbon assimilation and partitioning. This possibility requires further investigation.

Bunch number per shoot varied from 0.8 for Xynisteri (VX) in 2019 to 1.9 for Maratheftiko (MB) in 2017. Bunches per shoot in 2019 for all four varieties were similar and relatively low at 0.8-1.1. Xynisteri, however, was the lowest at 0.8. The bunch per shoot for all three seasons is nevertheless comparable with other studies: Freeman and Kliewer (1983) report non-irrigated Carignane with 1.5 bunches per shoot, while Guilpart *et al.* (2014) report Shiraz vines with between 1.3 and 2 bunches per shoot in their three-year study. In all three seasons, Xynisteri was less fruitful than Maratheftiko; that is, it had less bunches per shoot, but the bunches from Xynisteri tended to be larger. Xynisteri was also less fruitful than both Shiraz and Sauvignon blanc when grown in the same vineyard under the same environmental conditions. Further investigation is required to understand the reasons for such fertility differences between varieties.

Bunch number per vine in the study varied between seasons and varieties, ranging from 15.5 for MB to 30 for ZX in 2017. Bunch numbers for Xynisteri and Maratheftiko were similar to Shiraz and Sauvignon blanc in 2019; however, Xynisteri and Maratheftiko bunches were larger than the French varieties. In their study on the classification of reproductive performance of ten wine grape varieties grown on trellis in Australia, Dry *et al.* (2010) report that Shiraz and Sauvignon blanc averaged 28.5 bunches per metre of cordon.

This was similar to the 2019 trial with Shiraz 23.8 and Sauvignon blanc 23.6 bunches per metre of cordon.

Bunch weights for 2017 and 2018 were calculated using fruit weight per vine and bunches per shoot. Bunch sizes and yields per vine were lower in 2017 compared to 2018; this may have been due to the large difference in rainfall between these two seasons. Bunch weights ranged from 78.7 g for Maratheftiko (MB) in 2017 to 244.7 g for Xynisteri (ZX) in 2018. Xynisteri bunch weights were greater than Maratheftiko in all cases. Bunch weights were not available in 2019, but bunch volumes were calculated and Xynisteri (VX) had the largest volume (871.3 cm^3), followed by Maratheftiko (VM) (648.5 cm^3), Shiraz (VShz) (295.3 cm^3) and Sauvignon blanc (VSB) (208.1 cm^3) (bunch weights and yields were estimated using the vineyard owners' overall yield data for 2019, Table 7). The volume difference for Xynisteri and Maratheftiko was comparable to their weight difference in 2017 and 2018. Dry *et al.* (2010) also reported that Shiraz bunches were larger than Sauvignon blanc. This also demonstrates how much larger the two Cypriot varieties are when compared to both Shiraz and Sauvignon blanc grown under the same conditions.

The yield per hectare was estimated using vine density for each of the studied vineyards (Table 5). In 2017 and 2018, the Xynisteri vineyard, ZX, had greater yield per hectare than both Maratheftiko vineyards and the other Xynisteri vineyard. This is comparable to other non-irrigated studies with Guilpart *et al.* (2014) reporting yields of between 7.2 and 18.4 tonnes per hectare for non-irrigated and trellised Shiraz grown in southern France. While the region has similar annual rainfall to the test sites (750 mm), MJT of 22.6 is much lower and the planting density is similar (3333 vines/hectare). The lower MJT in France may be advantageous for higher yields when compared to the MJT of Cyprus. Intrigliolo and Castel (2009) investigating Tempranillo in Spain found that non-irrigated,

TABLE 5. Yield estimates tonne/hectare

Season	EX	ZX	MB	MT	VX	VM	VShz	VSB
2017	5.6	10.9	4.3	5.3				
2018	6.6	11.3	4.5	8				
2019					16.9	11	8.1	6.5

ZX, EX, VX-Xynisteri. MB, MT, VM-Maratheftiko, VShz-Shiraz, VSB-Sauvignon blanc.

trellised vines produced yields of between 4.5 and 14.1 tonnes per hectare over five years. This region, however, has less average rainfall (450 mm) and a lower MJT of 22.9, as well as a much lower planting density of 1666 vines per hectare. This would suggest that Xynisteri is capable of greater yields at a higher MJT when compared to other varieties grown in Western Europe with lower MJT and less dense vine spacing. Considering that the Cypriot vineyards are non-irrigated, the yields achieved in the hot environment are very promising and worth investigating in other hot climate regions of the world.

Shoot length ranged from a minimum of 115.5 cm for EX in 2018 to a maximum of 184.5 cm for MB in 2019. Maratheftiko shoot length and diameter were greater than for the other varieties in the study. Shavrukov *et al.* (2004) studied Chardonnay, Riesling, Exotic and Sultana and they reported shoot lengths ranging from 96.1 cm for Riesling to 113.3 cm for Sultana. Smart and Robinson (1991) state that there are no ideal values for shoot growth rate as it is highly variable and can depend on the variety and climate. Internode length ranged from 7.3 cm for Xynisteri (ZX) in 2018 to 13.5 cm for Maratheftiko (VM) in 2019. Utilising Smart and Robinson (1991) guidelines, this classifies Xynisteri and Maratheftiko as being moderate high vigour varieties when grown under these environmental conditions.

Bunch (inflorescence) length ranged from 10.45 cm for VSB in 2019 to 19.8 cm for EX in 2018. This compares with the study by Shavrukov *et al.* (2004), with inflorescence lengths ranging from 7.33 cm for Riesling to 27 cm for Exotic. Xynisteri and Maratheftiko bunches were larger and less compact than those of Shiraz and Sauvignon blanc. Both Maratheftiko and Xynisteri bunches have loose bunch architecture (Figure 2), which potentially has the advantage of reducing the risk of bunch rot (*Botrytis cinerea)* as reported by Molitor *et al.* (2014) in their study of Pinot gris and Riesling.

Internode length ranged from 7.3 cm for Xynisteri (ZX) in 2018 and 13.5 cm for Maratheftiko (VM) in 2019. Utilising Smart and Robinson (1991) guidelines, this classifies Xynisteri and Maratheftiko as being high vigour varieties when grown under these environmental conditions.

Overall, these results indicate that Xynisteri and Maratheftiko produce greater yields, bigger bunches and longer shoots than Shiraz and Sauvignon blanc in a hot climate. Beis and Patakas (2010) investigated indigenous Greek varieties Mavrodafni and Savatiano in Agrinio, Western Greece. Savatiano, a white variety, originating from a more arid environment was found to be more adapted to drought, while the red variety Mavrodafni was more sensitive to water stress. Agrinio has a climate more similar to Cyprus than other western European countries with an MJT of 25.9, but with more rainfall. They concluded that these two indigenous grapevine varieties may have evolved different drought adaptation strategies. They suggest that Savatiano may regulate stomatal closure more

FIGURE 2. Loose bunch architecture of Xynisteri (left) and Maratheftiko (right).

efficiently, while Mavrodafni displays greater chemical signalling (via nitric oxide in catalase up-regulation) (Beis and Patakas, 2010). These strategies may also warrant investigation for the Cypriot varieties in future studies.

3. Physiology measurements

3.1. Stem water potential (SWP)

Differences in midday leaf water potential were observed between varieties in all three seasons and at all sampling dates. Xynisteri (ZX) had the highest SWP in 2017 and 2018 (Figures 3a and 3b), while Xynisteri (VX) had the highest SWP in 2019, followed by Maratheftiko (VM), Shiraz (VShz) and Sauvignon blanc (VSB). Leaf water potential measurements concluded early for Sauvignon blanc (early August) and Shiraz (late August) in 2019, because of the earlier harvest (Figure 3c).

Viticulturists commonly use SWP to determine when to irrigate vines. Girona *et al.* (2006) defined SWP irrigation thresholds of -0.8 MPa for well-irrigated vines, -1.2 MPa for moderately stressed vines, and -1.5 MPa for severely stressed conditions. ZX had the highest SWP in 2017 and 2018 at all time points. Xynisteri also had the highest SWP in 2019 followed by VM, VShz and VSB. The poorest performing variety was VSB, ranging from -0.54 in early June to -0.99 in early August prior to harvest (VX at the same time was -0.8). At all times during the study, none of the Xynisteri or the Maratheftiko had an SWP below -1.2, thus they were only classed as moderately stressed according to the Girona *et al.* (2006) classification. These two varieties were also the last to be harvested in all three seasons: Maratheftiko in late September and Xynisteri in late September to early October.

Gonçalves *et al.* (2009) grew Touriga Franca in Portugal in the seasons 2004, 2005 and 2006, and they reported a mean midday SWP of -1.16 at veraison and between -1.33 and -1.56 at ripeness/harvest. Theodorou *et al.* (2013) studied dry grown Shiraz, Grenache, Xinomavro and Agiorgitiko in Greece in 2012 and reported mean SWP for non-irrigated vines as -2.10 for Grenache, -1.75 for Shiraz, -1.52 for Xinomavro and -1.56 for Agiorgitiko. The SWP results produced by non-irrigated Xynisteri and Maratheftiko were more similar to the results achieved by other studies using deficit irrigation (Koundouras *et al.,* 2009), in which 150 mm of irrigation during the growing season was applied and SWP values of between

-0.91 and -0.98 were obtained, as well as values ranging from -1.28 to -1.39 in non-irrigated vines. Theodorou *et al.* (2019) used deficit irrigation (50 % of evapotranspiration) and observed a SWP of between -1.18 and -1.21. The results from these studies, however, also showed that fully irrigated vines produce better SWP relative to non-irrigated Xynisteri and Maratheftiko.

It can therefore be suggested that in a dry grown environment, Xynisteri and Maratheftiko are potentially more capable of maintaining adequate SWP during the growing season, and late in the growing season long after Shiraz and Sauvignon blanc have been harvested.

3.2. Leaf stomatal conductance (SC)

Tomás *et al.* (2014) state that SC is commonly used to estimate the leaf Water Use Efficiency (WUE) of vines, as well as whole plant WUE. However, they also report that whole plant WUE estimates have discrepancies when attempting to scale up from leaf SC. In the present study all varieties exhibited a decreased rate of conductance throughout the season, as is to be expected with decreasing soil moisture throughout the summer season. Chaves *et al.* (2010) suggest that dramatic reductions in plant carbon assimilation may occur due to a severe decline in photosynthesis in Mediterranean environments, where temperatures and water deficits increase in parallel from spring to summer. However, in the present study, SC increased for Xynisteri in July of 2019. This may have been due to a large amount of rain (106 mm) that occurred in late June a few days prior to the scheduled testing. Overall mean SC ranged from 40.4 mmol/m²/s for EX prior to harvest in late September 2017 and 435 mmol/m²/s for ZX at flowering in early June 2018. These variations could be explained by the weather extremes across the three seasons. The driest season, 2017, had a total annual rainfall 146 mm below the long-term average, while in 2018, May and June received 259.4 mm of rain, which is 204.8 mm above the long-term average for those two months. In 2019, Maratheftiko showed the greatest resilience in terms of maintaining SC: mean SC ranged from 363 mmol/m²/s in early June to 194 mmol/m²/s in late September. Meanwhile, Xynisteri ranged from 303 mmol/m²/s in early July to 121 mmol/m²/s in late September. Shiraz performed in a similar way to Xynisteri with SC ranging from 362 mmol/m²/s in early June to 188 mmol/m²/s in late August. Mean SC for Sauvignon blanc ranged from 272 mmol/m²/s in early June to 182 mmol/m²/s in early August.
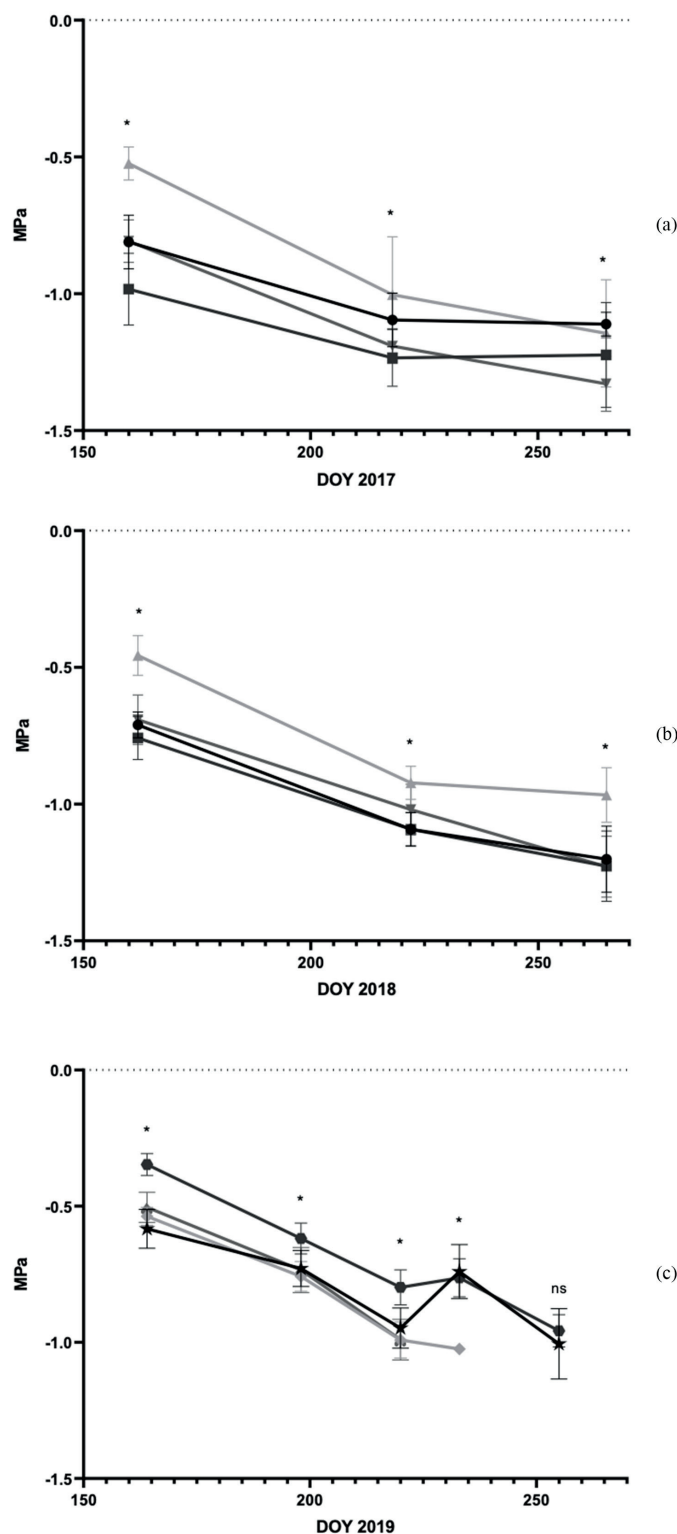
FIGURE 3. Relationship between midday stem water potential measurements (MPa) versus days of year (DOY).

(a) season 2017 ;

(b) season 2018, black circle (●) Maratheftiko Trellis (MT), dark grey square (■) Maratheftiko Bush (MB), light grey upward pointing triangle (▲) Xynisteri Z (ZX), medium grey downward pointing triangle (▼) Xynisteri E (EX) ;

(c) season 2019, black hexagon (⬢) Xynisteri (VX), black star (★) Maratheftiko (VM), dark grey spade (♠) Sauvignon blanc (VSB), Light grey diamond (♦) Shiraz (VShz). Statistically significant values are represented by: ns = not significant, * significant at P < 0.05
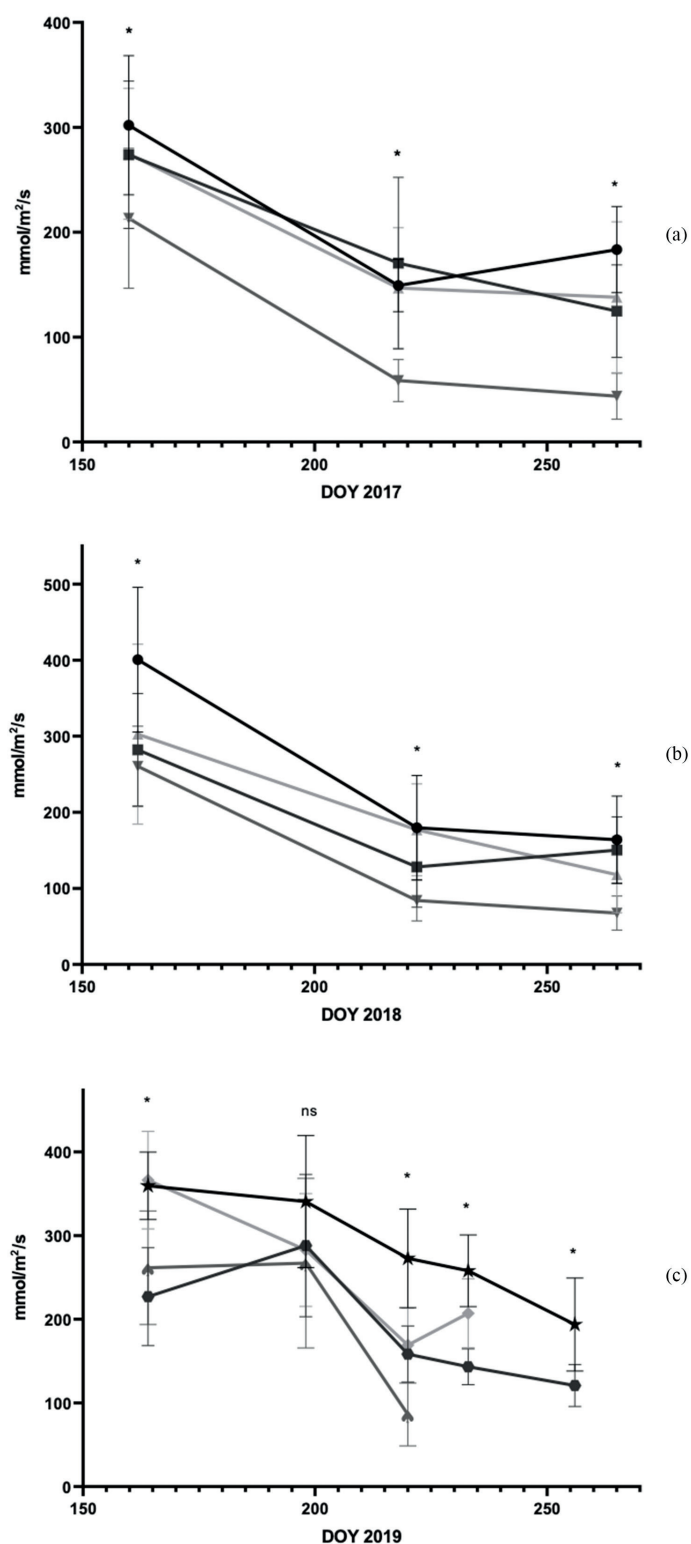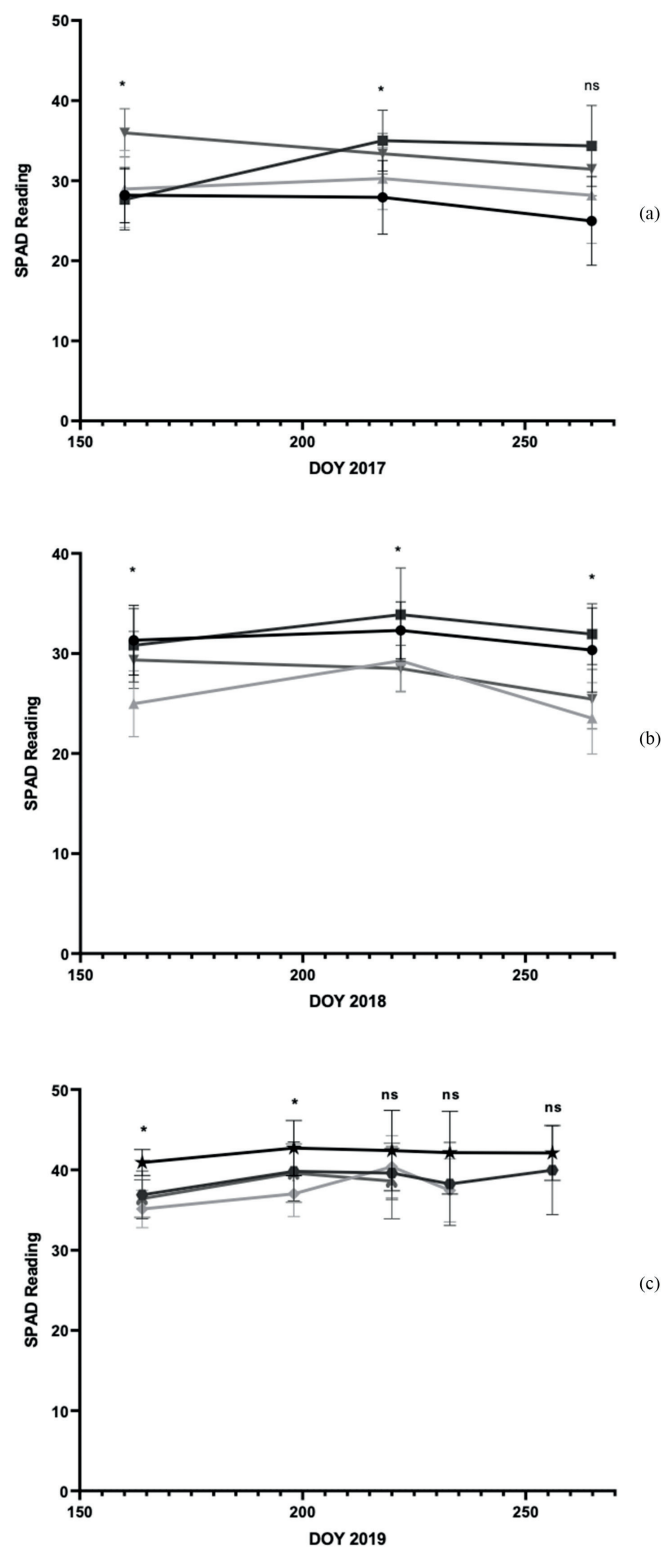
FIGURE 4. Stomatal conductance measurements at midday (mmol/m^2/s) versus days of year (DOY).

(a) season 2017 ;

(b) season 2018, black circle (●) Maratheftiko Trellis (MT), dark grey square (■) Maratheftiko Bush (MB), light grey upward pointing triangle (▲) Xynisteri Z (ZX), medium grey downward pointing triangle (▼) Xynisteri E (EX) ;

(c) season 2019, black hexagon (⬣) Xynisteri (VX), black star (★) Maratheftiko (VM), dark grey spade (♠) Sauvignon blanc (VSB), Light grey diamond (♦) Shiraz (VShz). Statistically significant values represented by: ns = not significant, * significant at P < 0.05.

FIGURE 5. SPAD reading at midday for chlorophyll content versus days of year (DOY).

(a) season 2017 ;

(b) season 2018, black circle (●) Maratheftiko Trellis (MT), dark grey square (■) Maratheftiko Bush (MB), light grey upward pointing triangle (▲) Xynisteri Z (ZX), medium grey downward pointing triangle (▼) Xynisteri E (EX) ;

(c) season 2019, black hexagon (⬢) Xynisteri (VX), black star (★) Maratheftiko (VM), dark grey spade (♠) Sauvignon blanc (VSB), Light grey diamond (♦) Shiraz (VShz). Statistically significant values represented by: ns-not significant, *- significant at P < 0.05.

The SC values from the study are comparable with the literature. Tomás *et al.* (2014) reported the mean SC for 74 varieties ranging from 40 mmol/m²/s for a white grape from Greece called Rosaki, to more than 600 mmol/m²/s for the Iranian table grape, Sefid Bidaneh cv (Aslanpour *et al.*, 2019). For non-irrigated Cabernet-Sauvignon grown in Greece, Koundouras *et al.* (2009) report an SC of 120 to 400 mmol/m²/s. In Portugal, Touriga Franca vines had an SC ranging from 103.5 to 784.6 mmol/m²/s during the period of ripeness to harvest (Gonçalves *et al.*, 2009), and in August (at harvest) when vines had overripe fruit, Semillon and Muscat blanc had an SC ranging from 230.2 to 347.4 mmol/m²/s (Dinis *et al.*, 2014). Studying Shiraz, Grenache, Xinomavro and Agiorgitiko in Greece, Theodorou *et al.* (2019) reported non-irrigated vines as having a mean SC of 40 mmol/m²/s for Grenache, 50 mmol/m²/s for Agiorgitiko, 90 mmol/m²/s for Shiraz and 150 mmol/m²/s for Xinomavro. As in the case of LWP, the results of SC for Xynisteri and Maratheftiko were more closely comparable to the results of deficit irrigation trials (Koundouras *et al.*, 2009; Theodorou *et al.*, 2019), but not as favourable as fully irrigated trials.

While SC is very dependent on soil water content/ status, and therefore affected by the climate of the particular season, we can conclude that all the Xynisteri and Maratheftiko vineyards were able to maintain SC across the growing period from early June to late September for all three seasons. In contrast, relative to the indigenous varieties, Shiraz and particularly Sauvignon blanc SC were impacted more severely throughout the 2019 season.

3.3. Chlorophyll Content

Steele *et al.* (2008) state that SPAD readings are adequately sensitive at around 35 (approximately 300 mg/m²) and their research demonstrated that grapevine leaves can have SPAD values of between 7 and 44 (63 to 576 mg/mm²). Taskos *et al.* (2014) concur with this, stating that SPAD meters were useful in assessing chlorophyll content and nitrogen in grape leaves; however, their results varied depending on variety, vineyard, phenology and canopy structure.

In July and August 2017, and for all time points in 2018, Maratheftiko had greater chlorophyll content than Xynisteri (Figure 5a and b); while in June and July 2019, Maratheftiko had greater chlorophyll content in June and July than Xynisteri, Sauvignon blanc and Shiraz

(Figure 5c). All measures values corresponded to the limits found by Steele *et al.* (2008) and Brunetto *et al.* (2012). Therefore, as chlorophyll concentration has been positively correlated with the rate of photosynthesis in other varieties (Lebon *et al.*, 2005), these findings suggest that Maratheftiko may have a greater photosynthetic capacity than the other plants.

Soil types in the wine growing regions of Cyprus are high in chalk, limestone and gypsum, thus having high calcium levels (Ladegaard-Pedersen *et al.*, 2020). Sabir *et al.* (2014) report that highly cultivated soil with high calcium levels can have a high pH, which in turn leads to a decrease in chlorophyll content. The fact that Maratheftiko, and to a lesser extent Xynisteri, have a higher chlorophyll content than Shiraz and Sauvignon blanc in such high calcium soils could indicate that they have adapted to cope with these soil types, therefore producing abundant chlorophyll for photosynthesis. For example, Cambrollé *et al.* (2014) demonstrated that a wild grapevine (*Vitis vinifera* ssp. *sylvestris*) was highly tolerant to lime stress and they determined that the exposure to very high calcium carbonate levels (60 %) induced nutrient imbalances and significantly inhibited photosynthetic function. This caused an overall reduction in carbon gain, high mortality, and a drastic reduction in the growth of the surviving plants. However, high to moderate (40- to 20 %) levels of calcium carbonate did not greatly affect the concentrations of iron, nitrogen, phosphorous and potassium in plant tissues. In addition, plant growth and photosynthetic function were also not drastically affected with these treatments. Future studies of Maratheftiko in particular, could explore this possibility further.

4. Stomatal density

Stomatal densities for Xynisteri ranged from 245 to 260/mm² and were higher than all other varieties in every season. Maratheftiko stomatal densities ranged from 215 to 235/mm² across the three seasons and were higher than the French varieties, apart from Shiraz in 2019 (Table 6). Semillon and Sauvignon blanc had the lowest densities of those studied (Table 6).

Gómez-del-Campo *et al.* (2015) state that the limits of stomatal density may be within 129-254/mm². However, in a later study, stomata density for the Greek variety Xinomavro was 280/mm² (Theodorou *et al.*, 2013). Rogiers *et al.* (2011a) believe from their

TABLE 6. Stomatal density (number of stomata per mm^2)

Variety	2017	2018	2019
Xynisteri	245.1a	252.7a	260.8a
Maratheftiko	215.4b	234.9a	230.2b
Shiraz		201.1b	213.2b
Cabernet Sauvignon		193.9b	
Chardonnay		171.8c	
Semillon		133.9d	
Sauvignon blanc			129.6c
Pr > F	< 0.0001	< 0.0001	< 0.0001

observations of Chardonnay that a stomata density of 320/mm^2 may represent the upper limits for *Vitis vinifera.* They also state that vines with limited water supply may have a lower stomatal density than vines with a non-limited water supply. Hopper *et al.* (2014), however, disagree, stating that Shiraz is less susceptible to the effects of water deficit than Cabernet-Sauvignon, despite having a lower stomatal density in their study.

The Cypriot varieties had high stomatal densities ranging from 215 to 261/mm^2 which compare more closely to varieties from warm climates; Examples include Trebbiano grown in Tuscany withhaving a stomata density of 205/mm^2 (Palliotti *et al.*, 2000), Trincadeira grown in Portugal with 250/mm^2 (Monteiro *et al.*, 2018), Malbec grown in a glasshouse (two air temperature regimes, high 45/22 °C and control temperature 35/20 °C) with 247/mm^2 (Galat Giorgi *et al.,* 2019), and several Portuguese varieties with between 200 and 250/mm^2 (Teixeira *et al.,* 2018). The results observed for Cabernet-Sauvignon, Chardonnay, Shiraz, Semillon and Sauvignon blanc are similar to those described in other studies (Rogiers *et al.,* 2009; Dinis *et al.,* 2014; Gonçalves *et al.,* 2009; Rogiers *et al.,* 2011b). No previously published data exists for stomatal density of Maratheftiko and Xynisteri, but indigenous varieties of neighbouring countries Greece and Turkey show they have similar stomatal density. The Greek varieties, Agiorgitiko and Xinomavro, had between 218-280/mm^2 (Theodorou *et al.*, 2019), and indigenous Turkish varieties ranged from 129 to 254/mm^2 (Eris and Soylu, 2015).

The leaves used to estimate stomatal density were collected in the first week of June for all three seasons. Interestingly, in 2018 and 2019, mean May temperatures were well above the long-term average of 28.3 °C and 29.3 °C (Figure 1).

This could explain why the stomatal densities for Xynisteri and Maratheftiko were higher at these times. Also leaf samples in 2017 and 2018 were taken from different vineyards than 2019. Rogiers *et al.* (2011a) demonstrated this effect with Chardonnay vines sampled in warmer climates.

Stomatal density, however, is not always directly related to the mechanism of drought tolerance. Boso *et al.* (2011) believe that the high stomatal density of Albarinho may be responsible for its high performance in the field, as it has an increased photosynthetic capacity. Xu and Zhou (2008) studied the grass, *Leymus chinesis,* and observed that a moderate water deficit led to an increase in stomata density, but severe water deficits led to an overall decrease in stomata density. Observations of drought causing an increase in stomatal density in some varieties and a decrease in other varieties imply that these observed differences in the anatomical response to drought among grape varieties could be associated with different adaptation strategies to water limitation (Theodorou *et al.*, 2013). Soar *et al.* (2006) suggest that one such strategy for coping with water limitation is root structure, concluding that drought tolerance is related to vine vigour and that varieties which have high vigour have the most extensive root systems. Some preliminary soil pits dug for this study showed that Xynisteri has a greater root density than other varieties; however, this information was not available for all the studied vineyards. Assessment of the root systems of Xynisteri and Maratheftiko is an area that requires further investigation. This study has found that Xynisteri and Maratheftiko have higher vigour than Shiraz and Sauvignon blanc when grown under the same environmental conditions, and, as such, could potentially have a larger root system, thus allowing these varieties to cope with drought, rather than relying on stomatal conductance alone.

In their research into prolonged drought stress, Gerzon *et al.* (2015) describe Grenache as isohydric and Shiraz as anisohydric. Isohydric vines are able to maintain constant low water potentials through rapid stomatal closure, while anisohydric vines only close stomata at very low water potentials (Gerzon *et al.*, 2015). These two functions, however, are not always distinguishable. Plants that are considered anisohydic, may show reduced stomatal conductance under certain conditions (Beis and Patakas, 2015). Rogiers *et al.* (2011b) and Chaves *et al.* (2010) concur by stating that the distinction between isohydric and anisohydric plants is not always clear, and that they may be able to switch between strategies depending on drought severity and environmental conditions. It was not within in the scope of this study to determine whether Xynisteri and Maratheftiko utilise isohydric or anisohydric strategies to cope with drought, but when the results are compared to that of Gerzon *et al.* (2015), who studied Grenache and Shiraz, we can posit that they are anisohydric. Further research is currently underway to investigate this.

CONCLUSION

From the data presented it can be concluded that the indigenous Cypriot varieties Maratheftiko and, in particular, Xynisteri are well adapted to a hot climate, continuing to perform well as the climate becomes hotter. Xynisteri and Maratheftiko achieve budburst earlier and are ready for harvest later than Shiraz and Sauvignon blanc, which could be advantageous for reducing harvest compression in hot climates and for promoting better wine quality.

Xynisteri had the greatest stomatal density, more shoots, more leaves, bigger bunches, higher yields, the highest leaf water potential at harvest and stomatal conductance equal to Maratheftiko, while both had greater stomatal conductance than Shiraz and Sauvignon blanc. Maratheftiko had the longest shoots and the largest shoot diameter, as well as the greatest chlorophyll content out of all four varieties. Xynisteri and Maratheftiko are classed as moderate to high vigour varieties. The higher yields and vigorous growth without irrigation of these Cypriot varieties indicate that they have potential to outperform other varieties in hot viticulture regions.

The purpose of this study was to provide a baseline understanding of the performance of Xynisteri and Maratheftiko, in comparison to each other and to Shiraz and Sauvignon blanc. This study has highlighted several positive aspects of Xynisteri and Maratheftiko performance, which warrant further investigation for their use in hot dry climates elsewhere and in comparison with other drought tolerant wine grape varieties.

A limitation of the study was that the vineyards were not all in precisely the same location, and there may be possible influences from other factors, such as the training system applied and soil water holding capacity. Therefore, the results are somewhat indicative and must be viewed with a degree of caution. Further studies utilising these four varieties under controlled conditions are currently being undertaken to eliminate the possibility of these confounding influences.

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