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The Suitability of Indigenous Cypriot Grape Varieties to Viticulture and Oenology in Australia

by

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Abstract

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. The majority of vineyards and wineries base their businesses on European grape varieties that traditionally do not have problems with water resources. This has led countries to investigate 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, producers have been seeking potential drought tolerant varieties 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 with a recent upsurge in interest and research into heat and drought tolerance and a return to cultivation of their indigenous varieties. To date there are at least 12 indigenous varieties that have been identified in Cyprus, but recent research indicates there could be more varieties and numerous clones in their germplasm.

The aims of this project were to investigate the potential of two indigenous grape varieties from Cyprus for use in Australian viticulture and oenology. They objectives to meet these aims included: (1) generate sensory and chemical profiles of commercial Cypriot wines made from the white grape Xynisteri and the red grapes Maratheftiko and Giannoudhi compared to Australian Shiraz, Pinot Gris and Chardonnay wines, (2) assess the Australian consumers' response to these wines, (3) investigate five potent thiols in Xynisteri, Maratheftiko, Giannoudhi, Pinot Gris, Chardonnay and Shiraz wines, (4) formulate a baseline understanding of the performance of the indigenous Cypriot white grape Xynisteri and the red grape Maratheftiko (*Vitis vinifera* L.), (5) compare these varieties to Shiraz and Sauvignon Blanc grown in a Cypriot vineyard, (6) assess the response of Xynisteri to different irrigation regimes and (7) compare the performance of Xynisteri, Maratheftiko, Shiraz and Sauvignon Blanc grown in pots with different irrigation regimes in Australia and Cyprus.

This research has addressed the seven aims by providing new knowledge on several aspects of Xynisteri and Maratheftiko grapevines and their wines. They include, the chemical composition and sensory attributes of wines made from these varieties. Consumers have demonstrated a liking for the wines and in some cases preferred these wines to wine made from more common varieties. Xynisteri was described sensorially as citrus, herbaceous, dried fruit, savoury, apple, pear, grass,

herbaceous with a full length of fruit and non-fruit flavours in the after taste. Maratheftiko was described sensorially as dried fruit, strawberry, cherry, jammy, confectionery, bitter, sweet, chocolate, herbaceous and with full length of fruit flavours in the after taste. For the first time, chemical analysis supported this sensory analysis with aroma compounds correlating to chemical compounds responsible for these aromas and tastes.

Varietal thiols are important compounds in certain varieties when fruity, tropical and citrus aromas are desired. This study measured the concentration of varietal thiols in these Cypriot wines and the concentration determined in these wines was comparable to those found in popular Australian wines such as Chardonnay and Sauvignon Blanc.

Xynisteri and Maratheftiko growing in non-irrigated vineyards in Cyprus were bench marked against more commonly grown varieties for the first time. Along with this, irrigation trials in Australia and Cyprus compared the vine growth response to different irrigation regimes and highlighted that the Cypriot varieties were better suited to heat and drought stress than more commonly grown varieties due to their stomatal density and stomatal conductance assisting in managing midday stem water potential under heat and drought stress. Xynisteri in particular, was able to produce large above and below ground biomass under all irrigation conditions. Maratheftiko achieved large above ground biomass also but less below ground biomass than Xynisteri. However, both Xynisteri and Maratheftiko had total biomass greater than Shiraz and Sauvignon Blanc.

This research has identified several aspects of the Cypriot varieties Xynisteri and Maratheftiko that may make them suitable for cultivation in Australia. Consumer trials indicated acceptance of wines made from these varieties, highlighting potential marketing opportunities to target markets. It has also provided information that will guide future research in terms of how these varieties perform in Australian commercial vineyards and the mechanisms by which they achieve their drought resilience.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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List of Publications

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Copper, A. W., Karaolis, C., Savvides, S., Bastian, S., Johnson, T., Koundouras, S. & Collins, C. (2020). **Vine performance benchmarking of indigenous Cypriot grape varieties Xynisteri and Maratheftiko.** OENO One, 54(4), 935–954.

Copper, A. W., Collins, C., Bastian, S. E. P., Johnson, T. E., & Capone, D. L. (2021). **Preliminary investigation of potent thiols in Cypriot wines made from indigenous grape varieties Xynisteri, Maratheftiko and Giannoudhi.** OENO One, 55(1), 223–234.

Copper, A. W., Koundouras, S., Bastian, S. E. P., Johnson, T. E. & Collins, C (2022). **Assessing the Response of *Vitis vinifera* L. cv. Xynisteri to Different Irrigation Regimes and Its Comparison to cvs. Maratheftiko, Shiraz and Sauvignon Blanc**. Agronomy, 12, 634.

Copper, A. W., Scharfbillig, R., Nguyen, T. P., & Collins, C. (2021). **Identifying lower limb problems and the types of safety footwear worn in the Australian wine industry: a cross-sectional survey**. Journal of Foot and Ankle Research, 14(1), 1-10.

Chapter 1. Introduction

1.1 Introduction

The changing climate of Australia is putting great pressure on the resources for sustainable viticulture. Many vineyards and wineries base their businesses on European grape varieties that traditionally do not have problems with water resources. It is therefore necessary for the Australian wine industry to investigate grape varieties that are indigenous to hot climates similar to Australia. The eastern Mediterranean island of Cyprus is one such place with 12 indigenous grape varieties that grow well in a hot climate with no irrigation.

Two of these varieties, the white grape Xynisteri and the red grape Maratheftiko, were chosen for this study due to their reported anecdotal drought tolerance. The vines were studied in Cyprus in vineyards and in pots to assess their phenological characteristics and their physiological responses to drought and heat stress. Wines of these varieties were assessed chemically and sensorially to determine their organoleptic attributes and the corresponding chemistry. Australian consumer response to these wines were also assessed to examine the potential acceptance of wines produced from these varieties in the Australian market.

Vines were imported into Australia from Cyprus in 2018 and underwent a quarantine period along with disease testing. Plant material was than propagated to allow potted irrigation trials to occur to compare to results of the Cypriot experiments. Finally, plant material has been propagated for future vineyard trials.

1.2 Objectives of the research

The objectives of this project were to investigate the potential of two indigenous grape varieties from Cyprus for the use in Australian viticulture and oenology. They included; (1) generate sensory and chemical profiles of commercial Cypriot wines made from the white grape Xynisteri and the red grapes Maratheftiko and Giannoudhi, (2) assess the Australian consumers' response to these wines relative to main stream varieties, (3) investigate five potent thiols in Xynisteri, Maratheftiko, Giannoudhi, Pinot Gris, Chardonnay and Shiraz wines, (4) formulate a baseline understanding of the performance of the indigenous Cypriot white grape Xynisteri and the red grape Maratheftiko (*Vitis vinifera* L.), (5) compare these varieties to Shiraz and Sauvignon Blanc grown in a Cypriot vineyard, (6) assess the response of Xynisteri to different irrigation regimes and (7) compare the performance of Xynisteri, Maratheftiko, Shiraz and Sauvignon Blanc grown in pots with different irrigation regimes in Australia and Cyprus.

1.3 Linking statement

The research presented in this thesis is ordered into chapters. The first chapter is an introduction to the topic followed by chapter 2 which presents a literature review of aspects pertinent to this study. Following this are four research chapters. These chapters are presented as manuscripts that have been published and are presented in the format required by the respective journals. The presentation of the individual manuscripts in this thesis reflect the timeline and evolution of this project.

Chapter 1 is a general introduction to the topic, its importance and indicates the novel addition this research makes to the field of viticultural science, oenology and sensory science.

Chapter 2 contains a thorough literature review which broadly addresses the past and present status of research in the various fields presented in this thesis. Since few studies have dealt with Cypriot grape varieties directly, the review also contains studies from other regions and their indigenous varieties.

Chapter 3 is a published paper that chemically assessed wines made from three Cypriot grape varieties and sensorially compared them to Australian wines. These wines were also assessed by consumers for their levels of liking. The paper is titled, 'Preliminary sensory and chemical profiling of Cypriot wines made from indigenous grape varieties Xynisteri, Maratheftiko and Giannoudhi and acceptability to Australian consumers.

Chapter 4 is a published paper that involved further chemical analysis of the same wines to identify and quantify the levels of aroma compounds, the polyfunctional thiols. The paper is titled, 'Preliminary investigation of potent thiols in Cypriot wines made from indigenous grape varieties Xynisteri, Maratheftiko and Giannoudhi'.

Chapter 5 is a published paper that assessed the phenology and physiology of Xynisteri and Maratheftiko and compared them to Shiraz and Sauvignon Blanc. The paper is titled, 'Vine performance benchmarking of indigenous Cypriot grape varieties Xynisteri and Maratheftiko'.

Chapter 6 is a published paper that assessed the effects of different irrigation regimes on Xynisteri in a Cypriot vineyard and potted irrigation trials of Xynisteri, Maratheftiko, Shiraz and Sauvignon Blanc in Cyprus and Australia. The paper is titled, 'Assessing the response of *Vitis vinifera* L. cv. Xynisteri to different irrigation regimes and its comparison to cvs. Maratheftiko, Shiraz and Sauvignon Blanc.'

Chapter 7 is a general discussion of the results reported in this thesis. This discussion combines the entire body of research through all previous chapters. Highlighted in the discussion are key findings, their implications and limitations encountered throughout this study. In addition, the general discussion outlines possible avenues for future research that would both complement the present study and make further advancements in relation to cultivation and wine making in Australia. These areas of investigation are considered independently and have been presented as such in the form suitable for publication in respective journals.

Appendix section was an additional study that surveyed Australian wine industry workers for the prevalence of lower limb injuries and the types of safety footwear worn. It builds on past master's thesis work in the fields of podiatry, biomechanics and occupational injuries. While conducting research in vineyards and wineries across Australia and Europe, anecdotal evidence of injuries and poor footwear emerged and a survey to investigate these issues in Australia was devised. The paper has been published and is titled, 'Identifying lower limb problems and the types of safety footwear worn in the Australian wine industry: a cross-sectional survey'.

Chapter 2. Background and Literature review

This literature review will focus on several main themes that emerged in the current literature. These themes relate to climate issues, drought, water resources, irrigation, grape varieties currently grown in Australia and Cyprus, alternative varieties, sensory attributes and consumer response to alternative varieties.

2.1 Climate issues in Australia

The observed climate in Australia and the rest of the world is undergoing rapid change. In 2019, the Australian climate was 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 Meteorology, 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).

These changes have made observable impacts on viticulture. Trends to earlier harvest date have been detected in 43 of 44 Australian vineyard blocks studied (Webb *et al.,,* 2013). These trends are partly due to warming climates, but also due to reduced water availability. Jarvis *et al.,,* (2018) report that unusually warm and dry spring conditions have been linked to earlier budburst, with a more rapid rate of growth and development for the remainder of the growing season, regardless of later temperatures.

Webb *et al.,,* (2013) say further climate change is expected over the coming decades. For most locations the best estimate of mean warming over Australia by 2030 is 0.7-0.9°C in coastal areas and 1-1.2°C inland. Annual precipitation is estimated to decrease by 2.5 to 5% in most regions of Australia except the northwest but could be as high as a 10% decrease in the south-west in winter and spring. Remenyi *et al.,* (2019) agree stating that hotter average temperatures, hotter summers, longer heatwaves, more frequent bush fires and changes to rainfall intensity and seasonality have already had impacts across the country, and these trends are expected to continue. Rapid and ongoing climate change has the potential to affect all aspects of the wine industry, including vineyard performance, pest and disease incidence, wine quality and market competitiveness. For example, they predict that the Barossa Valley region will become 1.3°C hotter and more arid in the next 20-30 years (Remenyi *et al.,* 2019).

Objectives to assist the wine industry in mitigating and adapting to these changes in climate include establishing adaptation scenarios for major wine regions based on changes to phenology and temperature tolerance of major varieties and future water demand and availability. Specifically evaluating the genetic diversity of the *Vitis* species through better suited *V. vinifera* varieties and clones, could be the first step to reducing the impacts of a warmer climate. Planting longer season varieties to achieve ripening at a desirable part of the season is another option. Also breeding among the *V. vinifera* varieties or outcrossing with other *Vitis* species could be undertaken to produce better adapted progeny, as in some regions, in the future, selection from existing varietal stock may not be adequate to fully avoid the negative impacts of climate change. One way to moderate the impacts of and adapt to climate change is to graft over or completely replant vineyards to grapes more closely adapted to the new climatic and weather conditions to produce grapes for premium wines (Mozell and Thach 2014). That is, wine grape cultivars better suited to the projected climate conditions need to be selected or alternatively climatically optimum sites need to be identified for growing particular cultivars (Webb 2011).

2.2 Limitations of current varieties

In 2021, the top three red varieties harvested in Australia by volume were Shiraz, Cabernet Sauvignon and Merlot, together accounting for 84% of the total red variety harvest. Among the white varieties, Chardonnay remains dominant with 45% of the total white variety harvest, Sauvignon Blanc 12% and Pinot Gris/Grigio 11% (Wine Australia Vintage report 2021). Therefore the 6 top wine varieties cultivated in Australia are French and come from a climate zone that is not traditionally hot and dry like the majority of Australia.

Lereboullet *et al.,* (2013) compared sites in France (Roussillon) and Australia (McLaren Vale). While they have similar homo-climes, the big difference is rainfall with on average the French region receiving almost 150mm more rain. Consequently, under Australian conditions, these French varieties must be irrigated for their survival and optimum production. It is predicted that by 2050 the harvest of Cabernet Sauvignon in Coonawarra could be 45 days earlier and Chardonnay 39 days earlier. Climate modelling in other wine growing regions has also shown that Chardonnay and Cabernet Sauvignon are likely to experience significant phenological changes. It is predicted that budburst could be 3 to 10 days earlier, harvest 8-27 days earlier and the harvest duration 4 to 20 days shorter (Webb *et al.,* 2007, Goodwin *et al.,* 2002). This can have a detrimental impact on the quality of the grapes with increasing sugar and decreasing acid levels and reduced anthocyanins and flavonoids. Increased temperatures can also disrupt the anthocyanin to sugar ratio in Shiraz and Cabernet Franc berries, with consequences for colour and alcohol balance in wine (Sadras and Moran 2012). Chardonnay vines have been shown to be influenced by increased temperatures at budburst, with a reduction in flower numbers of between 15 and 25% (Petrie and Clingeleffer 2005).

It is therefore necessary to find varieties better adapted to the changing climate of Australia.

2.3 Water use of current varieties

The amount of water used for irrigating Australian vineyards in 2019-20 was 469,300 ML up from 440,165 ML since 2015 and an increase of 22% since 2012 (Australian Bureau of Statistics 2021). Concurrently, growing season rainfall is predicted to decrease in many grape growing regions, including the Riverina, Murray Valley and Coonawarra. Margaret River is already often experiencing water shortages and is predicted to have a large reduction in growing season rainfall by 2050 (Hayman *et al.,* 2009). Other wine growing regions in the southwest of Western Australia such as Geographe and Blackwood valley are predicted to have an increase irrigation requirement from 17,300 ML in 2010 to 44,000 ML by 2030 (Ward and Campbell-Clause 2013). Most regions are predicted to become more arid between 2020 and 2040 (Remenyi *et al.,* 2019). This along with an expected increased demand for irrigation (between 2 and 33%) in regions such as Riverina and Murray Valley is predicted to add pressure to the wine industry and its current practices (Haymann *et al.,* 2008).

2.4 Irrigation water quality

Climate change is expected to reduce freshwater supplies in the world's arid and semi-arid irrigation regions. Water supplies are predicted to become scarce and the salinity of the irrigation water and its impacts are expected to rise (Connor *et al.,* 2012). The Padthaway wine grape region of south-eastern South Australia for example sources all its irrigation water from an unconfined aquifer that has a salinity range of 900-2000 mg/L (Degaris et al., 2015). Since 2004, the region has experienced below-average rainfall resulting in the groundwater level dropping and the salinity rising by up to 18mg/L per year (Degaris *et al.,* 2015). The general limits for irrigation of salt sensitive crops is reported to be between 650-1300mg/L (Queensland Government publications 2015). A combination of saline irrigation together with high rates of potential evapotranspiration, low rainfall and fine textured soils can concentrate salt within the root zone of a vineyard (Stevens *et al.,* 2011).

The Barossa Irrigation Limited (BIL) reported that in season 2020/21, 7,577 ML of water for irrigation was sourced from the Murray River, which is an increase of 9% since 2016/2017 (Schultz 2021). The salinity of the water used for irrigation can fluctuate depending on the river flows, in 2020/21 the average salinity was 325 µS/cm (195 mg/L) (Schultz 2021).

When saline water is used for irrigation it can lead to increased concentration of chlorine levels in wine. Some rootstocks such as 1103 Paulsen, Ramsey and 140 Ruggeri are able to exclude the uptake of sodium chloride, however when exposed to long-term stress they may lose this ability (Tregeagle *et al.,* 2006).

The use of partially saline irrigation water on Shiraz and Grenache grapevines has been shown to cause an increase in ion concentration within the laminae, petiole and berries of the grapevines, cause leaf burn and decrease the long-term viability of the grapevines (Degaris et al., 2016). Moderately saline irrigation water causes chlorine levels to increase along with potassium, (predominately in the reproductive organs) and sodium may be stored in the trunk of the vines (Degaris *et al.,* 2016). Similar results have been seen with Cabernet Sauvignon when irrigated with poor quality (saline) water. The berries and petioles were shown to have increased levels of sodium and chlorine (Biswas *et al.,* 2008). If this fruit is then used in wine production, the sensory attributes of the wines are seen to be affected by the salinity. That is, they can taste brackish, sea water-like and soapy. These are considered to be negative attributes and have been correlated with high levels of sodium, potassium and chlorine in wines (Miras-Avalos *et al.,* 2017).

The Australian Food standards code specifies an upper limit of 1000mg/L of sodium chloride and 607mg/L of free chlorine in wine. This level however may be insufficient; de Loryn *et al.,* (2013) evaluated the sensory thresholds and perception of sodium chloride in grape juice and wine. They found that experienced tasters in their group could detect the presence of sodium chloride in a single white or red wine at a level below the stipulated maximum legal limit of 1000mg/L in Australia. They also found that 25% of their tasters could recognise the salt taste within a concentration range that encompassed Australian legal limits.

Therefore, the long-term survival of the Australian wine industry would benefit greatly from varieties that require little to no irrigation for their production.

2.5 Alternative varieties in Australia

Wine Australia (National Vintage Report 2021) reports 17 red and 10 white wine grape varieties commonly grown in Australia (Table 1), the majority of these are of French or German origin and not from hot climate regions. Therefore, they may not be as suitable for the Australian climate as other alternative varieties.

Table 1: Grape varieties commonly grown in Australia.

Common Red Varieties	Common White Varieties
Barbera	Chardonnay
Cabernet Franc	Chenin Blanc
Cabernet Sauvignon	Colombard
Durif	Marsanne
Grenache	Pinot Gris (Grigio)
Malbec	Prosecco
Mataro/Mourvedre	Riesling
Merlot	Sauvignon Blanc
Montepulciano	Semillon
Muscat à Petits Grains Rouge	Traminer
Nero d'Avola	
Petit Verdot	
Pinot Noir	
Ruby Cabernet	
Sangiovese	
Shiraz	
Tempranillo	

There is however great confusion as to what constitutes an alternative variety and what is a common variety. Dry (2010) uses the Australian Alternative Wine Show (AAWS) definition for an alternative variety, that is, a variety that is not Cabernet Sauvignon, Chardonnay, Chenin Blanc, Colombard, Grenache, Merlot, Pinot Noir, Sauvignon Blanc, Semillon, Shiraz, Riesling or Verdelho. Therefore, the definitions of alternative and normal varieties can be quite fluid. Dry *et al.,* (2017) argue that the definition for alternative varieties is too "narrow", as it includes cultivars such as Petit Verdot, Muscat Blanc and Ruby Cabernet as "alternative" even though more tonnes of them are grown than some of the "traditional" varieties.

Dry (2010) states that the reasons why we require alternative varieties are that there is too much uniformity at the present time, i.e. we require a greater range of wine flavours, varieties better suited to particular wine styles e.g. rosé, better suited to climatic conditions, better marketing/promotional activities and most importantly, if the alternative varieties come from a hot climate region they will be better adapted to Australian conditions than existing varieties in terms of heat and drought tolerance.

To date little research has been published on these alternative varieties in Australia. The Murray Valley Winegrowers (MVWI) and GWRDC (Winckel 2012) conducted yield and disease susceptibility trials involving Arneis, Fiano, Vermentino, Sauvignon Blanc, Savignin, Viognier, Muscat Gordo Blanco, Pinot Gris, Graciano, Lagrein, Montepulciano, Pinotage, Tannat, Tempranillo, Shiraz and Cabernet

Sauvignon. They found from this group that the Italian varieties of Fiano, Vermentino and the Spanish variety Graciano were best suited to the Murray Valley. Vermentino produced the highest yield of 36 tonnes per hectare with low disease susceptibility. Fiano produced 22 tonnes per hectare with low disease susceptibility and Graciano produced 28 tonnes per hectare with low to moderate disease susceptibility. This would suggest that varieties from southern Mediterranean countries may be better suited than French and German varieties currently dominating Australian viticulture. No drought tolerance was investigated in these studies, however.

Wine Australia (National Vintage Report 2021) lists 'other' varieties grown in various Australian wine regions (Table 2). Consumer trials plus chemical and sensory analyses of some of these varieties has recently occurred, including Aglianico, Barbera, Durif, Graciano, Montepulciano, Negroamaro, Nero d'Avola and Touriga Nacional (Mezei *et al.,* 2021). Since the 1970's in Australia, the CSIRO have bred six alternative varieties that are reportedly drought and disease resistant (CSIRO 2021). Two of these varieties Tyrian and Cienna have been described as 'alternative' varieties (Wine Australia 2021). The other varieties include the white variety Taminga and the red varieties Mystique, Rubienne and Tarrango (CSIRO 2021).

It is only recently that Greek varieties have started to be imported to Australia, with Jim Barry wines introducing Assyrtiko in the Clare Valley and Yalumba Nursery importing 4 other Greek varieties for trials in South Australia. These varieties were selected as they are from regions with similar hot dry climates and are popular with consumers (Nick Dry Pers Comm 2017). The MJT in Athens for the period from 1897 to 2001 was 27.2°C with the highest MJT occurring in 2000 at 30.3°C, thus Greece is considered a hot climate zone similar to Southern Australia (Founda *et al.,* 2004).

No Cypriot grape varieties currently exist in Australia.

Table 2: Other/alternative grape varieties grown in Australia

Red varieties	White varieties
Aglianico	Albarino
Alicanté Bouschet	Arinto
Carignan	Arneis
Carmenère	Canada Muscat
Chambourcin	Clairette
Cienna	Fiano
Cinsault	Greco
Colorino	Grenache Blanc
Counoise	Gros Manseng
Dolcetto	Grüner Veltliner
Gamay	Moscato Giallo
Graciano	Marsanne
Isabella	Muscadelle
Lagrein	Palomino
Lambrusco	Pecorino
Marzemino	Pedro Ximenez
Mencia	Pinot Blanc
Mondeuse Noire	Rousanne
Muscat à Petits Grains Noirs	Savagnin
Nebbiolo	Sultana
Negroamaro	Topaque
Pinot Meunier	Verduzzo
Rubired	Vermentino
Sagrantino	
Saperavi	
Tannat	
Tarrango	
Teroldego	
Tinta Baroca	
Tinta Câo	
Touriga Nacional	
Tyrian	
Zinfandel/Primitivo	

2.6 Cypriot varieties

Karageorghis (1993) describes archaeological evidence for the existence of *Vitis vinifera* in Cyprus from between 4500-3900 BCE. Galet (1993) first described some of the indigenous Cypriot grape varieties with ampelographic studies in the 1980's and 1990's (Table 3). Later, work by Banilas *et al.,* (2009) stated that significant efforts had been made towards the characterisation of Cypriot cultivars and concluded that the knowledge on the local germplasm was far from complete. Recently, Grigoriou *et al.,* (2020) studied the germplasm of Cypriot varieties and found that was in fact the case. They revealed that the germplasm consists of a multi-clonal mixture, with many varieties unique to Cyprus. Future research with the germplasm has been planned and they predict more clones and varieties could be identified.

Table 3: List of indigenous Cypriot grape varieties

Cypriot Red Varieties	Cypriot White Varieties
Mavro	Xynisteri
Maratheftiko	Promara
Ophthalmo	Spourtiko
Giannoudhi	Kanella
Flouriko	Morokanella
Skouromavro	Katomylitiko
Rodhino	Michalias
Rozoudhi	Vasilissa
Maroucho	

Xynisteri and the red grape Mavro are the most widely planted grapes in Cyprus (Xynisteri 33% and Mavro 46% of total production). Mavro is however considered to be a low aromatic and lesser quality variety than the rarer Maratheftiko and is mainly used in the sweet wine Commandaria. Maratheftiko is considered a more floral variety capable of producing high quality wines (Vrontis and Paliwoda 2008).

Xynisteri is described as high yielding, well-adapted to poor soils and hot/dry climate producing wines with elegant aromas. Maratheftiko is considered to be the most promising red grape variety for producing high quality wines. It is a rare cultivar tolerant to various stresses, having black coloured and small size berries. It is however among the very few varieties in the world that is non-hermaphroditic, it is in fact female and for this reason is planted next to other vines for pollination. It has a natural propensity to severe flower abscission, resulting in thinly clustered grape bunches. These features collectively point to the hypothesis that Maratheftiko might be a wild form, rather than a well-established domesticated variety. Genetic comparison with wild *V. sylvestris* genotypes would help to address this question and aid to elucidate the origin of the other domestic cultivars (Banilas *et al.,* 2009).

At the beginning of this project in 2017, there had been minimal research on Cypriot grape varieties. Greenhouse gas emissions in Cypriot vineyards and the carbon footprint of indigenous and introduced grape varieties had been investigated (Litskas *et al.,* 2013, Litskas 2017). The phenolic content and antioxidant capacity of Cypriot wines (Galanakis *et al.,* 2015) and the authenticity of Cypriot wines using isotopic markers (Kokkinofta *et al*., 2017) were studied. Since then, multiple studies have occurred and much of it has been referred to in this project. The chemical composition and metabolic fingerprints of sun dried Xynisteri grape musts used in the renowned Commandaria wine have been studied extensively (Constantinou *et al.,* 2017, 2018 and 2019). The red variety Maratheftiko has been studied for its response to heat and drought stress in a potted and vineyard trial with different management strategies (Chrysargyris *et al.,* 2018a and Chrysargyris *et al.,* 2018b). Xynisteri has been compared to Chardonnay in similar potted and vineyard trials (Tzortzakis *et al.,* 2020, Chrysargyris *et al.,* 2020, Heyman *et al.,* 2021). The phenolic content and volatile compound chemistry of Maratheftiko wines from different regions and vintages has been analysed (Tsiakkas *et al.,* 2020). The soil bacterial community of Cypriot vineyards under different management regimes has been explored (Vink *et al.,* 2021) and finally an investigation in to regional "terroirs" has occurred via a metataxonomic analysis of grape microbiota during wine fermentation (Kamilari *et al.,* 2021). All of these studies are helping to build a big picture of the benefits of Cypriot grape varieties, however, the mechanism for their purported drought tolerance has not yet been uncovered.

2.7 Climate of Cyprus

The Cypriot Bureau of Meteorology has 12 climatological stations across the entire island and not all of them are in or near the five wine growing regions. Rainfall data is more readily available, however. Due to the sparsity of the Cypriot climatological stations, it was necessary to monitor specific vineyards involved in this study.

Rainfall in the five wine regions can vary between 350mm to 700mm per annum. The MJT for most of the wine regions is approximately 26°C, which classifies as very hot. This equates to Australian wine regions such as: the Riverland, Murrumbidgee Irrigation Area (MIA) and Roma in Queensland (Iland *et al.,* 2011).

2.8 Australian homo-climes

Weather station data from the Australian Bureau of Meteorology (BOM) indicate that equivalent homo-climes for temperature would be for example Renmark with a MJT 25°C but with significantly less rainfall at 250mm per annum.

2.9 Terroir and Cypriot soils

Terroir is the concept that connects the location (site) of a vine to its wine, essentially describing the characteristics a location. The components include mesoclimate, geology, soil properties, geomorphology and human interaction (Iland *et al.,* 2011).

van Leeuwen *et al.,* (2004) describe climate, soil and cultivar as the three main parameters of terroir, the effects of climate and soil on vine development and grape composition can be explained largely by their influence on vine water status. Soil influences vine water status through its water holding capacity and possibly its accessibility to the water table. Soil type can influence berry weight, berry sugar concentration and berry anthocyanin concentration. The best soil types are those in which water deficits result in earlier shoot growth slackening, reduced berry size, high grape sugar and anthocyanin concentration, thereby increasing grape quality potential (van Leeuwen *et al.,* 2004). The soils of Cyprus have been classified as mostly red, sedentary, alluvial and colluvial (Hadjiparaskevas 2002). Independent soil analysis undertaken by Stefanos Koundouras (pers comm 2014) found that 3 of the vineyards that were used in experiments (discussed in methods section below) were mainly sand, clay and loam with limestone gravel, as well as being highly calcareous. The nutritional and chemical status of these soils, when compared to standards reported by Lanyon *et al.,* (2004) are deficient in many of the nutrients considered necessary for healthy vine growth. Vink *et al.,* (2021) who recently studied Xynisteri, Maratheftiko and Chardonnay vineyards in Cyprus described the soils as calcaric, leptic (rocky), cambisols with a texture of clay loam, pH ranging from 7.3 to 7.9 and organic matter between 1.6 and 3.3%.

2.10 Irrigation and drought tolerance

The sustainability of grapevine production largely needs serious consideration regarding the environmental impact of the large amounts of irrigation volume and the foreseen increases of irrigation necessities according to future climate change scenarios (Medrano *et al.,* 2015). Systematic reviews of the literature have been conducted previously investigating improving water use efficiency of vineyards in semi-arid regions. They explored methods to investigate the effect of temperature on grapevine berry composition and the variability of water use efficiency in grapevines (Medrano *et al.,* 2015, Bonada and Sadras 2015, Tomas *et al.,* 2014). Overall, more than 60 trials were reviewed with the conclusion that many of these studies focus on the effect of temperature on pH, acidity, colour, phenolics, alcohol levels and wine quality. Trials have been conducted in field and in pots under controlled growing conditions such as a multi-chamber gas exchange system as described by Poni *et al.,* (2014). A general conclusion from these papers is that one method is not better than another; rather a combination of infield and potted trials is the most effective way of conducting irrigation/drought trials.

Many cultivars have been used in these trials including traditional French varieties such as Syrah/Shiraz, Cabernet Sauvignon, Semillon, Chardonnay, Merlot, Grenache, Pinot Noir and Sauvignon Blanc. These studies showed that reduced irrigation can increase anthocyanin/skin polyphenol levels in berries, reduce yield, decrease berry size, reduce net carbon exchange, decrease growth rate and reduce leaf water use efficiency (Tomas *et al.,* 2014, Kyraleou *et al.,* 2016, Koundouras *et al.,* 2008 and 2009, Edwards & Clingeleffer 2013, Petrie & Clingeleffer 2005, Escalona *et al.,* 2016 and Galbignani *et al.,* 2016).

Koundouras *et al.,* (2009) investigated the effects of irrigation on phenolic concentration and aroma potential of Cabernet Sauvignon grapes grown in a Greek vineyard. The decrease in water supply decreased berry size but did not affect the skin/pulp weight ratio but increased skin anthocyanin concentration, specifically malvadin-3-O-glucoside, and decreased the flavin-3-O-ol monomers in seed tissue. The decrease in irrigation also increased the aroma potential at harvest.

Other studies have used various table grapes, Spanish and Italian varieties, different *Vitis* species and also Greek varieties such as Agiorgitiko, Muscat of Alexandria, Muscat Blanc, Assyrtiko, Athiri, Roditis, Mavrodaphni, Xinomavro, Malagousia and Malvasia (Tomas *et al.,* 2014, Koufos *et al.,* 2014, Koundouras *et al.,* 2006 and Assimakopolou & Tsougriannis 2012).

Theodorou *et al.,* (2019) compared anthocyanin content and profile under variable irrigation regimes in four red grape cultivars, Greek varieties Agiorgitiko and Xinomavro alongside the French varieties Syrah and Grenache noir. The four cultivars had a similar response in terms of vigour and yield parameters, with values increasing with water supply. Anthocyanin concentration was maximised under non-irrigated conditions, but anthocyanin profile and relative distribution of single glucosides did not respond uniformly to irrigation in all cultivars. Especially with respect to the two indigenous Greek cultivars, Xinomavro seemed to favour the synthesis of more stable forms of anthocyanins under limited water supply, while for Agiorgitiko it had the opposite effect.

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. The later ripening indigenous Greek varieties experienced fewer impacts (potential alcohol increases and acidity decreases) due to temperature increases than the international varieties and were therefore potentially better adapted to future warmer climates. However, this study did not assess drought tolerance parameters, which is needed to better understand their potential.

In Cyprus, the red variety Maratheftiko has been studied for its response to heat and drought stress in both a potted and a vineyard trial with different management strategies (Chrysargyris *et al.,* 2018a and Chrysargyris *et al.,* 2018b). 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 of the berry characteristics; total soluble solids, phenolics and 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 utilisation of stomatal closure as an adaptive mechanism however this mechanism was not yet fully understood. Similarly, Xynisteri has been compared to Chardonnay in potted and vineyard trials (Tzortzakis *et al.,* 2020, Chrysargyris *et al.,* 2020, Heyman *et al.,* 2021). In the vineyard, Xynisteri 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. The authors suggested 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.

2.11 Sensory analysis

Sensory analysis has been described as involving the detection, discrimination and description of both the qualitative and quantitative sensory components of a consumer product by a trained panels of judges (Meilgaard *et al.,* 1991 cited in Danner *et al.,* 2018). The qualitative aspects of a product have been defined as the aroma, appearance, flavour, texture, aftertaste and sound properties of a product, which distinguish it from others. Sensory judges then quantify these product aspects in order to facilitate description of the perceived product attributes (Murray *et al.,* 2001 cited in Danner *et al.,* 2018). Traditionally, sensory evaluation of food products has been divided into two method groups: analytical tests, where trained individuals or panels are used to objectively evaluate sensory properties and affective tests, where consumers are used to determine product acceptance and preference (Stone and Seidel 1993, Lawless and Heymann 2010). Danner *et al.,* (2018) state that describing and quantifying the complex sensory properties of food and beverages to be an elaborate, time-consuming and expensive task, while Moussaoui and Varela (2010) believe that naïve consumers can be utilised to assess and quantify the sensory attributes of food and beverage products when given a suitable methodology.

Wine has been described as being comprised of hundreds of volatile aroma compounds and a diverse range of chemical constituents that potentially contribute to the wine flavour and aroma profile (Thorngate 1997). Danner *et al.,* (2018) agree stating that as well as wine being a natural product, the grape variety, variations in geographical growing region, production and processing techniques further add to the complexity of individual products and perceived differences in aroma profiles observed between styles.

The “Rate All that Apply” (RATA) method is one where the assessors are not only required to select attributes applicable to a product, but additionally indicate the perceived intensity of these sensory attributes using a rating scale (Ares *et al.,* 2014). The RATA method has demonstrated that using naïve consumers can result in very similar sample discrimination and sample configurations as descriptive analysis involving trained panellists when evaluating commercial wine samples (Danner *et al.,* 2018). The RATA method is also valid for rapid sensory profiling within industry and research applications which aim to describe the sensory characteristics of wines, making it particularly relevant when resources and time are limited, and/or additional consumer responses i.e., hedonic ratings or willingness-to-pay are of interest (Danner *et al.,* 2018).

2.12 Consumer sensory analysis and profiling

The long term aim of this work is to be able to produce quality wine sustainably that consumers like and want to buy and drink. It is therefore vital to ensure that the consumer actually likes the wine that will ultimately be produced from these varieties. Consumer acceptance testing or affective testing is one such method utilised for the measurement of liking or preference by consumers for a product through sensory evaluation. This can often involve a rating system such as the 9-point hedonic scale which is one of the most popular tools (Sidel and Stone 1993). The 9-point hedonic scale has been used routinely in food science for 60 years. It is used to assess liking and preference for various products, so the most liked can be selected for future development. It involves a line with 9 points equally spaced marked along it. Each mark relates to a consumer reaction ranging from extremely dislike at one end to extremely like at the other end. The participant samples the product then reads the related question and marks their liking along the scale (Wichchukit and O’Mahony 2016).

One of the aims of this project is to ascertain whether consumers like the wines made from these Cypriot varieties. It is also hypothesised that these varieties are drought tolerant and hence a more sustainable way of producing wine. Therefore, it is important to know whether the consumer has an interest in the sustainability of the product.

O’Rourke and Ringer (2016) analysed the impact of sustainability information on consumer purchase intentions in relation to a number of products. These products included personal care items, household chemicals, food and pet food. They concluded that sustainability information had, on average, no impact on direct users, demonstrating that simply providing more or better information on sustainability issues will likely have limited impact on changing mainstream consumer behaviour. Pomarici and Vecchio (2013) disagree having analysed Italian wine consumers, particularly the millennial generation and their attitudes to sustainable wine. They found that wines with labels listing sustainability features were accepted more than those without. The cohort most likely to display this behaviour were females aged 27-35 living in an urban area.

The concept of food neophobia that is the fear of new foods was described by Pliner and Hobden (1992). They state that humans, along with other omnivorous animals, have been characterised as being neophobic with respect to food. This reluctance to eat and/or avoidance of novel food is assumed to have adaptive value, serving a protective function in a potentially hostile food environment. Their work went on to develop the Food Neophobe Scale (FNS).

Ristic *et al.,* (2016) took this concept further and developed the Wine Neophobia Scale (WNS), that is, a scale to determine ones' level of fear of a new or unknown wine product. They ascertained that the degree of wine neophobia increased with age but decreased with higher education and greater income. The demographics where wine neophobes predominated were older than 55 years of age; having no tertiary education; and on average having an income less than AUD$75,000 annually. Wine neophilics were mostly younger than 54 years of age; tertiary educated and had an income greater than AUD$75,000.

Recently, Mezei *et al.,* (2021) investigated Australian consumers' responses to red wines made of lesser-known grape varieties with potential drought tolerance. They included Aglianico, Barbera, Durif, Graciano, Montepulciano, Negroamaro, Nero d'Avola and Touriga Nacional. The study revealed that the wines assessed possessed a vast range of sensory attributes to suit all tastes (from fruit-driven, smooth, red fruit predominant wines to more complex, savoury and oaky) and the wines were well liked by consumers. This indicates that while some wine consumers may have reservations about trying new and different wines, overall if the wines are made with desirable attributes, they will be well received by consumers regardless of the grape variety.

2.13 Summary

When this project commenced, there were very few studies involving the indigenous Cypriot grape varieties. In the last three years the topic has become more popular both in Cyprus and internationally. This literature review set the framework for the studies that followed with many of the more recent studies referenced in the publications. While the research identified in the literature has given some insight into Cypriot grape varieties, it has also identified some gaps in the knowledge that this project has attempted to address.

Chapter 3. Published Article 1: Preliminary sensory and chemical profiling of Cypriot wines made from indigenous grape varieties Xynisteri, Maratheftiko and Giannoudhi and acceptability to Australian consumers.

Statement of Authorship

Title of Paper	**Preliminary sensory and chemical profiling of Cypriot wines made from indigenous grape varieties Xynisteri, Maratheftiko and Giannoudhi and acceptability to Australian consumers**
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
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Contribution to the Paper	Designed and constructed the research experiments, analysed the data, drafted and constructed the manuscript		
Overall percentage (%)	90%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third on in this thesis. I am the primary author of this paper.		
Signature		Date	19/11/21

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Preliminary sensory and chemical profiling of Cypriot wines made from indigenous grape varieties Xynisteri, Maratheftiko and Giannoudhi and acceptability to Australian consumers

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ABSTRACT

Aim: The aims of this study were to (1) generate sensory and chemical profiles of commercial Cypriot wines made from the white grape Xynisteri and the red grapes Maratheftiko and Giannoudhi and (2) assess the Australian consumers' response to these wines.
Methods and Results: A Rate-All-That-Apply (RATA) method was used for sensory profiling of the wines (n=56 panellists on Xynisteri and n=60 on Maratheftiko and Giannoudhi) and to guide chemical analysis of flavour compounds. Chemical analysis involved quantitative analysis of aroma compounds by gas chromatography mass spectrometry (GC-MS) and non-targeted profiling of phenolic compounds (non-volatile secondary metabolites) using liquid chromatography mass spectrometry (LC-MS). Australian wine consumer's hedonic responses towards wines made from Cypriot grape varieties were also investigated. Consumers completed a questionnaire exploring their demographics, wine consumption habits, environmental/sustainability opinions and neophobic tendencies prior to the tasting. The first tasting (n=111 consumers) consisted of six commercial Xynisteri, one Australian Pinot Gris and one Australian unwooded Chardonnay wines. The second (n=114) consisted of three Maratheftiko, one Giannoudhi and one Australian Shiraz wines.
Conclusions: Principal Component Analysis (PCA) of the RATA study identified the following sensory characteristics for Xynisteri wine: stone fruit, dried fruit, citrus, herbaceous, grassy, apple/pear, confectionary, vanilla, creamy, buttery, wood, and toasty. Maratheftiko wines were described as woody, dried fruit, chocolate, herbaceous, confectionary, jammy, sweet and full bodied. Giannoudhi wine was described as woody, dried fruit, chocolate and full bodied. Chemical analysis identified 15 phenolic compounds in the white wine samples and 17 in the red wine samples, as well as 21 volatile/aroma compounds in the white wine samples and 26 in the red wine samples. These chemical compounds were then correlated with sensory data from the RATA and consumer hedonic responses using Agglomerative Hierarchical Clustering (AHC) and PCA to determine consumer liking drivers for the wines. Three clusters of consumers were identified for the white and red wines. The overall consumer means for liking indicated that Cypriot wines were liked similarly to Australian wines.
Significance and impact of the study: Australia's changing climate is placing great pressure on the resources for sustainable viticulture. Many vineyards and wineries base their businesses on European grape varieties traditionally grown in regions with abundant water resources. It is therefore necessary for the Australian wine industry to investigate grape varieties that are indigenous to hot climates similar to Australia. The eastern Mediterranean island of Cyprus is one such place with indigenous grape varieties that grow well in a hot climate without irrigation. These popular Cypriot wines have the potential to be popular with Australian consumers, thus offering new grape varieties to the Australian market that are better suited to the changing climate.

KEYWORDS

Rate-All-That-Apply (RATA), wine consumers, Gas Chromatography Mass Spectrometry (GC-MS), Liquid Chromatograph Mass Spectrometry (LC-MS), Partial Least Squares (PLS), Agglomerative Hierarchical Clustering (AHC), vineyard sustainability, Cypriot wines

INTRODUCTION

The climate in Australia and the rest of the world is undergoing rapid change. Since the middle of the 20th century, Australian temperatures have on average risen by about 1°C with an increase in the frequency of heat waves and a decrease in the number of frosts and cold days (Webb, 2011). These changes have made observable impacts on viticulture. Trends to earlier harvest maturity were observed in numerous regions across the country (Webb *et al.,* 2013). These trends are partly due to warming climates, but also due to reduced water availability. Jarvis *et al.* (2019) report that unusually warm and dry spring conditions have been linked to earlier budburst, with a more rapid rate of growth and development for the remainder of the growing season, regardless of temperatures later in the season.

Further climate change and rainfall reduction is expected over the coming decades (Johnson *et al.,* 2018). For most locations the best estimate of mean warming over Australia by 2030 is 0.7-0.9°C in coastal areas and 1-1.2°C inland and annual precipitation is estimated to decrease by 2.5 to 5% in most regions of Australia. Objectives to assist the wine industry in mitigating and adapting to these changes in climate include establishing adaptation scenarios for major wine regions based on changes to phenology and temperature tolerance of major varieties and future water demand and availability (Webb *et al.,* 2007).

Cyprus is reported to have the oldest wine tradition in the Mediterranean with more than 5,500 years of wine production with a vineyard area of approximately 7,000 hectares (Chrysargyris *et al.,* 2018b. It has been described by Evans (2009) and Lelieveld *et al.* (2016) as the cradle of viticulture and that this area is gradually and steadily becoming hotter and drier due to climate change. Many indigenous varieties of grapes originating from the region have been hand selected for millennia for their resistance to heat and drought (Fraga *et al.,* 2016; Patakas *et al.,* 2005). During the summer period, grapevines cultivated in the Mediterranean are often subjected to a combination of environmental stresses including strong winds, high air temperatures (heat waves) and soil/atmospheric water deficits (Beis and Patakas, 2012; Chrysargyris *et al.,* 2018a). There are more than 10 indigenous Cypriot grape varieties on the island, with many of them very well adapted to drought. They require less water and fertilisers when compared to introduced varieties and offer promising prospects for adaptation to climate change (Litskas *et al.,* 2017). This climate scenario of Cyprus is very similar to that of southern Australia and as such their indigenous varieties may also be a suitable strategy to mitigate climate change effects in Australian conditions. This study sought to analyse Cypriot wines made from indigenous grape varieties Xynisteri, Maratheftiko and Giannoudhi using chemical and sensory profiling. The white grape Xynisteri is the most widely planted white variety in Cyprus and is utilised for table wine, the sweet wine Commandaria and traditional sweets. Maratheftiko is considered a red floral variety capable of producing high quality wines and the rare Giannoudhi has been gaining popularity recently with the local market (Vrontis and Paliwoda, 2008). To date there is limited research on sensory and chemical profiling of wines made from Cypriot grape varieties. Research has mainly focused on investigating the chemical composition and metabolic fingerprints of sun dried Xynisteri grape musts (Constantinou *et al.,* 2017; Constantinou *et al.*, 2018a), the phenolic content and antioxidant capacity of Cypriot wines (Galanakis *et al.,* 2015) and the authenticity of Cypriot wines using isotopic markers (Kokkinofta *et al.,* 2006 and 2017).

There have been no consumer sensory studies on Cypriot wines to date. A consumer survey by Vrontis and Papasolomou (2007) suggested that there has been a shift in Cypriot consumer preference, with 87.2% of the 600 consumers surveyed preferring to drink wine made from the local varieties. Wine flavour and aroma were found to be the main drivers for purchasing wine made from local varieties, rather than more popular European varieties. Similar results have been noted with Greek consumers and Greek wines. Krystallis and Chrysochou (2010) studied consumer loyalty determinants in Greek wine varieties and found that 87% of those surveyed purchased Xinomavro and 89% purchased Agiorgitiko at an average frequency of six bottles a month.

The aims of this study were to (1) generate sensory and chemical profiles of commercial Cypriot wines made from the white grape Xynisteri and the red grapes Maratheftiko and Giannoudhi and (2) assess the Australian consumer's response to these wines that are very popular amongst wine consumers in Cyprus. This would enable the Australian wine industry to potentially introduce new grape varieties to the market that are both acceptable to consumers and better suited to the Australian climate.

MATERIALS AND METHODS

1. Wines

The wines used for both studies included four Cypriot Xynisteri 2016, one Cypriot Xynisteri 2015, one Australian Pinot Gris and one Australian Chardonnay 2017. The red wines were two Cypriot Maratheftiko 2015, one Cypriot Maratheftiko 2013, one Cypriot Giannoudhi 2014 and one Australian Shiraz 2014. The Cypriot wines were chosen as they were common brands and were spread across a range of price points (5-20 Euros). Some older wines and oaked aged wines were also chosen to assist in consumer preference for younger or older wine styles. The Australian wines were used as a reference to the otherwise unknown Cypriot varieties. They were also common brands readily available at wine retailers for between $20-$25 AUD. More detailed information on the wines used in this study is provided in Table 1.

2. Sensory analysis

The Rate-All-That-Apply (RATA) technique described by Danner *et al.* (2018) was utilised for sensory profiling of the wines. RATA is a rapid sensory profiling method with industry and research applications and aims to describe the sensory characteristics of wines, making it particularly relevant when resources and time are limited, and/or additional consumer responses i.e. hedonic ratings or willingness-to-pay are of interest (Ares *et al.,* 2014; Danner *et al.,* 2018). This method has demonstrated that using untrained consumers to evaluate commercial wine samples can result in very similar sample discrimination and sample configurations as descriptive analysis (DA) (Ares *et al.,* 2014).

RATA analysis of the white commercial wines occurred in November 2017 involving 57 tasters. The tasters were recruited from the School of Agriculture, Food and Wine staff members and post-graduate students who had previous experience in tasting and evaluating wines.

Nine wines were presented sequentially, monadic, blind and in a random order to the tasters to overcome serving order effects. Wines were served in International Standards Organisation (ISO) tasting glasses at 15°C. Tasters were required to select only the attributes that were applicable to the wine and additionally indicate the perceived intensity of these sensory attributes using a 7-point rating scale. Attributes included 3 colour, 22 aroma intensity, 3 taste, 22 flavour intensity, 6 mouthfeel intensity and 2 length of aftertaste questions (Supplementary Tables 1 and 2).

TABLE 1. Basic chemical, oak treatment and other information of wines used in sensory, consumer acceptance and chemical analysis.

Code	Wine	pH	TA	Alc %	Oak	Other
M1	Maratheftiko 2015	3.43	5.86	14.8	Yes	
M2	Maratheftiko 2013	3.62	5.45	13.2	Yes	
M3	Maratheftiko 2015	3.44	5.88	14.5	Yes	
SH	Shiraz 2014	3.57	6.13	14.5	Yes	
Yia	Giannoudhi 2014	3.65	5.5	13.4	Yes	
CH	Chardonnay 2017	3.33	7.35	12.9	No	
PG	Pinot Gris 2017	3.54	6.65	12.5	No	
X1	Xynisteri 2016	3.21	5.93	12.8	No	
X2	Xynisteri 2015	3.26	5.94	12.8	Yes	
X3	Xynisteri 2016	3.22	5.52	13.7	No	
X4	Xynisteri 2016	3.35	5.44	12.8	No	5% Muscat
X5	Xynisteri 2016	3.16	4.72	12.6	No	
X6	Xynisteri 2016	3.42	5.02	12.6	No	

Ethics approval for the sensory analysis was given by the University of Adelaide, approval number: H-2017-204. The tasting took place in the wine sensory lab at the Wine Innovation Central (WIC) building at the University of Adelaide Waite Campus. Results were collected using Red Jade sensory software.

RATA analysis of the red commercial wines involving 60 tasters occurred in July 2018 using the same protocols as 2017. The red wines were served at a room temperature of 22°C.

3. Consumer acceptance trials

Participants completed a questionnaire utilising a 9-point hedonic scale prior to the tasting. The questions explored their demographics, wine consumption habits, environmental/sustainability opinions and neophobic tendencies. The questions were taken directly from previously published and validated questionnaires. The questions came from: The Fine Wine Instrument (Johnson and Bastian, 2015), Wine Neophobe Scale (Ristic *et al.,* 2016) and The Concern About Sustainability questionnaire (Grunert *et al.,* 2014).

The white commercial wines (n=111) were assessed in December 2017 and the red commercial wines (n=114) in July 2018. Consumers were recruited from social media and the University of Adelaide registered taster database. Pre-requisites for consumers in the trial were to be over 18 years of age and consume wine at least once every 2 weeks.

As with the RATA trial, wines were presented sequentially monadic, blind and in a random order. During the tasting, the consumers were required to answer five questions on a 9-point Likert scale relating to their perception of the wine quality, how much they liked the wine, how likely they would be to recommend the wine, how likely they were to buy the wine again and how much they would pay for the wine.

4. Chemical analysis

Wine samples were analysed by the Australian Wine Research Institute (AWRI) and Metabolomics Australia at the Waite Campus (AWRI-Metabolomics South Australia, 2019). As this was a preliminary study, only a small number of wines were able to be imported to Australia quickly and easily with an aim to gain an initial understanding of the attributes of these wines and preliminary investigation of chemical compounds. Thus, only single measures were utilised in the chemical analysis.

4.1 Non-volatile profiling of secondary metabolites by Liquid Chromatography-Mass Spectrometry (LC-MS/MS), non-targeted analysis

The non-targeted method was developed to detect as many phenolic compounds as possible and was not specifically optimised for one class of phenols.

The sample set consisted of 13 samples (5 red wine and 8 white wine samples). Prior to analyses wine samples were submitted to a standard clean-up procedure using Strata-X reversed phase SPE cartridges. After conditioning the cartridge (1 mL methanol and 1 mL Milli-Q water), 2 mL of each sample were diluted with 8 mL of Milli-Q water and loaded on the cartridge. The eluted fraction was discarded, while compounds of interest were retained on the cartridge phase. Cartridges were then washed with 1 mL of aqueous solution of methanol (2%) and dried at full vacuum for 5 minutes. Analytes were eluted using 1 mL of methanol. The eluted fractions were collected in test tubes and methanol evaporated. The dried extracts were resuspended prior to analysis using 25 µL and 75 µL of solvent B (2% formic acid, 2% Milli-Q water, 40% acetonitrile in methanol) and solvent A (2% formic acid, 0.5% methanol in Milli-Q water) respectively. Chemical Analysis Separation was performed on an Agilent 1200SL High-performance liquid chromatography (HPLC) coupled to a Bruker MicroTOFQ-II. Samples were acquired in the MS negative mode. HPLC conditions included: injection volume 1 µL, flow rate 0.22 mL/min, column - Phenomenex Kinetex PFP 150mm x 2.1mm ID, oven temperature 30°C and DAD acquisition range 200-500 nm. MS conditions of the detector were: source temperature 200°C, capillary voltage 3500 V, end plate offset -500 V, nebuliser pressure 2.0 bar, dry gas flow rate 8.0 L/min, mass range 50-1650 m/z and acquisition rate 0.5 Hz.

A calibration solution of sodium formate (5 mM sodium hydroxide in 50% (v/v) 2-propanol) was introduced during LC-MS analysis via an inline post-column switching valve and sample loop. Using Bruker's Data Analysis (v4.0 SP4) software, mass spectra were calibrated in the range 100-1650 m/z from the sodium formate clusters using an enhanced quadratic algorithm. Each file was exported in the mzXML generic file format for further processing using R (statistical programming environment) v3.3.2 and Bioconductor v2.14 under a Debian Linux 64-bit environment. Analyses were divided into two batches (acquired within the same sequence), for white wines and red wines respectively. For each batch a Master Mix (a pooled mix of the samples) was prepared and several analytical replicates of the mix were acquired along the samples sequence. This was done to monitor the instrument performances along the instrument sequence. Each batch was processed using an R based script that allowed the extraction of all the molecular features from the data matrix. The term molecular feature describes a two-dimensional bounded signal: a chromatographic peak (retention time) and a mass spectral peak (m/z).

4.2 Quantitative analysis of fermentation products (aroma compounds) by Gas Chromatography/Mass Spectrometry (GC/MS)

The wine samples were diluted by factor 10. This was done to ensure that the concentrations of the detected analytes were within the instrument linear range. 1 mL of each sample was transferred into individual 20 mL vials containing 9 mL of buffer solution (pH 3.39) and 2 g of salt.

The analysis was performed on an Agilent 7890A gas chromatograph equipped with a Gerstel MPS2 multi-purpose sampler and coupled to an Agilent 5975C VL mass selective detector. Instrument control was performed with Agilent ChemStation E.02.00. The gas chromatograph was fitted with an Agilent DB-624UI column (30m x 0.25mm x 1.4µm). Helium (Ultra High Purity) was used as the carrier gas in constant flow mode. The oven temperature was started at 40°C, then increased to 60°C at 20°C/min (held for 14 mins) and followed by a series of temperature ramps. First ramp to 70°C at 10°C/min, second ramp to 80°C at 10°C/min, third ramp to 160°C at 20°C/min, and final ramp to 260°C at 10°C/min and held for 2 mins. The total run time was 45.5 mins. The vial and its contents were heated to 40°C for 5 minutes with agitation. The SPME fibre (polyacrylate) was exposed to the headspace in the sample for 15 minutes and was then desorbed in the injector (splitless mode) for 15 minutes. The injector temperature was set at 260°C. The mass spectrometer quadrupole temperature was set at 150°C, the source was set at 230°C and the transfer line was held at 260°C. Positive ion electron impact spectra at 70 eV were recorded in SIM and SCAN mode with solvent delay of 4 mins.

The raw data from Agilents' ChemStation software (v E.02.02.1431) were converted into MassHunter data files and processed using MassHunter Workstation Software for Quantitative Analysis (v B.04.00). The concentration of analytes in the samples are determined using stable isotope dilution analysis (SIDA) and are reported in µg/L. Aroma detection thresholds (DT) were determined from Wang *et al.* (2016), Waterhouse *et al.* (2016) and Gonzalez-Alvarez *et al.* (2011). Odour activity values (OAV) were calculated (concentration/DT).

4.3 Spectral analysis

The white wine samples underwent spectral analysis to determine Flavonoid Extractives, Total Hydroxycinnamates, Total Phenolics and Relative Brown colour. Procedures and conditions were based on standard techniques described by Cozzolino (2015).

4.4 Modified Somers and tannin assays

The red wine samples underwent modified Somers and tannin assays to determine Colour Density, Free Anthocyanins, Pigmented Tannin, Total Pigment, Percent of Pigmented Tannin and Total Phenolics. Procedures and conditions were based on standard techniques described by Mercurio *et al.* (2007).

5. Statistical analysis

Basic chemical data were processed with Microsoft Excel 2010. Chemical data are presented as mean values with standard deviation from replicate determinations. Sensory data and chemical data were analysed by one-way ANOVA (sample) using the statistical package XLSTAT (version 2018.7, Addinsoft SARL, Paris, France). The significantly different attribute means were subjected to Pearson's type Principal Component Analysis (PCA) using XLSTAT and partial least squares (PLS) regression using The Unscrambler (version 9.7, CAMO Software AS, Oslo, Norway) with

TABLE 2. Significant attributes identified by RATA in (a) white wine samples and in (b) red wine samples.

	Attribute	Code	Minimum	Maximum	Mean	Standard deviation	p-value
(a)	Colour brown	CB	0.71	1.66	1.02	0.30	<.0001
	Colour green	CGr	0.88	2.04	1.48	0.34	<.0001
	Colour yellow	CYe	2.95	4.56	3.67	0.56	<.0001
	Aroma apple pear	AA/P	1.98	2.80	2.34	0.33	0.050
	Aroma citrus	ACit	2.23	3.09	2.72	0.31	0.022
	Aroma dried fruit	ADrF	0.86	1.68	1.16	0.27	0.0419
	Aroma stone fruit	AStF	2.45	3.50	3.02	0.39	0.009
	Aroma confectionary	ACon	1.07	1.99	1.45	0.33	0.005
	Aroma tropical	ATr	2.16	3.46	2.76	0.41	0.0003
	Aroma floral	AFl	1.46	2.75	2.19	0.50	0.0001
	Aroma grass	AGr	0.32	1.07	0.77	0.25	0.0097
	Aroma herbal	AHe	0.60	1.09	0.82	0.21	0.0457
	Aroma butter	ABu	0.86	1.57	1.14	0.28	0.0286
	Aroma nutty	ANu	0.78	1.89	1.19	0.41	<.0001
	Aroma savoury	ASav	0.29	1.18	0.61	0.34	<.0001
	Aroma toast	ATo	0.48	1.29	0.91	0.27	0.0069
	Aroma wood	AWo	0.38	1.29	0.77	0.32	0.0001
	Aroma bread	ABr	0.57	1.50	0.98	0.33	0.0007
	Taste bitter	TB	1.68	2.39	2.15	0.23	0.0062
	Taste sweet	TSw	2.11	2.88	2.37	0.26	<.0001
	Taste acid	TA	3.65	4.45	3.99	0.23	0.0010
	Flavour stone fruit	FStF	2.52	3.32	2.89	0.30	0.0183
	Flavour confectionery	FCon	0.84	1.69	1.09	0.28	0.0009
	Flavour tropical	FTr	1.79	2.99	2.40	0.37	0.0011
	Flavour floral	FFl	1.25	2.39	1.79	0.44	0.0002
	Flavour nutty	FNu	0.83	1.77	1.18	0.29	0.0027
	Flavour toast	FTo	0.53	1.54	0.91	0.31	0.0003
	Flavour wood	FWo	0.45	1.19	0.72	0.26	0.0165
	Flavour vanilla	FVan	0.41	1.32	0.98	0.31	0.0023
	Flavour bread	FBr	0.48	1.39	0.94	0.30	0.0020
	Mouth feel alcohol	MFOH	3.21	3.89	3.62	0.22	0.0025
	Mouth feel astringent	MFAs	1.89	2.55	2.26	0.22	0.0045
	Mouth feel creamy	MFCr	2.02	2.88	2.47	0.29	0.0045
	After taste fruitlength	ATFL	3.68	4.25	3.94	0.22	0.0195
	After taste non-fruit length	ATNFL	3.34	4.12	3.77	0.24	0.0201
(b)	Colour red	CR	3.53	4.93	4.39	0.57	<.0001
	Colour purple	CP	1.38	4.92	2.75	1.72	<.0001
	Colour brown	CB	0.98	3.15	2.16	1.03	<.0001
	Aroma dried fruit	ADrF	2.08	3.15	2.67	0.45	0.0017
	Aroma jammy	AJ	2.37	3.22	2.69	0.34	0.0231
	Aroma confectionery	ACon	1.58	2.28	1.84	0.27	0.0541
	Taste bitter	TB	2.25	3.02	2.81	0.32	0.0025
	Taste sweet	TSw	2.15	2.80	2.49	0.24	0.0297
	Flavour dried fruit	FDrF	2.13	2.97	2.57	0.37	0.0051
	Flavour jammy	FJ	1.58	2.68	1.91	0.44	0.0001
	Flavour chocolate	FCh	1.05	1.80	1.51	0.31	0.0105
	Flavour herbal	FH	1.42	2.02	1.68	0.29	0.0175
	Flavourwood	FWo	2.13	2.95	2.58	0.33	0.0127
	Mouth feel bitter	MFB	3.98	4.47	4.31	0.21	0.0036
	Mouth feel astringent	MFAs	4.15	5.15	4.69	0.38	<.0001
	Mouth feel smooth	MFSm	3.05	3.90	3.37	0.35	0.0002
	Mouth feel rough	MFRo	2.98	3.95	3.57	0.39	<.0001

chemical parameters (x-variables) and RATA data (y-variables). All variables were standardised before analysis and significance p-values where p<0.05.

RESULTS

1. Sensory analysis

Panellists utilising the RATA technique identified 35 statistically significant attributes for the white wines and 17 for the red wine samples that defined the properties of the Cypriot wines (Tables 2 and 3). Figures 1 and 2 display the scores and loadings from the PCA of sensory data, chemical analysis and wine samples.

The white wine samples in Figure 1 show the first two principal components, which accounted for 73.05% of the variation in the data. The first principal component (x-axis, 44.5%) separated samples that were floral, tropical, sweet, confectionary, apple, pear, herbaceous, stone fruit, citrus, vanilla and creamy from samples that were woody, bread, nutty, buttery, dried fruit, alcohol, bitter and astringent. The second principal component (y-axis, 28.5%) separated samples that were floral, tropical, sweet, confectionary, apple, pear, citrus, herbaceous, stone fruit, vanilla and creamy from samples that were woody, bread, nutty, buttery, dried fruit, alcohol, bitter and astringent. Wines were well distributed within the four quadrants. The upper right quadrant contained X2, which was perceived as toasty, wood, nutty, creamy and vanilla. The upper left quadrant contained X4, X6, PG, CH which were perceived as apple, pear, grass, herbaceous, confectionary, sweet, tropical, floral, stone fruit, citrus, grass and herbaceous. The lower left quadrant contained X1 which was perceived as green in colour. The lower right quadrant contained X3, X5 which were perceived as woody, bread, toast, nutty, buttery, dried fruit, alcohol, bitter and astringent.

The red wine samples in Figure 2 show the first two principal components, which accounted for 79.19% of the variation in the data. The first principal component (x-axis, 45.83%) separated samples that were jammy sweet, chocolate, confectionery and dried fruit from samples that were woody, bitter, astringent, rough and herbaceous. The second principal component (y-axis, 33.36%) separated samples that were sweet, jammy, confectionery, bitter, astringent and rough from those that were woody, chocolate, dried fruit, smooth and had fruit driven after taste. Wines were well grouped in three quadrants with SH in the upper right quadrant perceived as jammy, sweet, smooth, dried fruit and chocolate. The lower right quadrant contained M1 and M3 which were perceived as confectionary, bitter, rough, astringent and herbaceous. The lower right quadrant contained M2 and Yia which were perceived as chocolate, dried fruit and wood.

2. Consumer acceptance

Agglomerative Hierarchical Clustering (AHC) was applied to the consumer data and revealed three clusters for the white and red wines.

The consumer means for liking before clustering revealed that the white wines were liked in the following order: PG, X4, CH, X3, X1, X2, X6, X5 driven by the attributes apple, pear, confectionery, sweet, floral, and tropical. Following clustering, the cohort in cluster 1 preferred X4, PG, X6, X2, X1, X5, X3 driven by the sensory attributes floral, tropical, sweet, confectionary, apple, pear, stone fruit, vanilla, creamy, woody, bread, nutty, buttery, dried fruit, alcohol, bitter and astringent. Cluster 2 preferred X2, PG, X1, CH, X3, X5 driven by the sensory attributes floral, stone fruit, vanilla, creamy, woody, bread, nutty, buttery, dried fruit, alcohol, bitter and astringent. Cluster 3 preferred CH, PG, X4, X5, X6 driven by the sensory attributes floral, tropical, sweet, confectionary, apple, pear, herbaceous, stone fruit, and citrus (Table 3).

The consumer means for liking before clustering revealed that the red wines were liked in the following order: SH, M3, M2, Yia, M1 driven by the attributes jammy, sweet, smooth and dried fruit. Following clustering, the cohort in cluster 1 were found to prefer M1, M3 driven by the sensory attributes sweet, jammy, confectionery and bitter. Cluster 2 preferred M2, SH, Yia driven by the attributes jammy, smooth, dried fruit, woody and chocolate. Cluster 3 liked all samples, but particularly M1, M3.

Analysis of the pre-tasting consumer questionnaire did not find any statistically significant relationships between the clusters and demographics, wine consumption habits, environmental/sustainability opinions, neophobic tendencies and wine acceptance. While the consumers in this trial were recruited from social media and the University of Adelaide volunteer taster database, it may be that the group were too homogenous to elicit any significant results.

TABLE 3. Sample, consumer means and clusters (C1, C2, C3) for (a) white wines and (b) red wines.

	Sample	Consumer mean	C1	C2	C3
(a)	CH	5.78	4.82	6.31	6.56
	PG	6.43	6.53	6.71	6.03
	X1	5.76	5.97	6.31	4.97
	X2	5.75	6.02	6.78	4.44
	X3	5.78	5.60	5.93	5.88
	X4	5.90	6.60	4.87	5.94
	X5	5.52	5.37	5.28	5.94
	X6	5.68	6.35	4.87	5.56
(b)	M1	5.80	5.79	4.25	7.05
	M2	6.00	4.46	7.09	6.65
	M3	6.20	5.59	5.50	7.19
	SH	6.50	5.46	7.09	6.88
	YIA	5.90	4.79	6.28	6.58

Overall however, the Cypriot wines were well liked by the Australian consumers in this study with the majority of mean liking scores greater than 5 on a 9-point hedonic scale.

3. Non-volatile profiling of secondary metabolites by Liquid Chromatography-Mass Spectrometry (LC-MS/MS), non-targeted analysis

As this was a preliminary study, it was decided to use non-targeted analysis of phenolic compounds. These normalised values were obtained by dividing the intensity value of each feature by the median intensity value across all features for that sample. The median value is the midpoint of all the feature intensities recorded separately for each sample. These values are reported as median normalised intensity values.

Analysis of the white samples identified 12 compounds and 3 unknown compounds (Table 4). Although not quantified, these phenolic compounds identified are consistent with the phenolic compounds identified in Xynisteri grape must by Constantinou *et al.* (2018a and b). PCA analysis in Figure 1 separated compounds caffeic acid, caffeic acid ethyl ester, coutaric acid A and epicatechin in the upper left quadrant correlating with PG, CH, X4, X6. The upper right quadrant contained fertaric acid and querctin-3-O-glucoronide (correlating to X2). The lower left quadrant contained catechin, ethyl gallate and gallic acid which correlated with X1 and the lower right quadrant contained caftaric acid, epigallocatechin and coutaric acid B with X3, X5.

To date only phenolic classes have been identified in Maratheftiko and Giannoudhi wines (Galanakis *et al.,* 2015). This study has confirmed the identity of these classes and has also identified 15 preliminary compounds and 3 unknown compounds for Maratheftiko and Giannoudhi (Table 4). PCA analysis in Figure 2 separated compounds laricitrin, epigallocatechin and syringetin-3-O-glucoside in the upper right quadrant correlating to SH. The upper left quadrant contained compounds epicatechin, procyanidin B1, fisetin and quercitin. The lower left quadrant contained compounds catechin, gallic acid, quercitin-3-galactoside, quercitin-3-O-glucoronide, caftaric acid, and coutaric acid a, correlating to M1, M3. The lower right quadrant did not contain any phenolic compounds and correlated to M2, Yia.

4. Quantitative analysis of fermentation products (aroma compounds) by GC/MS

Analysis identified 21 volatile/aroma compounds in the white wine samples and 26 compounds in the red samples. Compounds, concentrations and OAV are presented in Tables 5 et 6.

PCA analysis of the white wines in Figure 1 separated the volatile compounds into the following quadrants. The upper right quadrant contained ethyl hexanoate (apple), 2-

TABLE 4. Phenolic compounds (median normalised intensity values) identified in (a) white wines and (b) red wines by LC-MS/MS.

(a)	Class	Compound	CH	PG	X1	X2	X3	X4	X5	X6
	Hydrolysable tannin	Gallic acid	4.59	8.05	84.35	17.63	17.41	54.09	36.89	46.50
		Ethyl gallate	7.49	10.37	115.40	25.24	20.88	74.16	44.39	62.88
	Hydroxycinnamate	Caftaric acid	7.05	30.65	80.02	62.18	82.32	42.89	85.38	46.58
		Coutaric acid A	0.65	73.86	20.61	10.70	35.21	26.40	9.66	42.27
		Coutaric acid B	0.57	12.66	27.44	12.77	38.55	20.10	12.33	35.47
		Caffeic acid	120.29	129.25	55.07	70.09	22.37	20.66	73.66	22.55
		Caffeic acid ethyl ester	61.45	65.34	48.10	55.54	11.11	16.91	50.27	15.39
		Fertaric acid	0.59	1.32	1.01	3.55	0.56	0.29	1.47	1.50
	Flavan-3-ol	(+)-Catechin	0.18	0.10	1.54	0.24	1.99	1.17	0.71	1.56
		(-)-Epicatechin	14.79	28.56	15.15	10.68	6.85	7.68	7.59	14.41
		Epigallocatechin	2.50	6.14	28.44	16.75	9.26	12.58	11.73	20.47
	Flavanol	Quercetin-3-O-glucoronide	0.00	1.01	0.26	2.58	0.54	1.50	0.82	20.14
	Unknowns	C7 H12 O5	34.50	102.21	41.84	37.50	42.24	25.45	27.08	23.85
		C10 H11 NO4 S	6.70	4.81	46.94	28.92	177.76	6.42	1.97	126.89
		C15 H28 N2 O4	35.94	40.49	0.81	2.86	8.70	2.22	15.91	2.84

(b)	Class	Compound	M1	M2	SH	M3	Yia
	Hydrolysable tannin	Gallic acid	28.96	16.75	9.00	30.35	14.27
		Ethyl gallate	9.43	4.35	5.31	12.92	5.16
	Hydroxycinnamate	Caftaric acid	25.64	18.78	6.79	35.77	19.10
		Coutaric acid A	43.52	41.55	10.83	71.48	38.92
	Flavan-3-ol	(+)-Catechin	86.30	79.87	77.73	98.50	82.50
		(-)-Epicatechin	44.27	32.04	51.19	42.91	33.45
		Epigallocatechin	0.83	1.40	4.54	1.03	1.51
	Proanthocyanidin	Procyanidin B1 (1)	77.46	63.86	46.14	87.01	60.51
		Procyanidin B1 (2)	39.39	25.14	33.25	41.96	25.19
		Quercetin-3-O-glucoronide	48.83	35.75	1.61	54.99	36.34
		Quercetin-3-O-galactoside	43.47	3.96	0.03	21.19	9.63
		Syringetin-3-O-glucoside	22.47	21.04	47.29	21.29	24.03
	Flavanol	Quercetin	48.24	25.28	100.20	64.13	29.01
		Laricitrin	0.91	1.89	27.47	1.11	1.87
		Fisetin	15.73	1.31	9.44	16.98	1.92
	Unknowns	C15 H10 O8	6.58	11.69	50.00	9.58	10.91
		C16 H12 O7	6.65	6.16	40.55	9.46	7.30
		C30 H26 O13	44.18	31.52	23.16	48.51	30.20

methylpropanol and 3-methylbutanol (solvent) which correlated with X2. The upper left quadrant contained 3-methylbutyl acetate (banana), 2-methylpropyl acetate (banana), ethyl octanoate (pear, pineapple), ethyl butanoate (lactate), ethyl decanoate (floral), 2-phenylethyl acetate (stone fruit, floral), decanoic acid (fat), hexyl acetate (pear, apple), hexanoic acid (leafy, woody), hexanol (fruity) and octanoic acid (butter) which correlated with X4, X6, CH, PG. The lower left quadrant contained ethyl propanoate (fruity) which correlated with X1. The lower right quadrant contained 2-phenylethanol (honey), ethyl-3-methylbutanoate (fruity), butanoic acid (cheese), ethyl-2-methylpropanoate (sweet), ethyl acetate (acetone), acetic acid (vinegar), ethyl-2-methylbutanoate (strawberry), 3-methylbutanoic acid & 2-methylbutanol (solvent), 2-methylbutyl acetate (fruity), 3-methylbutyl acetate (banana), 2-methylbutanoic acid (cheese) and butanol (malty) which correlated with X3, X5.

PCA analysis of the red wines in Figure 2 separated the volatile compounds in the

TABLE 5. Volatile compounds identified in white wine samples.

Family	Compounds	CH	PG	X1	X2	X3	X4	X5	X6	DT	CH OAV	PG OAV	X1 OAV	X2 OAV	X3 OAV	X4 OAV	X5 OAV	X6 OAV
Acids	Acetic acid	82443	91940	247462	199352	206846	346071	23877	238876	20000	4,12	4,59	12,37	9,96	10,34	17,3	11,94	11,94
	Butanoic acid	1697	<LOQ	961	1262	1387	1326	1040	2205	200	8,49	nd	4,81	6,31	6,94	6,63	5,2	11,03
	Hexanoic acid	6071	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6138	420	14,45	nd	nd	nd	nd	nd	nd	30,69
	Octanoic acid	11988	6979	4211	7038	7112	6473	6311	10899	500	23,98	13,96	8,42	14,08	3,01	2,17	2,56	27,99
	Decanoic acid	4464	2836	681	937	1505	1086	1282	1378	1000	4,46	2,84	0,68	0,94	1,51	1,09	1,28	1,38
Alcohols	2-methylpropanol	13027	19865	13740	34016	20065	18302	15741	13993	40000	0,33	0,5	0,34	0,85	0,5	0,46	0,39	0,35
	3-methylbutanol	120594	150944	140360	209280	173834	154846	144071	150217	30000	4,02	5,03	4,68	6,98	5,8	5,16	4,8	5,01
	Hexanol	1801	2007	677	748	848	1326	524	1190	8000	0,23	0,25	0,08	0,09	0,11	0,17	0,07	0,15
	2-phenylethanol	14199	11929	35317	44604	35273	37277	25686	31378	14000	1,01	0,85	2,52	3,19	2,52	2,66	1,83	2,24
Acetate esters	2-methylpropyl acetate	28,8	64,1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1600	0,02	0,04	nd	nd	nd	nd	nd	nd
	3-methylbutyl acetate	2445	4109	220	186	470	377	385	1096	30	81,05	136,97	7,33	6,2	15,67	12,57	12,83	36,53
	Hexyl acetate	353	401	9,94	4,06	20,5	37,8	12,3	88,2	1500	0,24	0,27	0,01	0,001	0,01	0,03	0,01	0,06
	2-phenylethyl acetate	221	215	61,2	53,5	139	96,6	68,1	191	250	0,88	0,86	0,24	0,21	0,56	0,39	0,27	0,76
Ethyl esters	Ethyl acetate	25752	32238	32467	34440	38138	5539	51792	36331	15000	1,72	2,15	2,16	0,23	2,54	3,69	3,45	2,42
	Ethyl propanoate	153	119	150	126	144	162	130	153	1800	0,09	0,07	0,08	0,07	0,08	0,09	0,07	0,09
	Ethyl-2-methylpropanoate	28,4	39,5	118	281	211	204	130	92,7	15	1,89	2,63	7,87	18,73	14,07	13,6	8,67	6,18
	Ethyl butanoate	562	347	259	345	421	349	349	586	20	28,1	17,35	12,95	17,25	21,05	17,45	17,45	29,3
	Ethyl-3-methylbutanoate	<LOQ	<LOQ	43,3	65,6	61,7	41,8	44,4	31,8	3	nd	nd	14,43	21,87	20,57	13,93	14,8	10,6
	Ethyl hexanoate	1426	867	661	1060	1130	975	1014	1526	14	101,86	61,93	47,21	75,71	80,71	69,64	72,43	109
	Ethyl octanoate	1747	1101	827	1074	1257	1128	1131	1555	600	2,91	1,84	1,38	1,79	2,1	1,88	1,89	2,59
	Ethyl decanoate	669	466	145	114	259	206	243	235	200	3,35	2,33	0,73	0,57	1,3	1,03	1,22	1,18

All values reported in µg/L based on single measurements. DT (Detection Threshold), OAV (Odour Activity Value = concentration/DT).

TABLE 6. Volatile compounds identified in red wine samples.

Family	Compounds	M1	M2	SH	M3	Yia	DT	M1 OAV	M2 OAV	SH OAV	M3 OAV	Yia OAV
Acids	Acetic acid	903075	1411587	1253019	2842986	3892968	20000	45,15	70,58	62,65	142,15	194,65
	Propanoic acid	<LOQ	2908	5198	9561	6872	8000	nd	0,36	0,65	1,12	0,86
	Butanoic acid	2611	2036	2981	7189	4860	200	13,1	10,18	14,91	35,95	24,3
	3-methylbutanoic acid	<LOQ	<LOQ	<LOQ	3040	4815	30	nd	nd	nd	101,33	160,5
	Hexanoic acid	6249	5831	6967	16397	12961	420	14,88	13,88	16,59	39,04	30,86
	Octanoic acid	4170	3455	5088	11975	8651	500	8,34	6,91	12,11	28,51	20,6
	Decanoic acid	415	296	1124	2016	1288	1000	0,42	0,3	1,12	2,02	1,29
Alcohols	2-methylpropanol	101109	117621	129219	287726	265075	40000	2,53	2,94	3,23	7,12	6,63
	Butanol	3420	4131	7422	9504	9713	590	5,8	7	12,58	16,11	16,46
	3-methylbutanol	472190	566188	636626	1383105	1393035	30000	15,74	18,87	21,22	46,1	46,43
	2-methylbutanol	161333	195019	215352	462861	472136	1200	134,44	162,52	179,46	385,7	393,45
	Hexanol	2333	2252	8848	12285	6114	8000	0,29	0,28	1,11	1,54	0,76
	2-phenylethanol	100558	107505	117513	248898	267200	14000	7,18	7,68	8,39	17,78	19,09
Acetate esters	3-methylbutyl acetate	570	859	613	1858	1998	30	19	28,63	20,43	61,93	66,6
	2-methylbutyl acetate	87	159	109	274	422	10	8,7	15,9	10,9	27,4	42,2
	Hexyl acetate	4,32	<LOQ	7,84	26	10,9	1500	0,001	nd	0,01	0,02	0,01
	2-phenylethyl acetate	70,1	106	58,8	244	275	250	0,28	0,42	0,24	0,98	1,1
Ethyl esters	Ethyl acetate	132458	213643	214802	446547	540668	15000	8,83	14,24	14,32	29,7	36,04
	Ethyl propanoate	389	431	967	1244	1170	1800	0,22	0,24	0,54	0,69	0,65
	Ethyl-2-methylpropanoate	478	628	825	1229	1579	15	31,87	41,87	55	81,93	105,27
	Ethyl butanoate	586	433	738	1814	1052	20	29,3	21,65	36,9	90,7	52,6
	Ethyl-2-methylbutanoate	80	103	161	177	268	1	80	103	161	177	268
	Ethyl-3-methylbutanoate	132	218	300	355	520	3	44	72,67	100	3,55	173,33
	Ethyl hexanoate	1141	796	1294	2894	1815	14	81,5	56,86	92,43	206,71	129,64
	Ethyl octanoate	925	762	1206	2739	1430	600	1,54	1,27	2,01	4,57	2,38
	Ethyl decanoate	97,5	73,2	350	463	186	200	0,49	0,37	1,75	2,32	0,93

All values reported in µg/L based on single measurements. DT (Detection Threshold), OAV (Odour Activity Value = concentration/DT).

following ways. The upper left quadrant contained ethyl decanoate (pear), hexanol (fruity), decanoic acid (fatty), hexyl acetate (cherry) and ethyl octanoate (pear). The upper right quadrant contained ethyl propanoate (fruity), propanoic acid (pungent) and butanol (solvent) which correlated with SH. The lower left quadrant contained ethyl hexanoate (strawberry), butanoic acid (cheese), hexanoic acid (woody/leafy), octanoic acid (butter) and ethyl butanoate (strawberry) which correlated with M1, M3. The lower right quadrant contained ethyl-2-methylbutanoate (strawberry), 3-methylbutanol & 2-methylbutanol (solvent), ethyl-2-methylpropanoate (sweet), ethyl-3-methylbutanoate (fruity), 2-methylbutyl acetate (fruity), 2-phenylethyl acetate (plum), 3-methylbutyl acetate (banana), 3-methylbutanoic acid (cheese), 2-methylpropanol (solvent), ethyl acetate (fruity), acetic acid (vinegar), 2-phenylethanol (rose, honey), 2-methylpropyl acetate (banana, cherry), 2-methylpropanoic acid (cheese) and 2-methylbutanoic acid (fruity) which correlated with M2, Yia.

5. Spectral analysis and modified Sommers and tannin assays

There have been limited studies on the phenolic content of Cypriot wines, however, our results in Table 7 for total phenolics mirror the work done by Galanakis *et al.* (2015). The only measure that stands out is the total phenolics for X1 at 423.35 mg/L which is very high for a white wine, levels are generally around 200 mg/L (Waterhouse *et al.,* 2016). This is however consistent with the high levels of phenolic compounds such as ethyl gallate, gallic acid and epigallocatechin identified for this wine in the non-volatile profiling of secondary metabolites by LC-MS/MS, non-targeted analysis.

6. Relating wine composition and sensory data by PLS regression

Volatile composition, basic chemical parameters and sensory data determined for eight white and five red wines were analysed through PLS regression to explore their underlying relationship. This PLS approach has been used successfully to evaluate mixed sensory and chemical data sets in Sauvignon Blanc wines (Benkwitz *et al.,* 2012). The first two principal components explained 60% of the variation in white wine composition (x-variables) and 62% of the variation in sensory properties (y-variables). In the red wine samples, the first two principal components explained 79% of the variation in wine composition (x-variables) and 58% of the variation in sensory properties (y-variables).

White wines (Figure 3a and 3b) were separated on the left side of the plot (PG and CH) based on characteristics such as stone fruit, sweet,

TABLE 7. Phenolic and anthocyanin composition of (a) white wine samples and (b) red wine samples.

(a)	Wine code	Total phenolics mg/L (GAE per a.u. @280 nm)	Flavonoid extractives mg/L	Total hydroxycinnamates mg/L
	CH	86.5	35.75	34
	PG	68	0.25	46
	X1	423.3	365	39
	X2	86	33.75	35
	X3	53.75	11.5	28
	X4	30.1	80	32
	X5	84.5	30.25	34
	X6	124.3	68	37

(b)	Wine code	Free anthocyanins mg/L	Total tannins mg/L	Total phenolics mg/L (GAE per a.u. @280 nm)
	M1	136	3220	2075
	M2	154	2360	1775
	SH	127	2030	1625
	M3	186	2430	1825
	Yia	147	2510	1825

All values reported in mg/L based on single measurements.

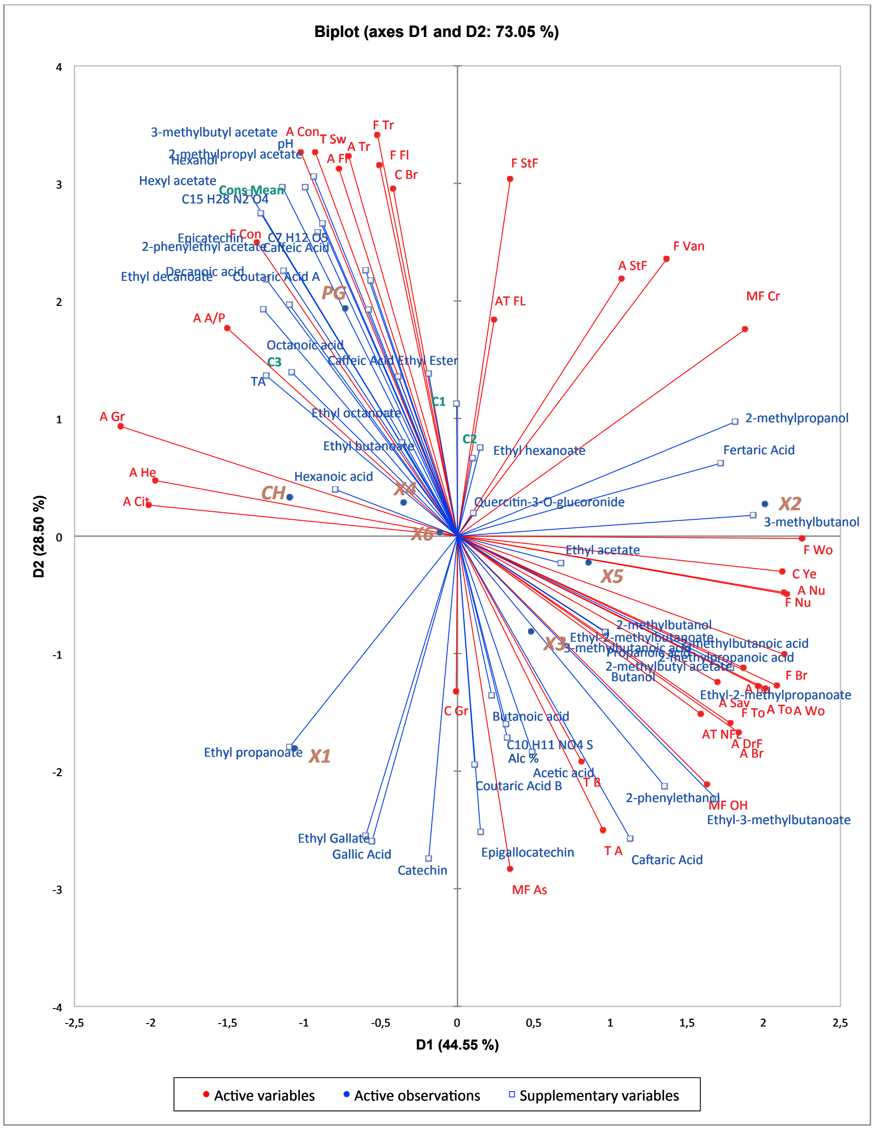


FIGURE 1. PCA biplot of white wine samples generated from correlation with chemical compounds and sensory attributes.

Sensory attributes (red), Chemical compounds (blue), Wines (orange), Consumer mean and Clusters (green). Colour Brown (CB), Colour Green (CGr), Colour Yellow (CYe), Aroma Apple Pear (AA/P), Aroma Citrus (ACit), Aroma Dried Fruit (ADrF), Aroma Stone Fruit (AStF), Aroma Confectionary (ACon), Aroma Tropical (ATr), Aroma Floral (AFl), Aroma Grass (AGr), Aroma Herbal (AHe), Aroma Butter (Abu), Aroma Nutty (ANu), Aroma Savoury (ASav), Aroma Toast (ATo), Aroma Wood (AWo), Aroma Bread (ABr), Taste Bitter (TB), Taste Sweet (TSw), Taste Acid (TA), Flavour Stone Fruit (FStF), Flavour Confectionery (FCon), Flavour Tropical (FTr), Flavour Floral (FFl), Flavour Nutty (FNu), Flavour Toast (FTo), Flavour Wood (FWo), Flavour Vanilla (FVan), Flavour Bread (FBr), Mouth Feel Alcohol (MFOH), Mouth Feel Astringent (MFAs), Mouth Feel Creamy (MFCr), After Taste Fruit Length (ATFL), After Taste Non-Fruit Length (ATNFL).

confectionery, tropical, floral, herbaceous, citrus, apple and pear. These characteristics correlated with fruity aroma compounds such as hexanol, hexyl acetate, 3-methylbutyl acetate and 2-methylpropyl acetate. Wines on the right side of the plot (X1, X2, X3, X5, X5, X6) had more astringent, bitter, savoury, bread, wood, toasty, alcohol characteristics. In particular X2, X3, X5 in the upper right quadrant exhibited more developed, secondary characteristics associated with oak intervention and ageing. These characteristics correlated with compounds such as 2-phenylethanol, ethyl-3-methylbutanoate, ethyl-2-methylpropanoate, 3-methylbutanol and 2-methylpropanol. X1, X4, X6 in the lower right quadrant were associated with bitterness, astringency and green characteristics, which correlated to compounds such as ethyl acetate,

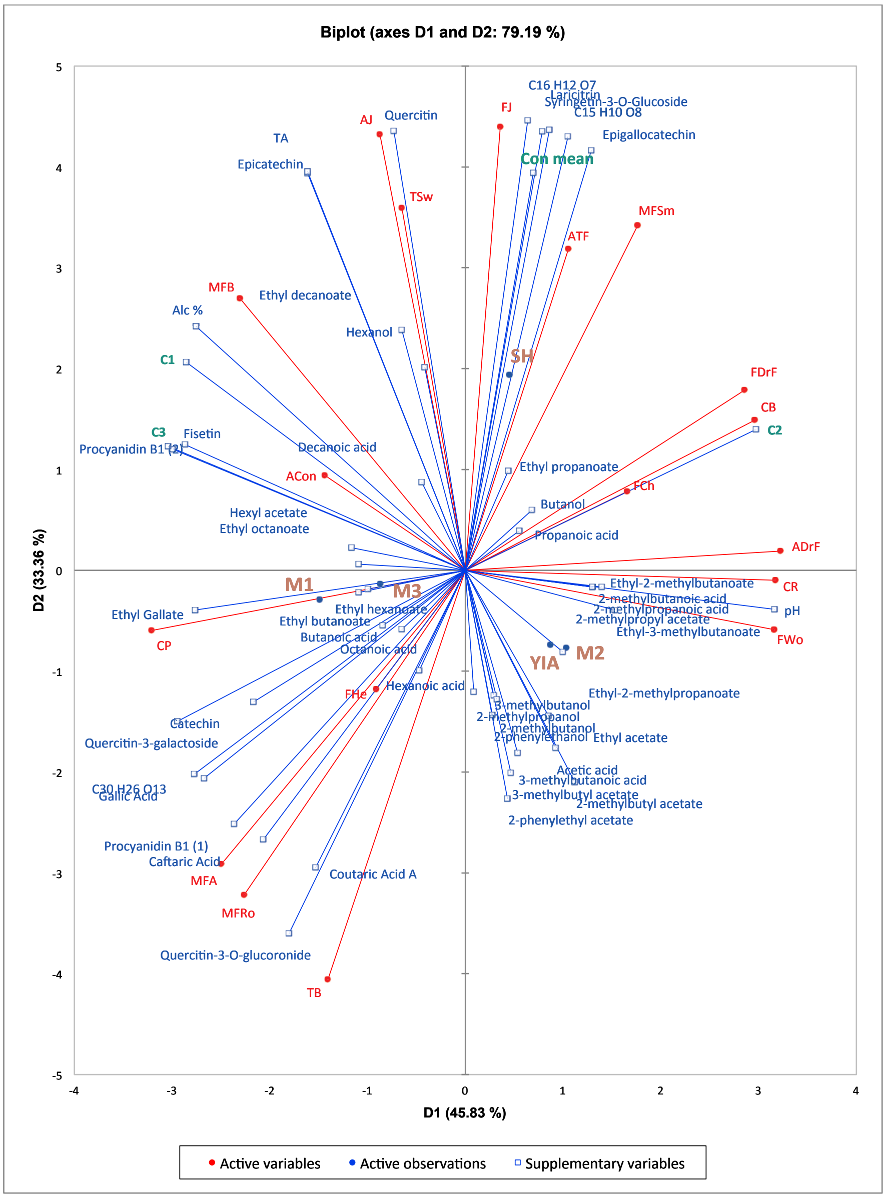


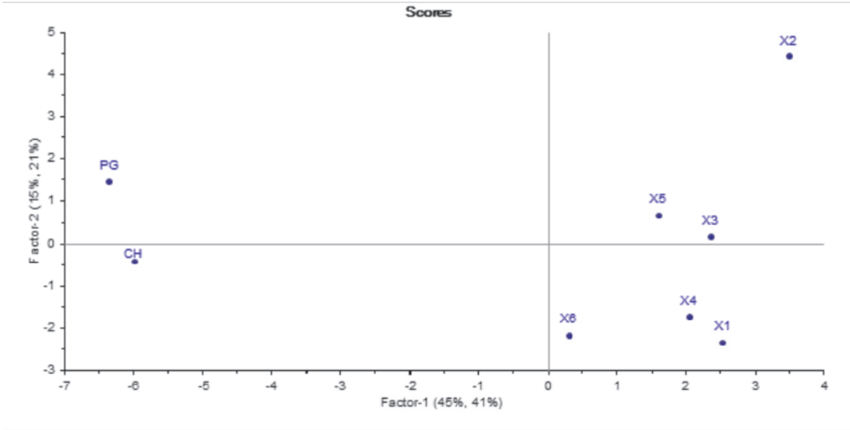
FIGURE 2. PCA biplot of red wine samples generated from correlation with chemical compounds and sensory attributes.

Sensory attributes (red), Chemical compounds (blue), Wines (orange), Consumer mean and Clusters (green). Colour Red (CR), Colour Purple (CP), Colour Brown (CB), Aroma Dried Fruit (ADrF), Aroma Jammy (AJ), Aroma Confectionery (ACon), Taste Bitter (TB), Taste Sweet (TSw), Flavour Dried Fruit (FDrF), Flavour Jammy (FJ), Flavour Chocolate (FCh), Flavour Herbal (FHe), Flavour Wood (FWo), Mouth Feel Bitter (MFB), Mouth Feel Astringent (MFAs), Mouth Feel Smooth (MFSm), Mouth Feel Rough (MFRo).

ethyl propanoate, butanoic acid, acetic acid, catechin, epigallocatechin and coutaric acid.

Red wines (Figure 4a and 4b) were separated on the left side of the plot (M1, M3) based on characteristics such as bitterness, astringency, herbal and confectionary, while wines on the right side of the plot (M2, Yia, SH) were separated based on characteristics such as toast, woody, dried fruit, jammy, sweet and fruity after taste. M3 in the upper left quadrant correlated to compounds such as hexyl acetate, ethyl octanoate, ethyl hexanoate, butanoic acid, hexanoic acid and octanoic acid. Sample Yia, which was close to the centre line in the upper right quadrant, correlated with propanoic acid, butanol, ethyl-2-methylbutanoate, ethyl-2-methylpropanoate, ethyl-3-methylbutanoate, acetic acid and ethyl propanoate. SH was associated with compounds such as epigallocatechin, laricitrin, quercetin and syringettin-3-O-glucoside. M2 in the lower right

(a)



(b)

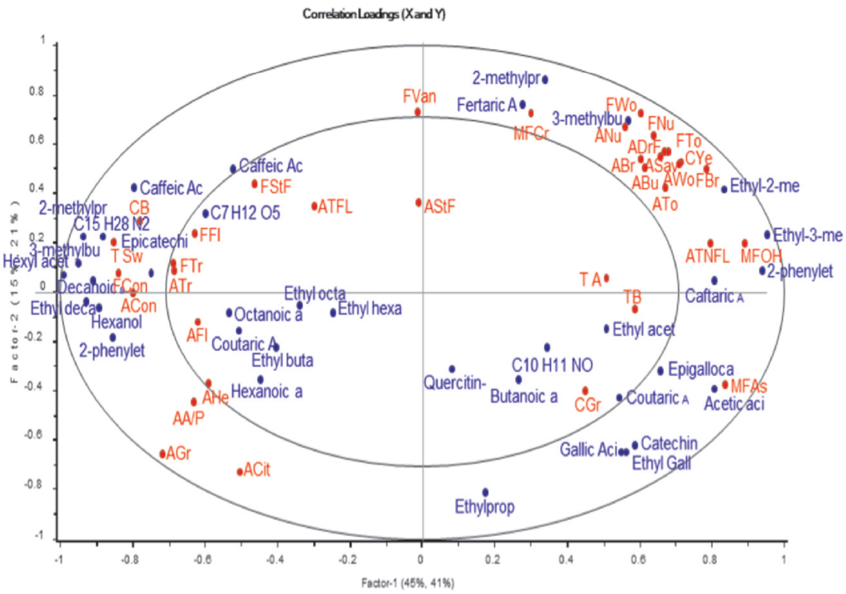


FIGURE 3. (a) PLS Regression plots of standardised volatile aroma compounds in white wines.
(b) Correlation loadings between chemical (blue) and sensory (red) data, 50% (inner), 100% (outer) explained variance limits.

Colour Brown (CB), Colour Green (CGr), Colour Yellow (CYe), Aroma Apple Pear (AA/P), Aroma Citrus (ACit), Aroma Dried Fruit (ADrF), Aroma Stone Fruit (AStF), Aroma Confectionary (ACon), Aroma Tropical (ATr), Aroma Floral (AFl), Aroma Grass (AGr), Aroma Herbal (AHe), Aroma Butter (Abu), Aroma Nutty (ANu), Aroma Savoury (ASav), Aroma Toast (ATo), Aroma Wood (AWo), Aroma Bread (ABr), Taste Bitter (TB), Taste Sweet (TSw), Taste Acid (TA), Flavour Stone Fruit (FStF), Flavour Confectionery (FCon), Flavour Tropical (FTr), Flavour Floral (FFl), Flavour Nutty (FNu), Flavour Toast (FTo), Flavour Wood (FWo), Flavour Vanilla (FVan), Flavour Bread (FBr), Mouth Feel Alcohol (MFOH), Mouth Feel Astringent (MFAs), Mouth Feel Creamy (MFCr), After Taste Fruit Length (ATFL), After Taste Non-Fruit Length (ATNFL).

quadrant correlated with epicatechin and M1 in the lower left quadrant correlated with quercetin, fisetin and procyanidin B1.

DISCUSSION

In summary this was the first detailed sensory, chemical and consumer study of wines made from the indigenous Cypriot grape varieties Xynisteri, Maratheftiko and Giannoudhi. This work has built on previous work from other authors (Constantinou *et al.,* 2017; Constantinou *et al.,* 2018a; Constantinou *et al.,* 2018b; Galanakis *et al.,* 2015; Kokkinofta *et al.,* 2017).

For a better understanding as to why these wines are liked by Australian consumers, it is necessary to try and understand the relationship between sensory compounds and quality. Sáenz-Navajas *et al.* (2015) have previously studied

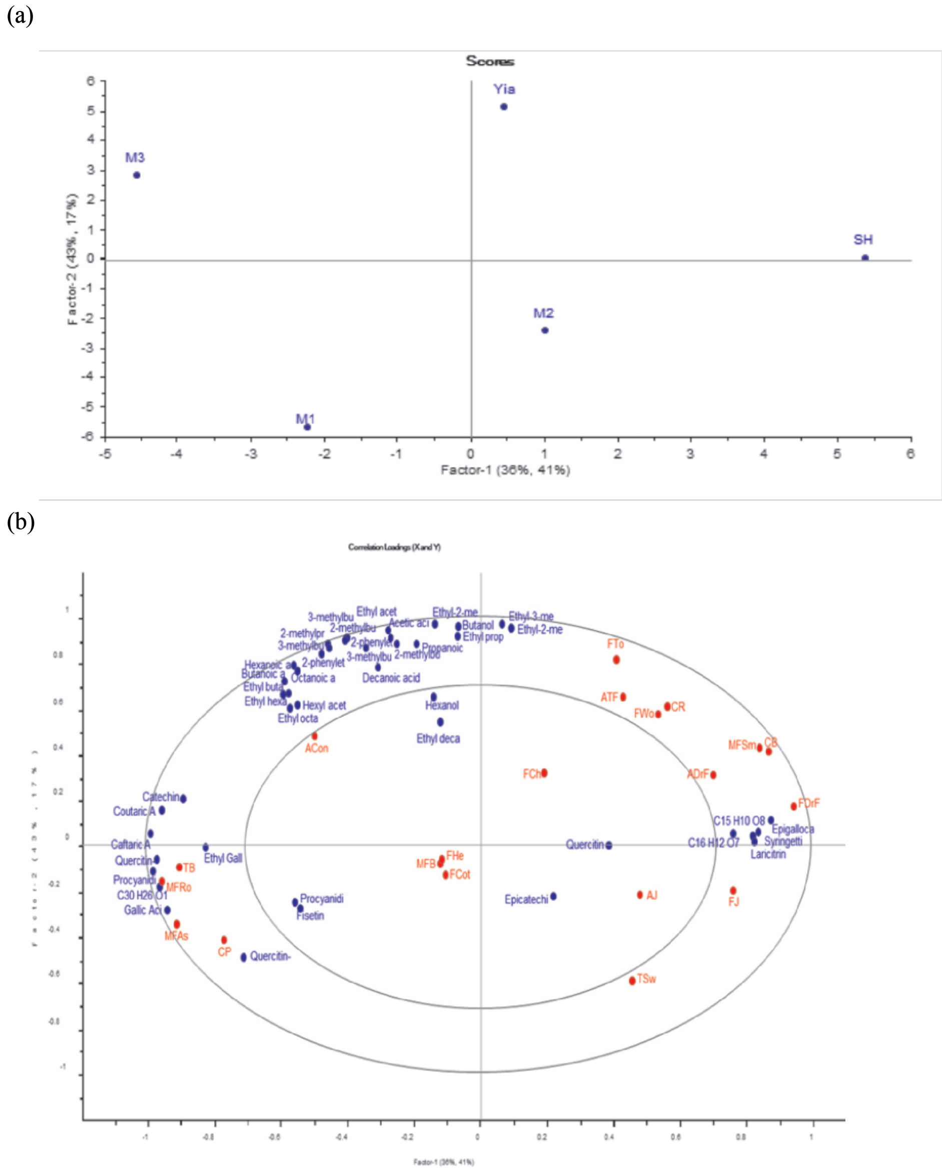


FIGURE 4. (a) PLS Regression plots of standardised volatile aroma compounds in red wines.
(b) Correlation loadings between chemical (blue) and sensory (red) data 50% (inner), 100% (outer) explained variance limits.
Chemical compounds (Blue), Sensory attributes (Red). Colour Red (CR), Colour Purple (CP), Colour Brown (CB), Aroma Dried Fruit (ADrF), Aroma Jammy (AJ), Aroma Confectionery (ACon), Taste Bitter (TB), Taste Sweet (TSw), Flavour Dried Fruit (FDrF), Flavour Jammy (FJ), Flavour Chocolate (FCh), Flavour Herbal (FHe), Flavour Wood (FWo), Mouth Feel Bitter (MFB), Mouth Feel Astringent (MFAs), Mouth Feel Smooth (MFSm), Mouth Feel Rough (MFRo).

sensory active compounds in red wine (predominately Tempranillo and Grenache) that influence wine experts and consumers perception of quality. They found that there was a difference between consumers and experts in terms of relating sensory compounds and wine quality. Their consumers linked high quality with oak ageing and leather-like compounds, while the wine experts linked high quality with red fruity aromas (Sáenz-Navajas *et al.,* 2015). A study by Johnson *et al.* (2013) involving wine experts concur with Sáenz-Navajas *et al.* (2015), with wine experts preferring berry fruit, spice, red fruit, dark fruit and oak characteristics to developed and savoury characteristics in Shiraz wines. Likewise, Niimi *et al.* (2018) had difficulties predicting wine quality from sensory profiling wines. Winemakers were consistently able to sort Cabernet-Sauvignon wines based on quality but found that Chardonnay wines were poorly discriminated in both sensory profiles and quality.

When relating wines made from the indigenous Cypriot varieties to other varieties, the following characteristics have been explored in terms of being positive or negative: for white wine King *et al.* (2010) explored Sauvignon Blanc wines made with different yeast strains. They found that flavours such as bruised apple, cooked, estery and floral aromas were not well liked while the box hedge/cat urine aromas were liked by both consumers and winemakers. Ali *et al.* (2011) studied the sensory attributes of Riesling and Mueller Thurgau. Their 'superior' wines were found to contain high levels of amino acids (proline and arginine), organic acids (malic and tartaric) and phenolic compounds (quercetin, catechin and epicatechin). Poor quality wines contained higher levels of lactic, acetic, and succinic acids, as well as amino acids (threonine and alanine) and phenolic compounds (caffeic acid, gallic acid and vanillic acid). Riesling was found to have higher levels of catechin, epicatechin, caftarate and coutarate. González-Álvarez *et al.* (2011) explored the sensory and chemical profile of wines made from the Spanish white variety Godello. They found that the sensory descriptors with the highest intensity were fruity (apple, citrus), floral aromas and herbaceous notes. The chemical compounds attributed to these compounds were ethyl esters, acetates, fatty acids and terpenes. Danish researchers Liu *et al.* (2015) analysed sensory and chemical composition of Solaris wines and found that 3-methyl-1-butanol, 3-methylbutyl acetate, ethyl acetate and ethyl hexanoate are important amongst the 79 compounds identified. Acetates and ethyl esters of fatty acids were correlated with floral and fruity aromas. The positive sensory attributes were described as floral and fruity (peach/apricot, Muscat, melon, banana and strawberry) while the negative attributes were described as chemical, wood and rooibos/smoke.

Many of these positive attributes have also been identified from our analysis of Xynisteri which was described sensorially as citrus, herbaceous, bitter, astringent, creamy, alcohol, dried fruit, bread, savoury, toast, wood, nutty, apple, pear, grass, herbaceous with a full length of fruit and non-fruit flavours in the after taste. Some of these attributes such as toast, wood, creamy and nutty however, are related to the wine making process and the use of oak barrels and are not grape variety attributes. Chemical analysis supported sensory analysis with aroma compounds of ethyl propanoate (fruity), 2-phenylethanol (honey), ethyl-3-methylbutanoate (fruity), ethyl acetate (acetone), ethyl-2-methylpropanoate (sweet), 3-methylbutanol & 2-methylbutanol (solvent), hexanoic acid (leafy, woody), ethyl octanoate (pear, pineapple), hexanoic acid (leafy, woody) and ethyl butanoate (lactate) identified in wines. Phenolic compounds of catechin, caftaric acid, epigallocatechin, coutaric acid B, epigallocatechin, ethyl gallate and gallic acid and have been associated with quality in Riesling wines (González-Álvarez *et al.,* 2011).

Shiraz is the most widely planted and consumed red variety in Australia; it was therefore chosen to assist in benchmarking the red Cypriot varieties (Australian Bureau of Statistics, 2015). Shiraz sensory quality has been described by Li *et al.* (2017) as having aromas of red fruit, dark fruit, and confectionary, as well as flavours of jam, and high intensity along with five palate attributes: sweetness, palate fullness, astringency, surface coarseness, and hotness. These characteristics have been linked to ethyl acetate, ethyl 2-methylpropanoate, 2-methyl-propyl acetate, ethyl butanoate, ethyl 3-methylbutanoate, ethyl hexanoate, ethyl lactate, ethyl octanoate, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol (Li *et al.,* 2017).

When comparing the phenolic content of Cypriot varieties to Greek varieties Agiorgitiko, Xinomavro and Mandilaria, the Cypriot varieties have an equivalent total phenolic content to Agiorgitiko and less phenolics than Xinomavro and Mandilaria and have been shown to be less astringent than these two varieties (Kallithraka *et al.,* 2011). The same can be said for total tannins, Maratheftiko and Giannoudhi exhibit equal or less total tannins than Greek varieties Araklinos, Bakouri, Fidia, Karvounaris, Kotselina, Limniona, Mavrotragano, Nerostafilo, Papadiko and Thrapsa (Kallithraka *et al.,* 2015).

Koussissi *et al.* (2007) employed a sensory profiling of aroma in Greek wines using a rank rating technique. They investigated Agiorgitiko, Xinomavro, Syrah and Cabernet Sauvignon and found that Agiorgitiko wines differentiated from the other wines by aroma characteristics of floral, vanilla, caramelised (confectionery), fruity and berry. Xinomavro has been linked to high astringency and bitter/sour taste (Koussissi *et al.,* 2003). Cypriot red wines, Maratheftiko and Giannoudhi therefore compare favourably with common European varieties and less common Greek varieties being described sensorially as dried fruit, jammy, confectionery, bitter, sweet, chocolate, herbaceous, woody, astringent and rough with full length of fruit flavours in the after taste. The Cypriot wines were also assessed to have aroma compounds that contributed to the above attributes, that is: strawberry, sweet, fruity, banana, cherry, pear, woody/leafy, and butter. As with the Xynisteri wines, the attributes of buttery and wood are due to the use of barrels in the wine making process and are not direct varietal attributes.

It is also worth noting that due to the small number of wine samples available for this preliminary study, it is difficult to make in depth comparisons with the more common European varieties. However, when we consider these quality parameters above and the consumer data generated in this study, we can speculate that the wines made from Cypriot varieties are comparable to common Australian wines and potentially similar to other quality European wines made from varying grape varieties.

These studies have provided us with useful information which will be followed up with further in-depth studies to investigate specific phenolic compounds by LC-MS/MS (targeted, quantitative analysis) as well as analysis of thiols and terpenes with repeated measures, along with further quantitative analysis of specific aroma compounds by GC/MS with repeated measures. Further RATA studies of Cypriot wines may involve research wines made from different locations and standardised wine making techniques to eliminate any wine making influence on the sensory analysis.

We believe that these studies have given wine producers in Australia and Cyprus further insight into a few of the popular Cypriot grape varieties and how Australian consumers might respond to these wines in the market place. Considering the similar climates of Australia and Cyprus, it is also predicted that these Cypriot grape varieties will be a source for environmentally sustainable wines which require less resources and aid in the future adaptation of the wine industry to a changing climate.

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Chapter 4. Published Article 2: Preliminary investigation of potent thiols in Cypriot wines made from indigenous grape varieties Xynisteri, Maratheftiko and Giannoudhi.

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Overall percentage (%)	65%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

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Preliminary investigation of potent thiols in Cypriot wines made from indigenous grape varieties Xynisteri, Maratheftiko and Giannoudhi

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ABSTRACT

Polyfunctional thiols have previously been shown to be key aroma compounds in Sauvignon blanc and more recently in Chardonnay wines. Their role in other wine varieties such as those made from three popular indigenous Cypriot grape varieties has remained unexplored. As an extension of a previous project that profiled the sensory and chemical characteristics of Cypriot wines and their comparison to Australian wines, this study aimed to investigate five potent thiols in Xynisteri, Maratheftiko, Giannoudhi, Pinot gris, Chardonnay and Shiraz wines.

Wines were analysed utilising Stable Isotope Dilution Assay (SIDA) with derivatisation and High-Performance Liquid Chromatography–Tandem Mass Spectrometry (HPLC-MS/MS). The varietal thiols measured were 4-methyl-4-sulfanylpentan-2-one (4MSP) that has an aroma of "boxwood" and "cat urine" at high concentration, 3-sulfanylhexan-1-ol (3SH) which has been described as having a "grapefruit/tropical fruit" aroma, and 3-sulfanylhexyl acetate (3SHA) that has also been described as having an aroma of "passionfruit". Additionally, two other potent thiols were measured including benzyl mercaptan (BM) that has an aroma of "smoke and meat" and furfuryl thiol (FFT) that has been described as having a "roasted coffee" like aroma. The reason these thiols are known as potent thiols are due to their very low aroma detection thresholds in the low ng/L (ppt) range. Of the thiols that were measured, 3SH was the only varietal thiol detected in the red wine samples. All of the white wine samples contained 3SH, BM and 3SHA, whereas 4MSP was only detected in Pinot gris and three Xynisteri wines. The potent thiol, FFT, was detected only in the Chardonnay and four of the Xynisteri wines. Interestingly the thiols that were present in the samples were found at concentrations above their aroma detection thresholds (determined in hydroalcoholic solutions), especially 3SH which was found in an order of magnitude above its aroma detection threshold. These findings provide early knowledge of the presence of these thiols in Cypriot wines, compared with Australian wines and establish any relationships between this chemical data with previous wine sensory profile data.

KEYWORDS

varietal thiols, sensory analysis, chemical analysis, consumer preference, Xynisteri, Maratheftiko, Giannoudhi

INTRODUCTION

The indigenous grape varieties of Cyprus have become the focus of increasing interest in recent times, with several studies investigating the chemical profiles of their juice, must and wines (Copper *et al.,* 2019; Constantinou *et al.,* 2019; Galanakis *et al.,* 2015; Tsiakkas *et al.,* 2020; Kokkinofta *et al.,* 2014). These studies have mainly reported on the phenolic compounds, volatile compounds and sensory characteristics. Varietal thiols are one such group of aroma compounds that have not been examined in wines made from Cypriot grape varieties to date. One possible reason for this is that polyfunctional thiols have a low sensory threshold and are present at trace concentrations (nanograms per litre). They are missed in conventional wine analysis as it is almost impossible for them to be detected directly with Gas Chromatography/Mass Spectrometry (GC/MS) and require specialised methods (Capone *et al.,* 2018). The polyfunctional thiols 4-methyl-4-sulfanylpentan-2-one (4MSP), 3-sulfanylhexan-1-ol (3SH) and 3-sulfanylhexyl acetate (3SHA) are one such group of compounds. More recently, Capone *et al.* (2015) have developed a simplified method whereby the free thiols, including benzyl mercaptan (BM) and (furan-2-yl)-methanethiol also referred to as 2-furfurylthiol (FFT), are derivatised and then analysed using HPLC-MS/MS.

These compounds are potent with extremely low detection thresholds and have been described as possessing aromas of "boxwood", "cat urine", "passionfruit", "grapefruit", "tropical fruit", "smoke" and "roasted coffee" (Dubourdieu and Tominaga, 2009; Table 1). They have been shown to be important aroma compounds in Chardonnay, Sauvignon blanc, Chenin blanc, Grechetto, Pinot noir, Pinot gris, Riesling, Gewürztraminer, Sylvaner and some French red wine blends (Capone *et al.,* 2015; Capone *et al.,* 2018; Coetzee *et al.,* 2018; Maslov-Bandić *et al.,* 2018; Cerreti *et al.,* 2017; Rigou *et al.,* 2014). Thiols have also been identified in indigenous Italian varieties Trebbiano di Lugana (Mattivi *et al.,* 2012) as well as Catarratto Bianco Comune and Grillo (Fracassetti *et al.,* 2018).

King *et al.* (2011) reported that not only did all three thiols (3-SH, 3-SHA and 4-MSP) contribute to the "tropical" and "cat urine/sweaty" aroma in wine, in combination with 3-isobutyl-2-methoxypyrazine (IBMP), they can contribute to a "cooked green vegetal" aroma in Australian Sauvignon blanc wines.

Interestingly, Australian consumers preferred wines containing a combination of varietal thiols and IBMP (a cluster containing 43 %) compared with wines possessing higher "tropical" and "confectionery" aromas (a cluster containing 31 %) (King *et al.,* 2011). Given Australian consumer's liking of "tropical" and "confectionery" characters (Copper *et al.,* 2019) and that they are important in other varieties, their contribution in Cypriot varieties remains unexplored. Therefore, this study aimed to assess the thiol composition of the indigenous Cypriot varieties, which in the future could allow producers to tailor their products to suit consumer preferences.

TABLE 1. Polyfunctional thiols in wine, their characteristic aromas and their aroma detection thresholds (determined in hydroalcoholic or model solutions).

Thiols	4MSP	3SHA	3SH	BM	FFT
Sensory descriptor	Boxwood, blackcurrant	Passionfruit, tropical, boxwood	Grapefruit, tropical, passionfruit	Smoke, toasty, struck flint	Roasted Coffee
Odour detection threshold (ng/L)	0.8	4	60	0.3	0.4
Concentration range in commercial Chardonnay wines (ng/L)	0[a]–23[b]	0[a]–100[b]	10[b]–1368[a]	30–40[c]	14[c]
Concentration range in commercial Sauvignon Blanc wines (ng/L)	0–88[d]	0–106[d]	350–5664[d]	0.6–5.5[d]	1–36[d]

a: Capone *et al.* (2018); b: Mateo-Vivaracho *et al.* (2010); c: Gambetta *et al.* (2014); and d: Capone *et al.* (2015). 4MSP: 4-methyl-4-sulfanylpentan-2-one; FFT: furfuryl thiol; 3SH: 3-sulfanylhexan-1-ol; BM: benzyl mercaptan; 3SHA: 3-sulfanylhexyl acetate.

MATERIALS AND METHODS

1. Wine samples

The wines used in this study were previously reported Copper *et al.* (2019). They included 2016 Xynisteri (n = 5), 2015 Xynisteri (n = 1), 2017 Australian Pinot gris (n = 1) and 2017 Australian Chardonnay (n = 1). The red wines 2015 Maratheftiko (n = 2), 2013 Maratheftiko (n = 1), 2014 Cypriot Giannoudhi (n = 1) and 2014 Australian Shiraz (n = 1). The wines were chosen as they were common brands, readily available at wine retailers and were spread across a range of price points (5-20 € for Cypriot wines. $20-$25AUD for Australian wines). Further detailed information on the wines are provided in Table 2.

2. Chemicals and materials

Analytical reagents were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia) unless otherwise specified. Unlabelled and deuterium-labelled standards were synthesised as previously described in Capone *et al.* (2015). This included: 3-SH, [$^2H_{10}$]-3-sulfanylhexan-1-ol (d_{10}-3-SH), 3-SHA, [$^2H_5$]-3-sulfanylhexyl acetate (d_5-3-SHA), 4-MSP, [$^2H_{10}$]-4-methyl-4-sulfanylpentan-2-one (d_{10}-4-MSP) (Howell *et al.,* 2004; Swiegers *et al.,* 2007; Pardon *et al.,* 2008), [$^2H_5$]-2-furfurylthiol (d_5-FFT) and [$^2H_5$]-benzyl mercaptan (d_5-BM) (Capone *et al.,* 2015). Bond Elut C18 (500 mg, 6 mL) solid-phase extraction (SPE) cartridges were purchased from Agilent Technologies (Mulgrave, Vic, Australia).

3. Chemical analysis of wine thiols

Samples were prepared as described by Capone *et al.* (2015), an aliquot of wine (20 mL) was added into a 22 mL glass screw cap vial with a Teflon lined cap, labelled standards, d_{10}-4-MSP, d_{10}-3-SH, d_5-3-SHA, d_5-FFT and d_5-BM, each with a final concentration of 500 ng/L was added. An addition of ethylenediaminetetraacetic acid disodium salt (20 mg), 50 % acetaldehyde (80 µL) and freshly thawed 4,4′-dithiodipyridine reagent (10 mmol, 200 µL) proceeded. After 30 min, the sample was passed through a pre-conditioned Bond Elut C18 cartridge (6 mL, 500 mg, Agilent Technologies, Forest Hill, Vic., Australia) as previously detailed. The eluate was collected, concentrated and reconstituted with 10 % ethanol (200 µL) and analysed on an Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA, USA) using a 250 × 2.1 mm i.d., 5 µm, 100-Å Alltima® C18 column (Grace Davison Discovery Sciences, Rowville, Vic., Australia) coupled to an Agilent 6410A Triple Quad MS (Agilent, Santa Clara, CA, USA) in electrospray ionisation mode as described in Capone *et al.* (2015). Data acquisition was performed using Agilent Mass Hunter Workstation software (version 10.0). For accurate quantification, duplicate standards in the appropriate wine matrix were prepared at the time of analysis with 4-MSP at concentrations of 0, 11.5, 38, 50, 75 and 100 ng/L; 3-SH at 0, 620, 1824, 2400, 3650 and 5000 ng/L; 3-SHA at 0, 120, 380, 500, 775 and 1025 ng/L; FFT and BM at 0, 12.5, 37.5, 50, 200 and 400 ng/L.

TABLE 2. Basic wine chemical data, oak treatment use and other information of wines used in thiol analysis.

Code	Vintage	Wine	pH	TA (mg/L)	Alc (%)	Oak
M1	2015	Maratheftiko	3.43	5.86	14.8	Yes
M2	2013	Maratheftiko	3.62	5.45	13.2	Yes
M3	2015	Maratheftiko	3.44	5.88	14.5	Yes
SH	2014	Shiraz	3.57	6.13	14.5	Yes
Yia	2014	Giannoudhi	3.65	5.5	13.4	Yes
CH	2017	Chardonnay	3.33	7.35	12.9	No
PG	2017	Pinot Gris	3.54	6.65	12.5	No
X1	2016	Xynisteri	3.21	5.93	12.8	No
X2	2015	Xynisteri	3.26	5.94	12.8	Yes
X3	2016	Xynisteri	3.22	5.52	13.7	No
X4	2016	Xynisteri	3.35	5.44	12.8	No
X5	2016	Xynisteri	3.16	4.72	12.6	No
X6	2016	Xynisteri	3.42	5.02	12.6	No

X4-contained 5 % Muscat in addition to Xynisteri. Table adapted from Copper *et al.* (2019).

Duplicate quality control checks were also prepared with 4-MSP at concentrations of 0, 25, 50, 75 and 100 ng/L; 3-SH at 0, 1200, 2400 and 5000 ng/L; 3-SHA at 0, 250, 500 and 1025 ng/L; FFT and BM at 0, 25, 50 and 400 ng/L.

4. Sensory analysis

The Rate-All-That-Apply (RATA) technique was utilised for descriptive sensory profiling of the wines. RATA is a rapid sensory profiling method used to describe the intensity of only the sensory characteristics actually perceived in wines employing a rating scale (Danner *et al.,* 2017). Wines were presented sequentially, monadically, blind and in a random order to the tasters to overcome serving order effects. Wines were served in clear International Standards Organisation (ISO) tasting glasses at 15 °C. Tasters were required to select only the attributes that were applicable to the wine and additionally indicate the perceived intensity of these sensory attributes using a seven-point rating scale as previously reported by Copper *et al.* (2019).

5. Statistical analysis

Basic chemical data were processed with Microsoft Excel 2010. Chemical data are presented as mean values with standard deviation from duplicate determinations and analysed by one-way ANOVA (sample) using XLSTAT (version 2018.7, Addinsoft SARL, Paris, France). The significantly different means of the chemical and sensory data were subjected to partial least squares (PLS) regression using The Unscrambler (version 10.5.1, CAMO Software AS, Oslo, Norway) with chemical parameters (X-variables) and sensory data from Rate-All-That-Apply (RATA) (Y-variables). All variables were normalised before analysis and significance set at $p < 0.05$.

RESULTS AND DISCUSSION

The only thiol detected in red wines was 3SH. Maratheftiko (M3) had a significantly higher concentration than the other two Maratheftiko wines (M1 and M2) and was similar in concentration to Shiraz (SH) and Giannoudhi (Yia) (Table 3). The levels of 3SH ranged from 164–172.8 ng/L, which was lower than that detected by Rigou *et al.* (2014) in French red wine blends (Syrah, Grenache, Mourvedre, Cinsault, Carignana, Grenache noir) which ranged from 678–11,487 ng/L. Regardless of this, the concentration of 3SH measured in the samples was above the aroma detection threshold (60 ng/L in hydroalcoholic solution), meaning this thiol could contribute to “blackcurrant” aroma as described by Rigou *et al.* (2014) or as “berry”, “jam”, “earthy”, “plum” and “soy” aromas as recently reported by Garrido-Bañuelos *et al.* (2020). Although these compounds were identified in Bordeaux red wines several years ago, there is limited quantitative and sensory data available for red varieties, most likely due to previous difficulties in measuring these compounds at threshold concentrations (Capone *et al.,* 2015). Recently, Mafata *et al.* (2018) quantified 4 thiols (3SH, 3SHA, 4MSP and FFT) using ultraperformance convergence chromatography-tandem mass spectrometry in South African Shiraz, Cabernet-Sauvignon and Pinotage, the reported 3SH levels for all three varieties ranged from 76–363 ng/L.

TABLE 3. Concentration of thiols in red and white wine samples (ng/L).

Code	4MSP	3SHA	3SH	BM	FFT
M1	nd	nd	165b	nd	nd
M2	nd	nd	164b	nd	nd
M3	nd	nd	172a	nd	nd
SH	nd	nd	167ab	nd	nd
Yia	nd	nd	167ab	nd	nd
	nd ≤ 1	nd ≤ 3		nd ≤ 2	nd ≤ 3
CH	nd	47b	486d	9.9a	3.7c
PG	3.6cd	54a	663a	7.7b	nd
X1	nd	39d	642b	6.7cd	nd
X2	nd	39d	444f	7.1c	12.9a
X3	3.9bc	39d	426g	8b	nd
X4	5.8a	40d	546c	5.7e	4c
X5	4.2b	39d	407h	6e	5.1b
X6	3.5d	42c	464e	6.5d	3.4c
	nd ≤ 3.4				nd ≤ 3

Different letters next to the concentration of each compound indicate significant differences ($p < 0.05$), between white or red wines.

Concentrations containing the same letters are not statistically significantly different. nd — not detected. M1 = 2015 Maratheftiko; M2 = 2013 Maratheftiko; M3 = 2015 Maratheftiko; SH = 2014 Shiraz; Yia = 2014 Giannoudhi; CH = 2017 Chardonnay; PG=2017 Pinot gris; X1 = 2016 Xynisteri; X2 = 2015 Xynisteri; X3, X4, X5, X6 = 2016 Xynisteri.

4MSP: 4-methyl-4-sulfanylpentan-2-one; FFT: furfuryl thiol, 3SH: 3-sulfanylhexan-1-ol; BM: benzyl mercaptan; 3SHA: 3-sulfanylhexyl acetate.

In the white wine samples, 4MSP was only detected in Pinot gris (PG) and four Xynisteri wines. Xynisteri (X3, X4, X5) had the highest levels, Pinot gris had the second-lowest levels with Xynisteri (X6) the lowest, ranging from 3.5-5.8 ng/L. These were all above the detection threshold of 0.8 ng/L (in hydroalcoholic solution) and above the 1 ng/L threshold (determined in a dearomatised white wine, with added flavour compounds), reported by Mateo-Vivaracho *et al.* (2010) required to contribute to "green" and "fruity" aromas.

The highest level of 3SHA was detected in Pinot gris followed by Chardonnay (CH), whereas all Xynisteri were present at lower concentrations. 3SHA ranged from 38.6–53.7 ng/L which was above the detection threshold and consistent with levels seen by Coetzee *et al.* (2018) in South African Sauvignon blanc wines, which ranged from 23–151 ng/L.

3SH was highest in Pinot gris followed by Xynisteri (X1) and ranged from 426.7–663.7 ng/L and were both above the detection threshold. This range was similar to those reported by Coetzee *et al.* (2018) in South African Sauvignon blanc.

BM was highest in Chardonnay followed by Xynisteri (X3) then Pinot gris, which ranged from 5.7–9.9 ng/L this concurred with Capone *et al.* (2015) who reported that Chardonnay and Pinot gris had higher levels when compared to some sparkling wines and may contribute to "smoky", "struck flint" characteristics. Capone *et al.* (2015) also suggested that BM may be a varietal character of importance to Pinot gris. Consequently, BM has the potential to be important in Xynisteri wines as well and warrants further investigation.

FFT was only detected in Chardonnay and four Xynisteri wines and ranged from 3.4–12.9 ng/L. The highest level was in Xynisteri (X2) and may be attributable to its fermentation and ageing in oak barrique. Capone *et al.* (2015) reported that Chardonnay wines aged in oak also had higher levels of FFT and attributed this to it being a Maillard reaction product and associated with exposure to toasted oak. Interestingly, no FFT was detected in any of the red wine samples despite being aged in oak, possibly due to the binding of melanoidin and FFT as has been shown to occur in coffee (Hofmann and Schieberle, 2002).

Volatile composition, basic wine chemical parameters and sensory data from the previous work by Copper *et al.* (2019) were re-analysed with thiol data included. PLS regression was utilised to explore the underlying relationship and determine if the model was improved with the inclusion of the thiol data. In the red wine samples, the first two factors explained 75 % of the variation in wine composition (x-variables) and 63 % of the variation in sensory properties (Y-variables), Figure 1a and b. The Maratheftiko sample M3 had the highest concentration of 3SH and was correlated with the attributes of smooth mouthfeel, fruity after taste, jammy aroma and jammy palate, which are similar to the blackcurrant aromas descriptor reported by Rigou *et al.* (2014).

In the white wines, the first two factors explained 58 % of the variation in the chemical composition (x-variables) and 61 % of the variation in sensory properties (y-variables), Figure 2a and b. However, utilising three factors explained an additional 12 % of the sensory variation (data not shown), now explaining 73 % of the sensory difference. Including the additional thiols slightly improved the previous model reported by Copper *et al.* (2019) from 60 % (x-variables) and 62 % (y-variables). Xynisteri (X2) correlated with FFT and "bread", "wood", "toasty" and "buttery" characteristics and these associations can be further demonstrated in Figure 3, by the regression coefficients for "bread" and "wood" aroma and flavour as well as "butter" aroma at or very close to 0.1. The remaining Xynisteri wines (X1, X3, X4, X5, X6) correlated with 4MSP aroma and had more astringent and bitter characteristics. Pinot gris and Chardonnay correlated with BM, 3SHA and "stone fruit", "sweet", "confectionery", "tropical", "floral", "herbaceous", "citrus", "apple" and "pear" characteristics. 3SH in the lower right quadrant did not correlate with any of the wines, but correlated with "grassy", "floral", "apple/pear", "confectionery" and "citrus" aromas. Regression coefficients of "grassy", "citrus" and "herbal" aroma showed a strong association with 3SH (Figure 4a, 4c, 4d, respectively), whereas "tropical" aroma was strongly associated with 3SHA (Figure 4b).

The thiols that were detected in all the Cypriot white wines were above threshold levels; however, the Australian Pinot gris and Chardonnay had better correlation with the desirable thiol 3SHA and its "passionfruit/tropical" characteristics. One explanation for this could be the age difference of the wines. Apart from Xynisteri (X2), which was aged in oak for 6 months, the remaining Xynisteri wines were at least six months older

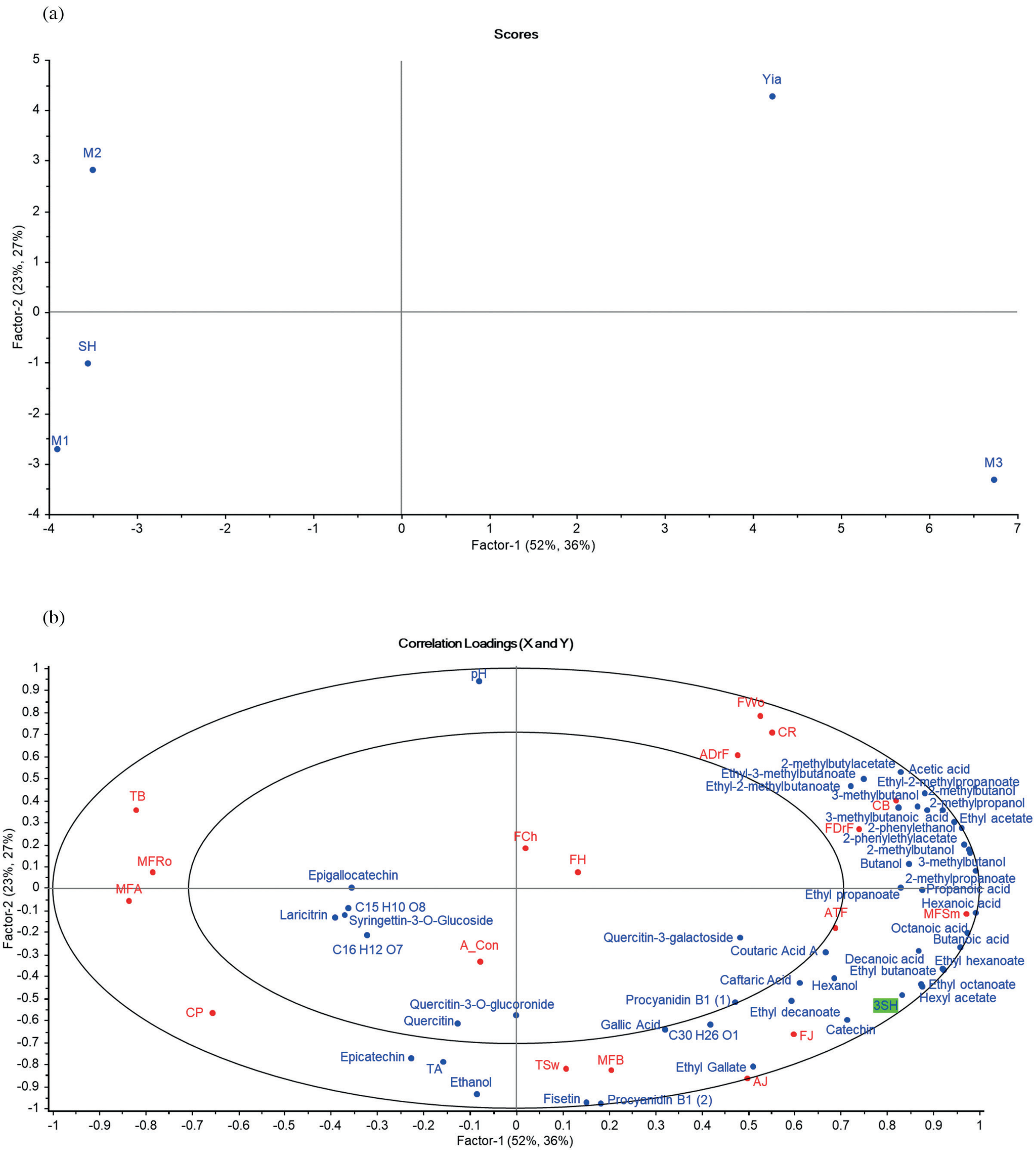

FIGURE 1. Adapted from Copper et al. (2019). (a) PLS Regression plots of standardised volatile aroma and thiol compounds (green) in red wines. (b) Correlation loadings between chemical (blue) and sensory (red) data 50 % (inner), 100 % (outer) explained variance limits.

Chemical compounds (Blue), Sensory attributes (Red). Colour Red (CR), Colour Purple (CP), Colour Brown (CB), Aroma Dried Fruit (ADrF), Aroma Jammy (AJ), Aroma Confectionery (ACon), Taste Bitter (TB), Taste Sweet (TSw), Flavour Dried Fruit (FDrF), Flavour Jammy (FJ), Flavour Chocolate (FCh), Flavour Herbal (FH), Flavour Wood (FWo), Mouth Feel Bitter (MFB), Mouth Feel Astringent (MFA), Mouth Feel Smooth (MFSm), Mouth Feel Rough (MFRo), After Taste Fruit (ATF). 3SH: 3-sulfanylhexan-1-ol. Maratheftiko (M1, M2, M3), Giannoudhi (Yia), Shiraz (SH).

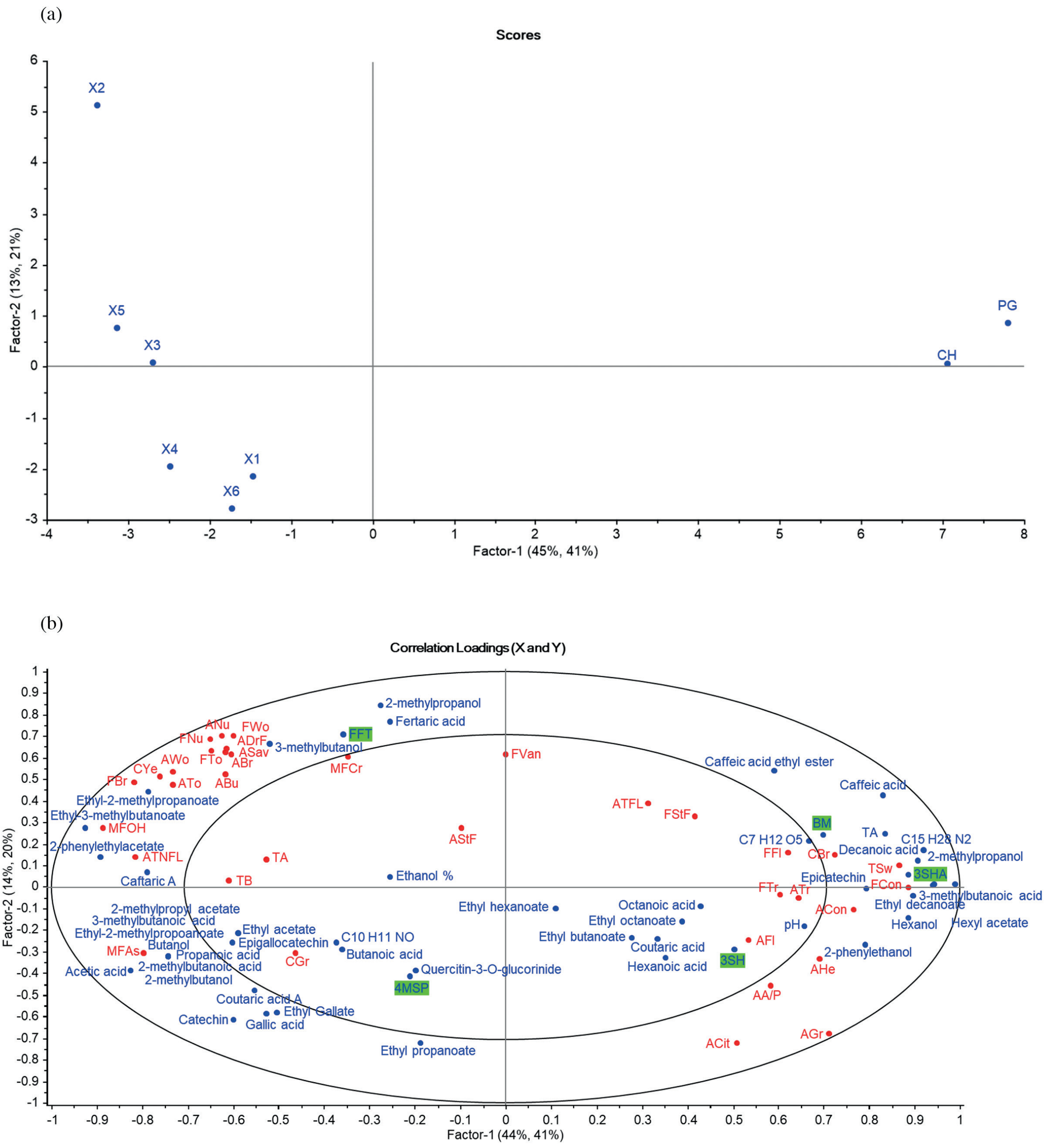

FIGURE 2. Adapted from Copper et al. (2019). (a) PLS Regression plots of standardised volatile aroma and thiol compounds (green) in white wines. (b) Correlation loadings between chemical (blue) and sensory (red) data, 50 % (inner), 100 % (outer) explained variance limits.

Colour Brown (CB), Colour Green (CGr), Colour Yellow (CYe), Aroma Apple Pear (AA/P), Aroma Citrus (ACit), Aroma Dried Fruit (ADrF), Aroma Stone Fruit (AStF), Aroma Confectionery (ACon), Aroma Tropical (ATr), Aroma Floral (AFl), Aroma Grass (AGr), Aroma Herbal (AHe), Aroma Butter (Abu), Aroma Nutty (ANu), Aroma Savoury (ASav), Aroma Toast (ATo), Aroma Wood (AWo), Aroma Bread (ABr), Taste Bitter (TB), Taste Sweet (TSw), Taste Acid (TA), Flavour Stone Fruit (FStF), Flavour Confectionery (FCon), Flavour Tropical (FTr), Flavour Floral (FFl), Flavour Nutty (FNu), Flavour Toast (FTo), Flavour Wood (FWo), Flavour Vanilla (FVan), Flavour Bread (FBr), Mouth Feel Alcohol (MFOH), Mouth Feel Astringent (MFAs), Mouth Feel Creamy (MFCr), After Taste Fruit Length (ATFL), After Taste Non-Fruit Length (ATNFL). 4MSP: 4-methyl-4-sulfanylpentan-2-one, FFT: furfuryl thiol, 3SH: 3-sulfanylhexan-1-ol, BM: benzyl mercaptan, 3SHA: 3-sulfanylhexyl acetate.

Xynisteri (X1, X2, X3, X4, X5, X6), Pinot gris (PG), Chardonnay (CH)

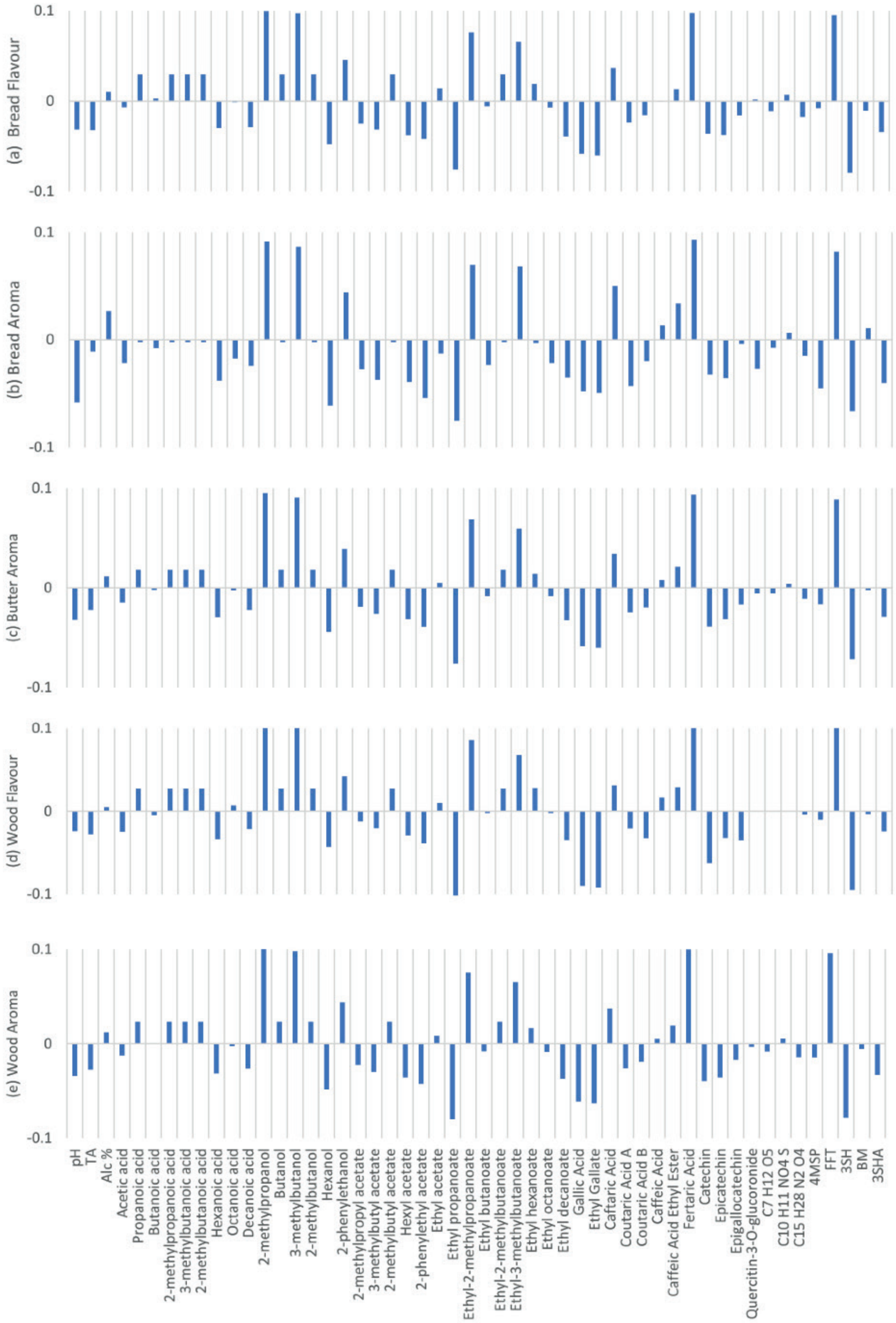

FIGURE 3. Regression coefficients from partial least squares model, relating relative volatile composition for the white wines: (a) bread favour, (b) bread aroma, (c) butter aroma, (d) wood aroma and (e) wood flavour.

4MSP: 4-methyl-4-sulfanylpentan-2-one; FFT: furfuryl thiol; 3SH: 3-sulfanylhexan-1-ol; BM: benzyl mercaptan; 3SHA: 3-sulfanylhexyl acetate.

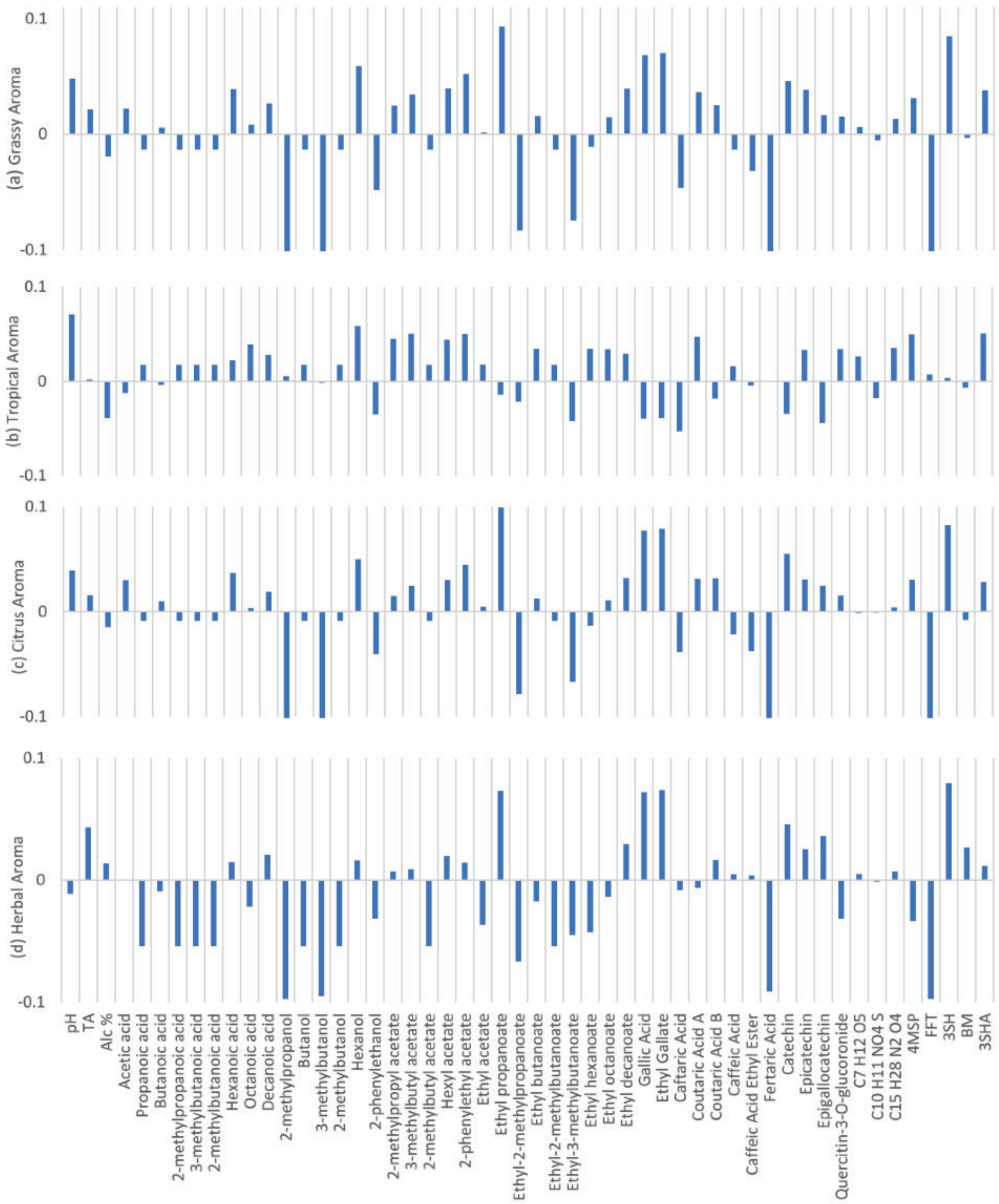

FIGURE 4. Regression coefficients from partial least squares model, relating relative volatile composition for the white wines: (a) grassy aroma, (b) tropical aroma, (c) citrus aroma and (d) herbal aroma.

4MSP: 4-methyl-4-sulfanylpentan-2-one; FFT: furfuryl thiol; 3SH: 3-sulfanylhexan-1-ol; BM: benzyl mercaptan; 3SHA: 3-sulfanylhexyl acetate.

than the Australian wines, having been bottled in late 2016 or early 2017, alternatively, this may also be attributable to masking/suppression and synergistic effects as has been shown previously in Chenin blanc (Wilson *et al.,* 2019) and in red wine (Garrido-Bañuelos *et al.,* 2020). Herbst-Johnstone *et al.* (2011) report that thiol concentrations in Sauvignon blanc wines were not stable and that between 62 % and 76 % of 3SHA had been lost seven months after bottling. Therefore, for the preservation of these compounds, bottle storage conditions can be an important issue. Thiol concentration in wines can also be altered pre-fermentation for example they can be enhanced with particular treatments of grapes and must prior to fermentation. Additionally, Capone *et al.* (2012) report that storing fruit at 10 °C prior to crushing can lead to an increase in 3SH precursors. Chen *et al.* (2019) also demonstrated that freezing grapes and musts to –20 °C prior to fermentation dramatically increased varietal thiol precursors and thiol levels in the finished Sauvignon blanc wine. Furthermore, Maggu *et al.* (2007) demonstrated that increasing skin contact time, less clarified juice (higher turbidity) and a greater press pressure also resulted in higher concentrations of 3SH precursors, most likely due to a greater concentration of precursors located in the skins (Roland *et al.,* 2011).

CONCLUSION

This study measured the concentration of varietal thiols in a small selection of Cypriot wines and the concentration determined in these wines was comparable to those found in popular Australian wines such as Chardonnay and Sauvignon blanc. These varietal thiols are important compounds in certain varieties when "fruity", "tropical" and "citrus" aromas are desired. While this study was a preliminary investigation, it highlights the importance of thiols in white wines, however, their role in red wines is not well understood.

Further investigation into the role of thiols in Cypriot wines could involve analysis of a much greater number of samples and vintages to evaluate its distribution. Winemaking practices that affect thiols, such as the handling of the grapes prior to fermentation, could be applied to these varieties to be able to meet the desired wine style, whether it be to enhance or reduce these characteristics.

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Chapter 5. Published Article 3: Vine performance benchmarking of indigenous Cypriot grape varieties Xynisteri and Maratheftiko.

Statement of Authorship

Title of Paper	Vine performance benchmarking of indigenous Cypriot grape varieties Xynisteri and Maratheftiko
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
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Principal Author

Name of Principal Author (Candidate)	Alexander Copper		
Contribution to the Paper	Designed and constructed the research experiments, analysed the data, drafted and constructed the manuscript		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	19/11/21

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Supervised development of work and manuscript evaluation		
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Signature		Date	30/7/2021

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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

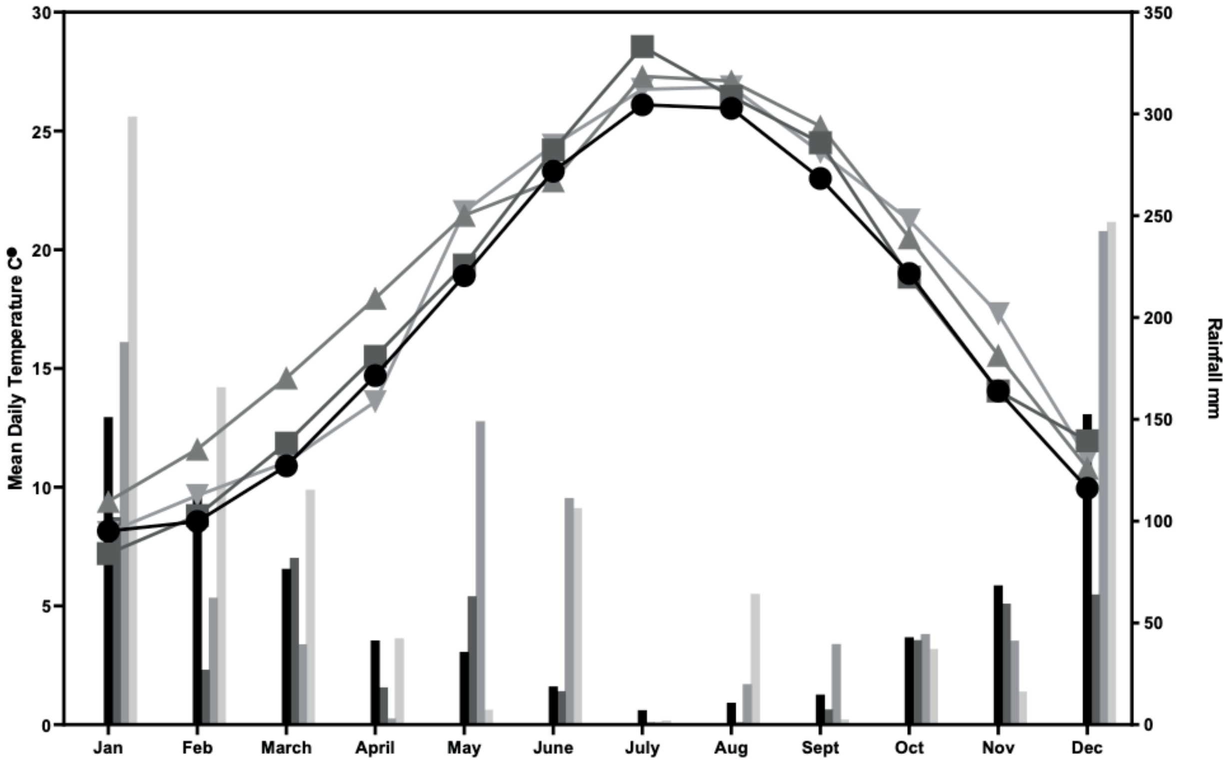

FIGURE 1. Mean daily temperature and rainfall per month for the three seasons studied compared to the long-term average.

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 (cm^3)
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/m^2/s for EX prior to harvest in late September 2017 and 435 mmol/m^2/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/m^2/s in early June to 194 mmol/m^2/s in late September. Meanwhile, Xynisteri ranged from 303 mmol/m^2/s in early July to 121 mmol/m^2/s in late September. Shiraz performed in a similar way to Xynisteri with SC ranging from 362 mmol/m^2/s in early June to 188 mmol/m^2/s in late August. Mean SC for Sauvignon blanc ranged from 272 mmol/m^2/s in early June to 182 mmol/m^2/s in early August.

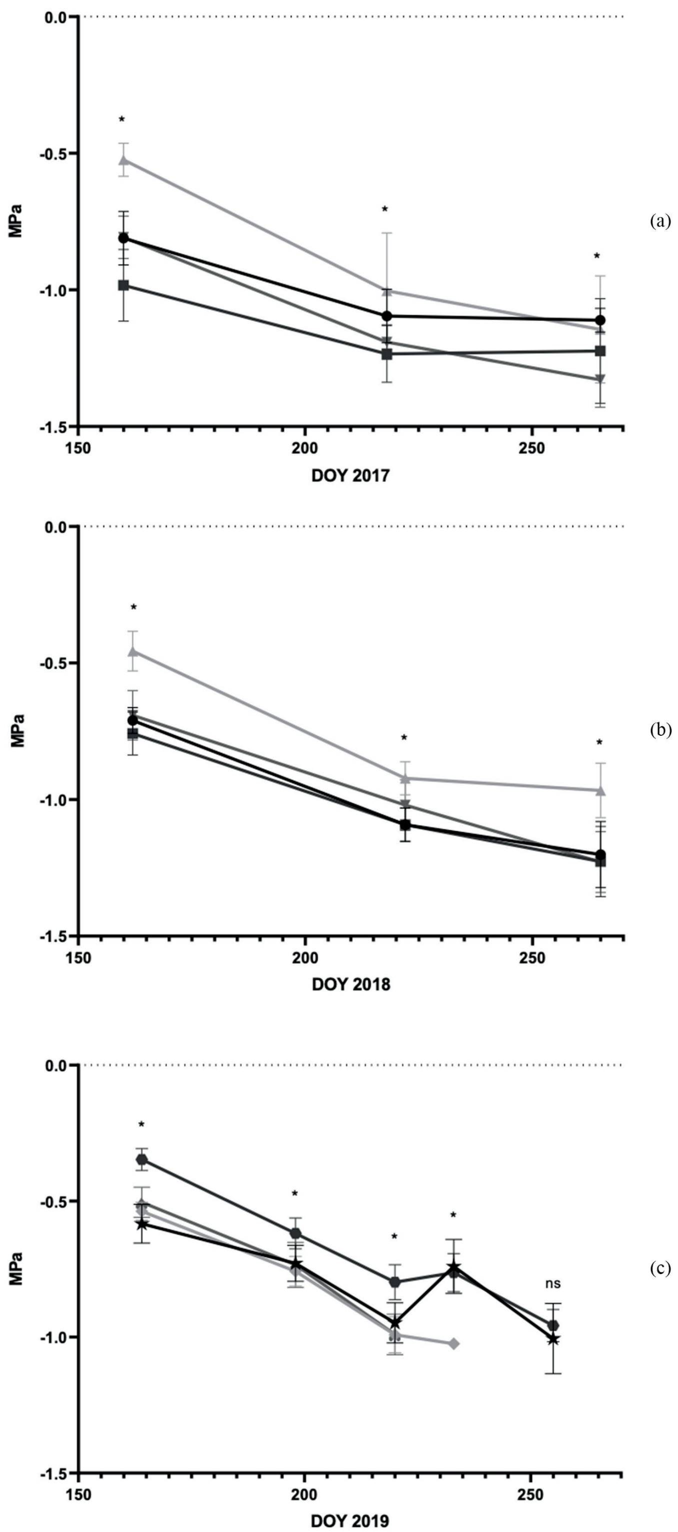

FIGURE 3. Relationship between midday stem water potential measurements (MPa) versus days of year (DOY).

(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 $P < 0.05$

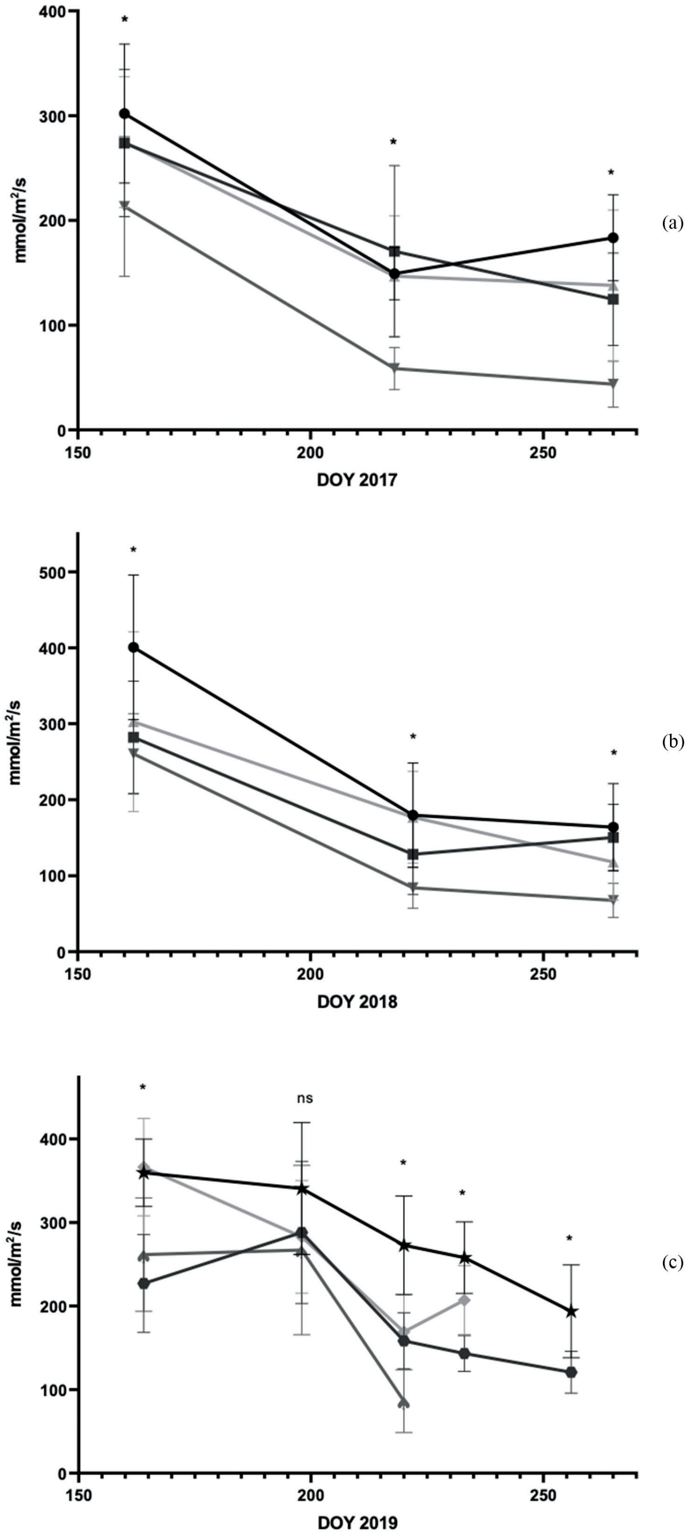

FIGURE 4. Stomatal conductance measurements at midday (mmol/m²/s) versus days of year (DOY).

(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 $P < 0.05$.

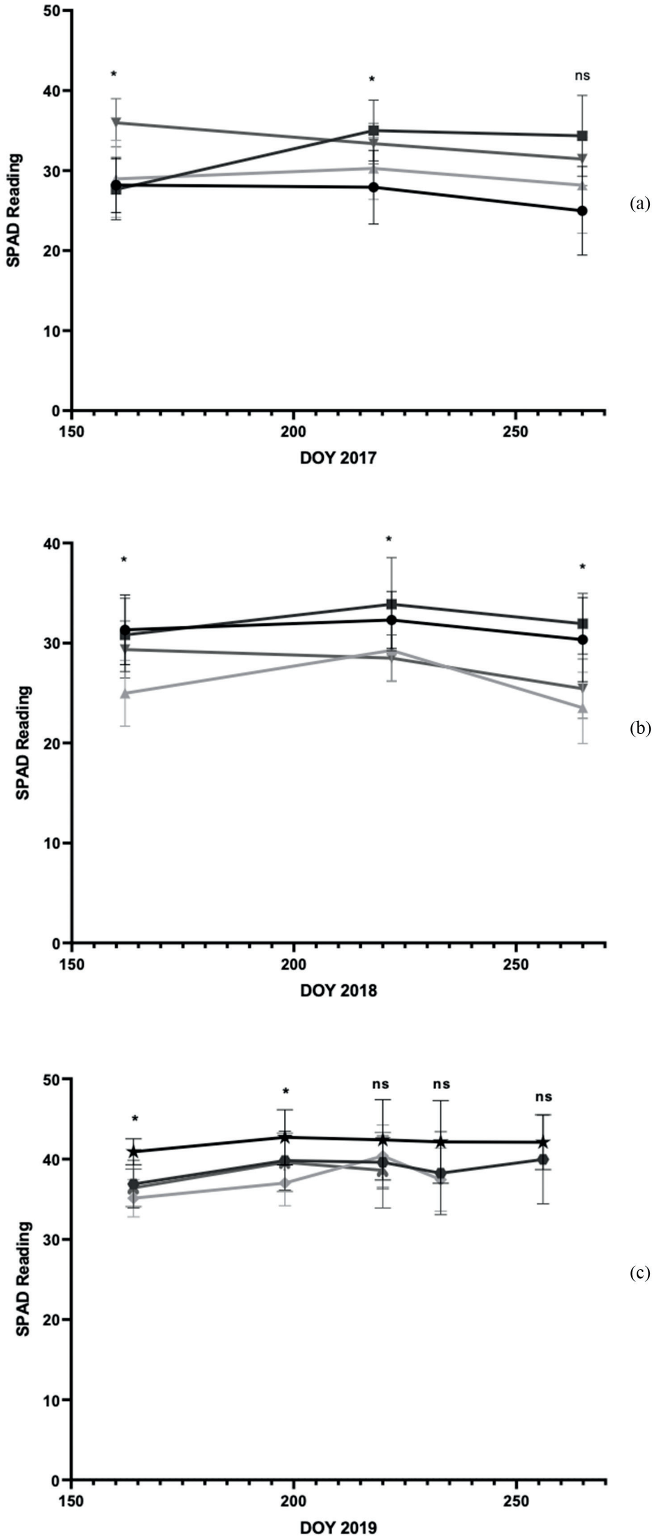

FIGURE 5. SPAD reading at midday for chlorophyll content versus days of year (DOY).

(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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Chapter 6. Published Article 4: Assessing the response of *Vitis vinifera* L. cv. Xynisteri to different irrigation regimes and its comparison to cvs. Maratheftiko, Shiraz and Sauvignon Blanc.

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Contribution to the Paper	Designed and constructed the research experiments, analysed the data, drafted and constructed the manuscript.		
Overall percentage (%)	90%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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Assessing the Response of *Vitis vinifera* L. cv. Xynisteri to Different Irrigation Regimes and Its Comparison to cvs. Maratheftiko, Shiraz and Sauvignon Blanc

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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. This study investigated whether two Cypriot varieties (Xynisteri and Maratheftiko) responded differently to Shiraz and Sauvignon Blanc grown under different irrigation regimes (full, 50% and 25%). Irrigation trials were established in Cyprus in 2019 and in Australia in 2020/2021. Vine growth and physiology and fruit composition (field trial only) measurements were recorded. The trial in Cyprus in 2019 demonstrated that for all three irrigation regimes, Xynisteri had higher stem water potential, stomatal conductance, chlorophyll and greater biomass than Sauvignon Blanc under all irrigation regimes. In 2020/2021, Xynisteri had a greater biomass than Maratheftiko and Sauvignon Blanc, with Shiraz having the lowest. Under reduced irrigation, Xynisteri and Maratheftiko had higher stem water potential, stomatal conductance and chlorophyll content than Shiraz and Sauvignon Blanc. 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.

Keywords: Cyprus; indigenous grape varieties; vine growth; vine physiology

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1. 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 [1–15]. 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 [16] with a recent upsurge in interest and research into heat and drought tolerance and a return to the cultivation of their indigenous varieties [17–20]. Studies have involved investigating the indigenous Cypriot red variety Maratheftiko by conducting a trial that compared tillage and no tillage with irrigated and non-irrigated treatments [21]. 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. Additionally, 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 [22].

In a vineyard and in a potted trial, the performance of Xynisteri and Chardonnay were compared under different irrigation and tillage regimes [23,24]. 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 [25]. Historical data in Greece was reviewed and 16 indigenous Greek and 13 international varieties cultivated across 14 different regions were assessed for harvest dates, potential alcohol and titratable acidity levels. The study 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 may be potentially better adapted to future warmer climates [25].

These studies highlight the possibility of indigenous varieties from the Eastern Mediterranean being cultivated with reduced irrigation and being suitable for wine producers growing grapevines in areas where water is limited. 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 [26]. Volumetric water content is commonly used in potted trials and has been used to study Maratheftiko grown in small (8 L) containers [24]. Volumetric water content is believed to be 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 [27]. ET estimates also assume that the crop has access to unlimited water resources, which is often not the case in container-grown crops [28]. Midday leaf water potential has been studied for its use in scheduling deficit irrigation of vineyards, and it has been concluded that the method can increase the precision of irrigation with highly repeatable results [29]. To date, no studies have looked at the specific irrigation rates required for the optimal growth of Xynisteri and Maratheftiko, and their irrigation limits remain largely unknown.

Therefore, the aims of this exploratory 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. Thereby testing the hypothesis that Cypriot cultivars show good agronomic and physiological behaviour under semi-arid and hot conditions.

2. Materials and Methods

2.1. 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, South Australia,

Australia. Both potted trials were performed under field conditions rather than controlled environments. Trial one was established in a commercial Xynisteri vineyard, latitude 34°53′ N longitude 32°99′ E and elevation 840 m. The vineyard was planted at a density of 3300 vines per hectare, with 1.5 m vine spacing by 2 m row spacing. The soil was calcaric, leptic (rocky), cambisol with a texture of clay loam, pH 7.6 and organic matter 2.8%. 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 L per vine/0.14 mL per hectare), 50% (22 L 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 has identified the possibility of different clones (biotypes) within the germplasm of Xynisteri from different regions [17]. 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 latitude 34°58′ S longitude 138°38′ E, soil type was hard pedal red duplex with clay to 300 cm.

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 four nodes and were approximately 20 cm 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 L 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 [30]. Prior to the trial commencing, all pots received 8 L 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 L per vine, 50% was 4 L per vine and 25% was 2 L 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.

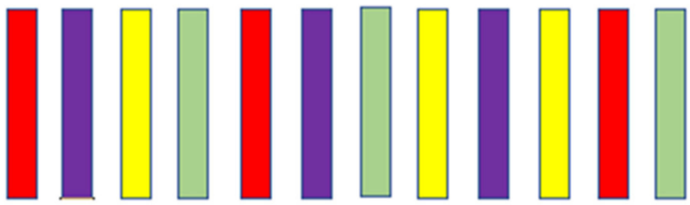

Figure 3. Randomised block design of pots in South Australia in 2020/2021. Green—Xynisteri. Yellow—Sauvignon Blanc. Red—Maratheftiko. Purple—Shiraz. n = 7 vines per block.

2.2. Measurements

2.2.1. 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. 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, 0.02 km from the trial site).

2.2.2. Vine Performance Measures

Vine growth measurements were made at flowering (EL–21), 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).

2.2.3. Physiology Measures

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, 2020/2021.

Site	Start Date	End Date	Total Days	Measurements
Cyprus vineyard trial 2019	11 June (EL–21)	26 September (EL–38)	107	5
Cyprus potted trial 2019	19 July (EL–33)	24 September (EL–38)	67	6
Australian potted trial 2020/2021	16 December (EL–33)	27 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 measurement, in order to allow leaf water potential to equilibrate [31]. After the equilibration period, the leaves were cut with a sharp blade and the stem water potential measured. A maximum of 60 s 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 [32].

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 [33] using a SPAD 502 Meter 2900 (Minolta, Tokyo, 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 2020/2021.

2.2.4. Stomatal Density

Stomatal density was determined by selecting one leaf per vine from the varieties using a modified method described in Hilu and Randall [34]. 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. The 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 and Sauvignon Blanc, and mean values for each variety were determined.

2.2.5. Statistical Analysis

Measurements were analysed 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 using Tukey's Honest Significance Difference (HSD) post hoc tests. Two-way ANOVA was used to assess the effects of irrigation, variety and their interaction (irrigation * variety) on root, shoot and leaf mass in the potted trials.

Two fixed factors (time and irrigation) and one interaction factor (time * irrigation) were analysed using the repeated measure ANOVA Restricted Maximum Likelihood (REML) method. p-values < 0.05 were considered significant. The REML method was employed as it allows for changing variances and is commonly used in experiments where some treatments (for example, different spacings, crops growing over time, treatments that include a control) have a changing variance structure [35].

3. Results

3.1. 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 is 129 mm (1 April–30 September). The long-term average MJT for Adelaide is 22.6 °C and growing season rainfall is 140 mm (1 October–31 March).

The potted trial site in 2019 received 49 mm of rain during the testing period (July–September average 33 mm) and the 2020/2021 trial site received 127 mm (December–February average 42 mm) during the testing period. The mean daily temperature for the 2019 testing period was 24 °C and 21 °C in 2020/2021 (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).

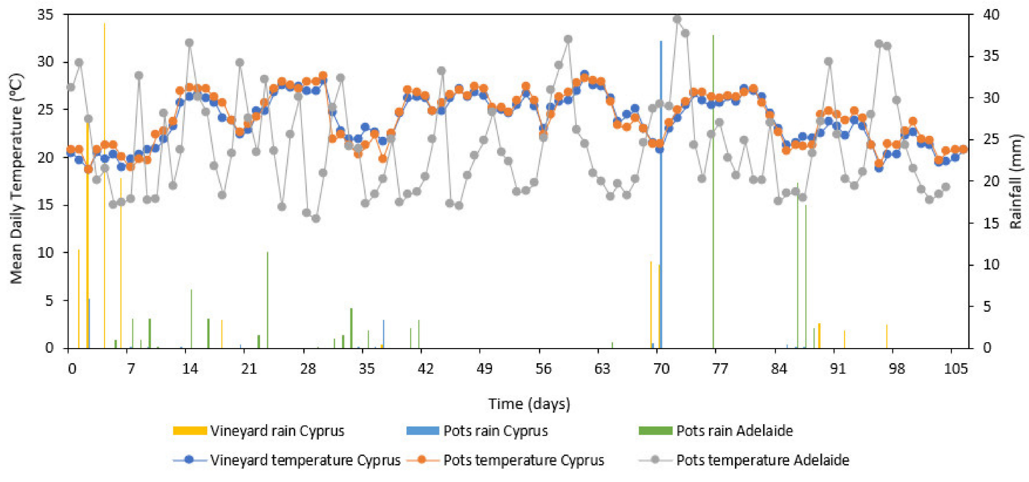

Figure 4. Climate and rainfall data for Cyprus Xynisteri vineyard site (Kato Mylos), Cyprus potted trial (Omodhos) and Adelaide Waite Campus, Australia for the testing periods 2019 and 2020/2021.

In 2020/2021, 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 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 [36]. 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 [37].

Growing Season Temperature (GST) in south-east Australia has previously been investigated and it has been concluded that traditional growing season temperature thresholds may not be suitable for Australian wine regions and that latitude adjustments are necessary to improve growing season models in Australia [38]. While suggesting the use of a hot region classification being GST of 19–22 °C and a very hot region being 22–24 °C [38]. 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 2020/2021 season Adelaide was only classified as hot.

3.2. Vine Growth and Physiology Measurements

3.2.1. 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–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 previously reported [39]. 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 [40]. 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.

Treatment	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 L, 50%—22 L, Nil—no irrigation, ns—not statistically significant different. Shoot, bunch number, bunch length and bunch width are means of $n = 12$ vines.

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 [39]. 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 and the Greek varieties Agiorgitiko and Xinomavro [21,41]. In 2019, the vineyard region received 194 mm of rain in the growing season (April–September). However, 106 mm of the rain occurred in early June and 34 mm occurred during two episodes in August, which may have influenced the results, especially when considering the long-term average growing season rainfall is 129 mm.

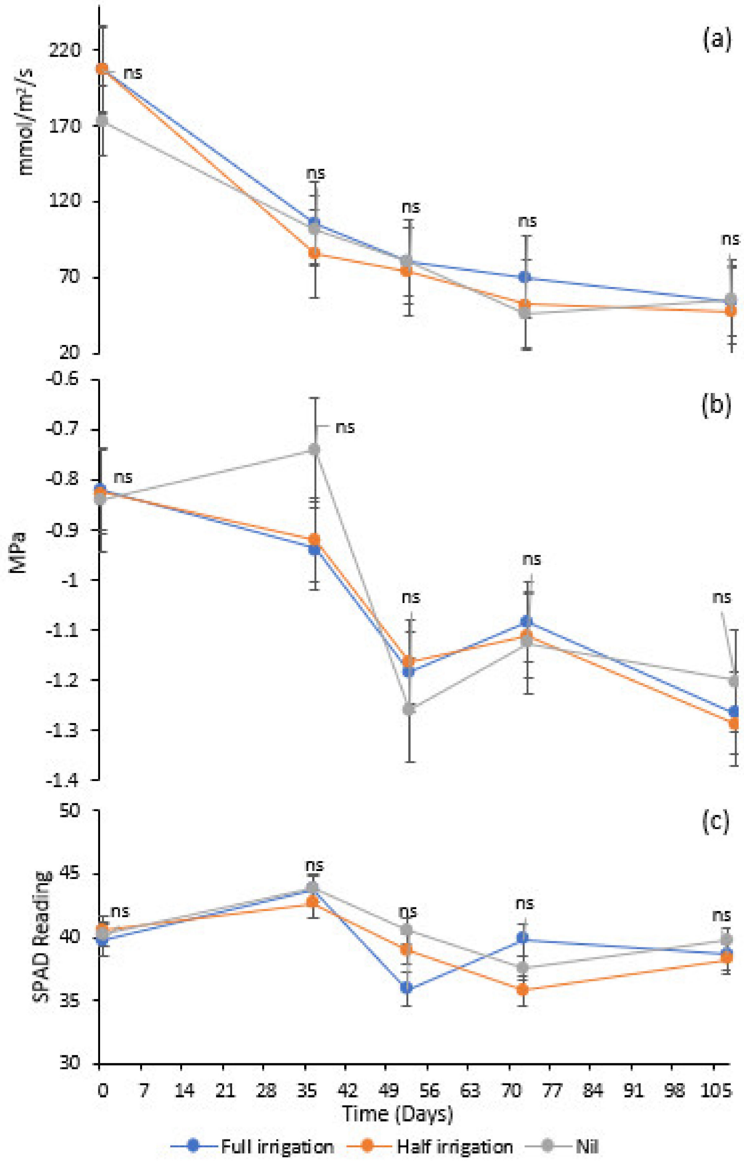

Figure 5. Vine leaf physiological measurements for 107–day test period in commercial Cypriot Xynisteri vineyard 2019. (**a**) stomatal conductance (mmol/m^2/s), (**b**) stem water potential (MPa), (**c**) SPAD reading-indicative chlorophyll content. Full irrigation–44 L/vine, Half irrigation–22 L/vine, 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, ns—not statistically significant.

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 [29]. Therefore, it is difficult to conclude whether the testing period rainfall had an impact on the results or not.

3.2.2. 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, vineyards that produce long shoots, large leaves and extensive lateral growth are reported as having high vigour [42]. This high vigour growth can have an impact on the canopy density and the exposure of fruit to sunlight and the resultant wine composition.

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	0.3	3.9	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	1.6	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 L, 50%—22 L, Nil—no irrigation. ns—not statistically different. Different letters indicate significant differences $p < 0.05$. Each data point is a mean of $n = 12$ samples. Means were separated by ANOVA using Tukey's test. * indicates significance at $p < 0.05$, ns = not significant.

Table 4. Vine growth assessments at flowering for all varieties in potted trials in season 2019 and 2020/2021.

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	235 [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
2020/2021				
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 2020/2021 with 2 shoots per vine.

In 2020/2021, 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 previous field trial data where Xynisteri had the longest shoots and the largest shoot diameter and Maratheftiko had the least leaves per shoot and longest internode length [39].

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 2020/2021 season.

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.

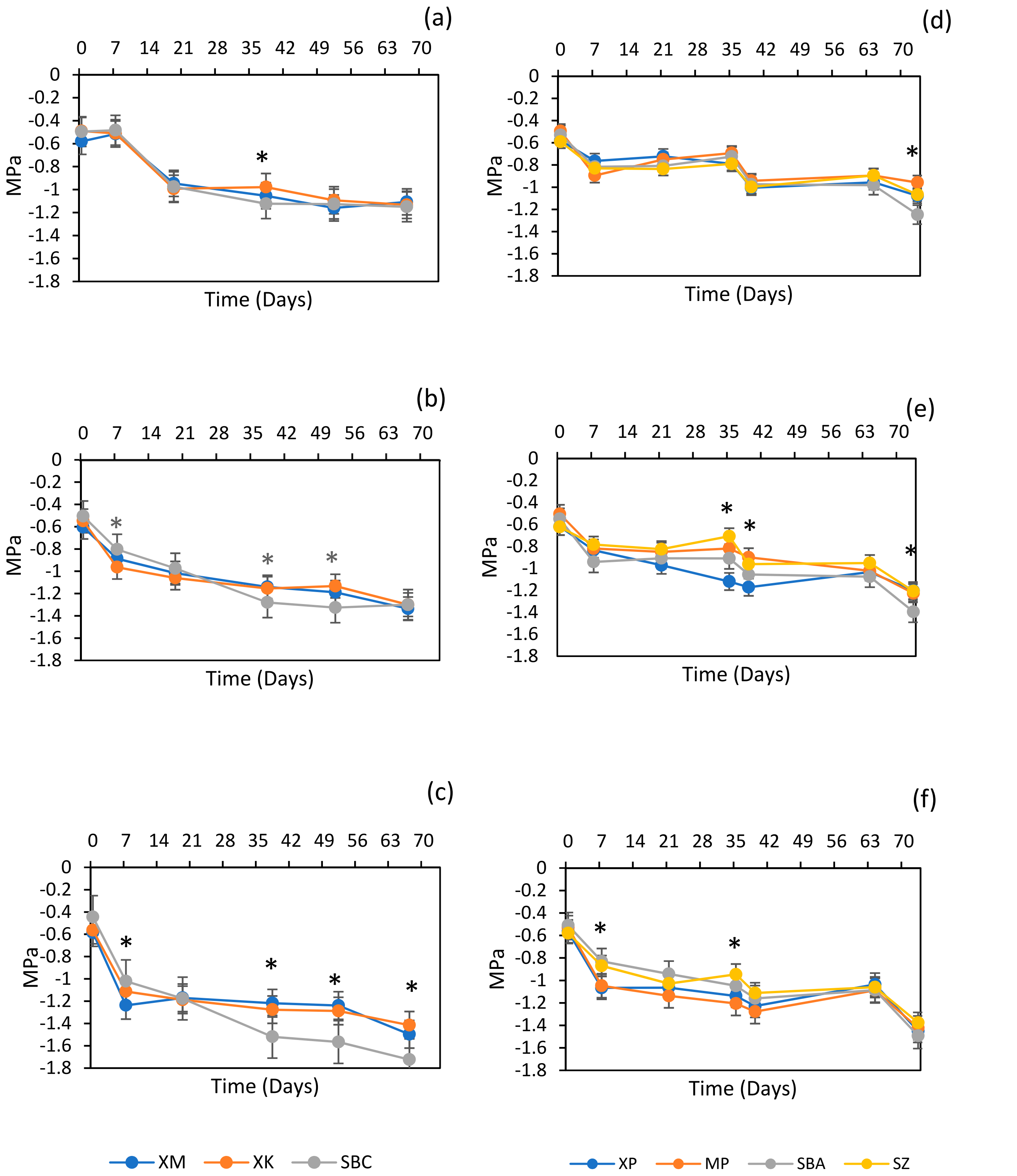

Figure 6: Stem water potential (MPa) measures for potted trials for seasons 2019 and 2020/21 XM- Xynisteri Mandria, XK- Xynisteri Kathikas, XP- Xynisteri Paphos, MP- Mavro Paphos, SBC- Sauvignon Blanc Cyprus, SBA- Sauvignon Blanc Adelaide, SZ- Shiraz. (a) Full irrigation 2019, (b) 50% irrigation 2019, (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 $p < 0.05$.

Figure 6. Stem water potential (MPa) measurement for potted trials for seasons 2019 and 2020/2021. XM—Xynisteri Mandria, XK—Xynisteri Kathikas, XP—Xynisteri Paphos, MP—Mavro 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 2020/2021, (e) 50% irrigation 2020/2021, (f) 25% irrigation 2020/2021. Each data point are means of $n = 10$ vines in 2019 and $n = 7$ vines in 2020/2021. Bars indicate the standard error. Means were separated by ANOVA using Tukey's test. * indicates 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
2020/2021		
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

* indicates significance at $p < 0.05$.

In 2020/2021 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, stem water potential decreased the most at the end of the trial, while under 25% irrigation, stem water potential decreased earlier and mid-way through the trial. While the results for 2020/2021 were not conclusive, findings for 2019 were similar to those previously reported, that is, Xynisteri had higher stem water potential than Maratheftiko and Shiraz, while Sauvignon Blanc had the lowest stem water potential [38].

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. Stomatal conductance between 50–150 mmol/m^2/s is considered the threshold for severe water stress [43]; using this classification, XK and SBC were stressed from day 39 and XM from day 38.

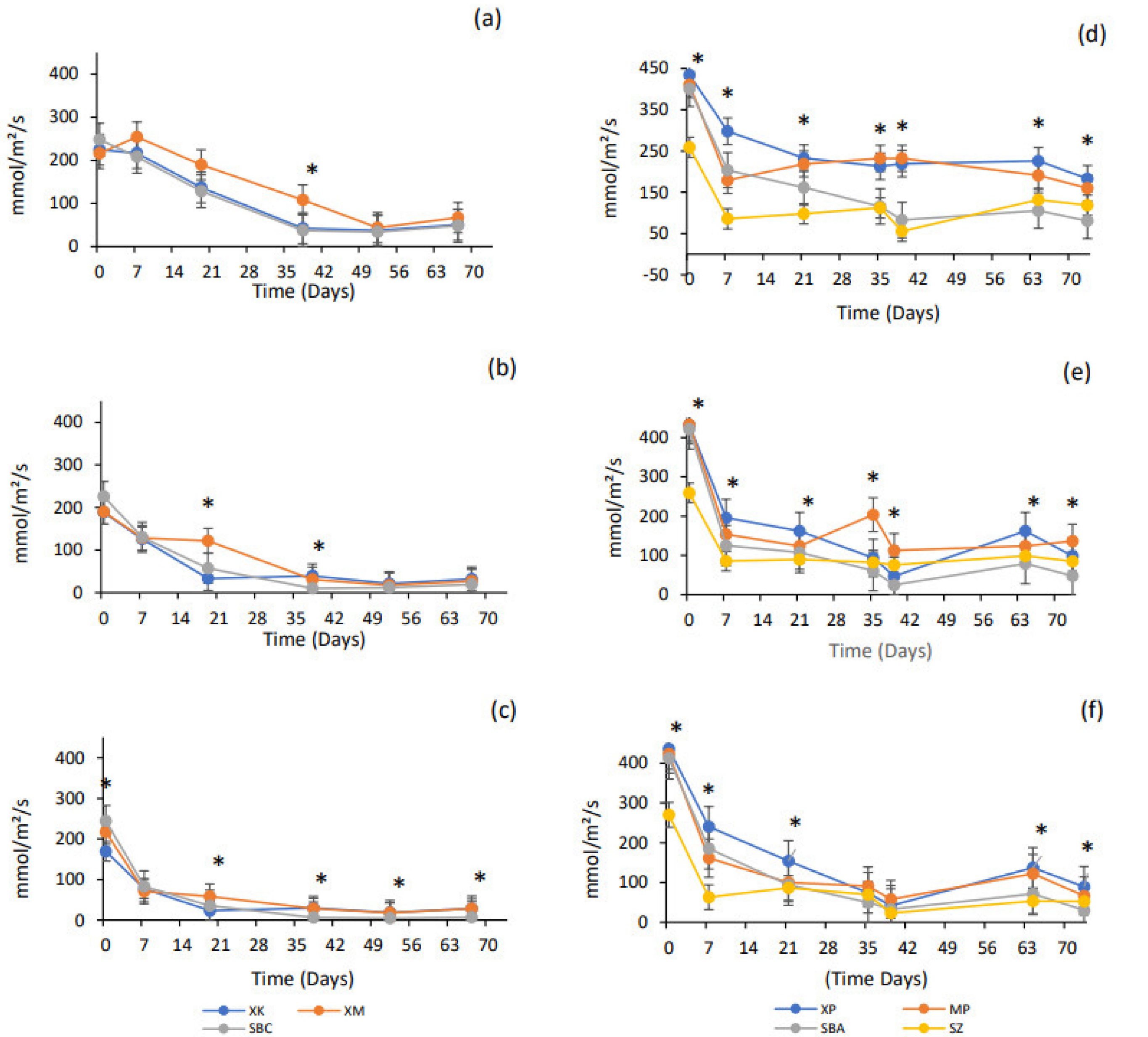

Figure 7. Stomatal Conductance (mmol/m^2/s) measures for potted trial for seasons 2019 and 2020/2021. XM—Xynisteri Mandria, XK—Xynisteri Kathikas, XP—Xynisteri Paphos, MP—Maratheftiko Paphos, SBC—Sauvignon Blanc Cyprus, SBA—Sauvignon Blanc Adelaide, SZ—Shiraz. (**a**) Full irrigation 2019, (**b**) 50% irrigation 2019, (**c**) 25% irrigation 2019, (**d**) Full irrigation 2020/2021, (**e**) 50% irrigation 2020/2021, (**f**) 25% irrigation 2020/2021. Each data point are means of n = 10 vines in 2019 and n = 7 vines in 2020/2021. Bars indicate the standard error. Means were separated by ANOVA using Tukey's test. * indicates significance at $p < 0.05$.

Under 50% irrigation, XM and XK had higher stomatal conductance than SBC at days 19 and 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 than 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 over the testing period.

In 2020/2021 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, and 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.

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 previously reported, indicating that Xynisteri and Maratheftiko had greater stomatal conductance than Shiraz and Sauvignon Blanc in a vineyard trial [39]. All the varieties in this study showed a reduction in stomatal conductance over time but at differing rates.

SPAD readings in both seasons ranged between 15 and 40, which is consistent with previous studies that have reported that SPAD readings are adequately sensitive at around 35 (chlorophyll content approximately 300 mg/m^2) [44]. Previous research has also 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 [44]. SPAD readings are reported as being proportional to the amount of chlorophyll present in the leaf and that converted SPAD values only differ from photometric measurements of solvent-extracted chlorophyll by 6%, while also being a non-destructive method suitable for preserving the leaves of plants being studied [45].

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

In 2020/2021, 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–f). Repeated ANOVA indicated that SPAD readings were significantly affected by time only (Table 5).

This again concurs with previous research that demonstrated that Xynisteri and Maratheftiko had higher chlorophyll content than Shiraz and Sauvignon Blanc [39]. However, Maratheftiko grown in a vineyard demonstrated higher chlorophyll content than Xynisteri, which was not the case with this potted trial.

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 2020/2021, 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 two-way ANOVA was applied to the root, shoot and leaf mass data to assess for the effects of variety, irrigation and their interactions, only the interaction of the shoot mass of Xynisteri and full irrigation in 2019 was significant ($p < 0.0001$). In 2020/2021, root mass was explained by variety for Xynisteri ($p < 0.0001$) and the interaction of the root mass of Xynisteri and minimal irrigation ($p = 0.035$).

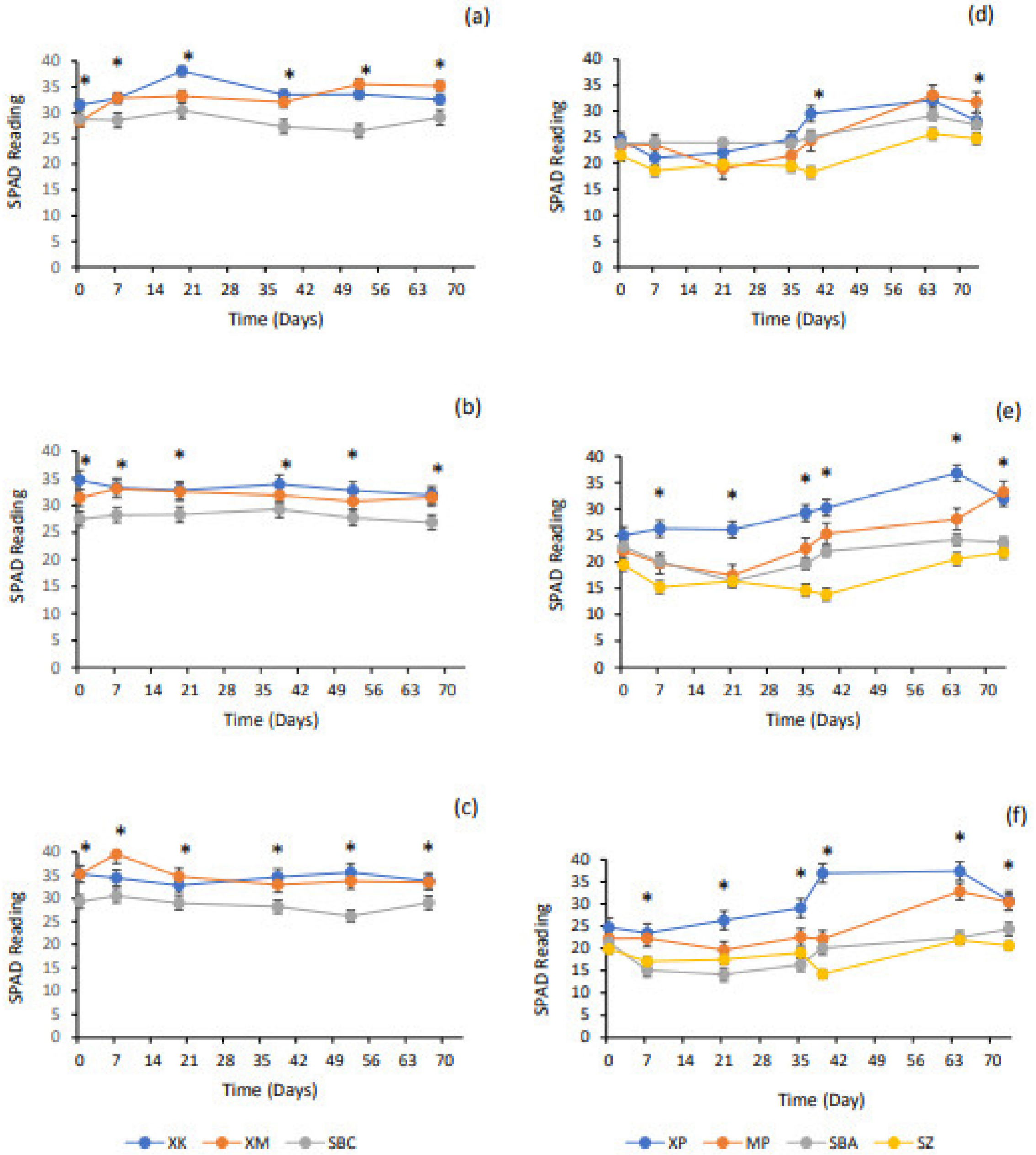

Figure 8. SPAD reading (indicative chlorophyll content) measures in potted trials for seasons 2019 and 2020/2021. XM—Xynisteri Mandria, XK—Xynisteri Kathikas, XP—Xynisteri Paphos, MP—Maratheftiko Paphos, SBC—Sauvignon Blanc Cyprus, SBA—Sauvignon Blanc Adelaide, SZ—Shiraz. (**a**) Full irrigation 2019, (**b**) 50% irrigation 2019, (**c**) 25% irrigation 2019, (**d**) Full irrigation 2020/2021, (**e**) 50% irrigation 2020/2021, (**f**) 25% irrigation 2020/2021. Each data point are means of $n = 10$ in 2019 and $n = 7$ in 2020/2021, Bars indicate the standard error. Means were separated by ANOVA using Tukey's test. * indicates significance at $p < 0.05$.

Table 6. Fresh root, shoot and leaf mass (EL 38 Harvest) measures for potted trials for seasons 2019 and 2020/2021.

Mass (gm)	Root			Shoot			Leaf		
IR	Full	50%	25%	Full	50%	25%	Full	50%	25%
Cyprus 2019									
XM	693 [b]	582 [ab]	387 [ab]	264 [b]	204 [a]	112 [b]	243 [b]	184 [a]	102 [ab]
XK	939 [a]	643 [a]	486 [a]	377 [a]	234 [a]	180 [a]	359 [a]	208 [a]	156 [a]
SBC	493 [c]	352 [b]	182 [b]	109 [c]	93 [b]	63 [c]	129 [c]	93 [b]	48 [b]
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]	458 [a]	411 [a]	342 [a]	357 [a]	291 [a]	259 [a]
MP	620 [b]	567 [b]	539 [b]	425 [ab]	366 [ab]	296 [ab]	252 [ab]	240 [ab]	201 [ab]
SZ	592 [b]	445 [b]	320 [c]	299 [b]	286 [b]	238 [ab]	215 [b]	154 [c]	137 [b]
SBA	610 [b]	494 [b]	443 [b]	307 [b]	274 [b]	206 [b]	236 [b]	205 [bc]	140 [b]
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 L per pot per week, 50% = 4 L per pot per week, 25% = 2 L 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 2020/2021. Means were separated by ANOVA using Tukey's test. Different letters next to the measures indicate significant differences $p < 0.05$.

In both seasons, the root mass for Xynisteri was greater than the shoot and leaf mass (aboveground biomass), while Maratheftiko, Shiraz and Sauvignon Blanc had similar root and aboveground biomass ratios. However, in the cooler 2020/2021 season, root and shoot/leaf masses were higher than the warmer 2019 season.

3.2.3. Stomatal Density

In 2019, XK and XM had greater stomatal density than SBC. The stomatal density of Xynisteri was the highest (similar to previous findings [39]), followed by MP, SZ and SBA (Table 7) in 2020/2021. 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 2020/2021 growing season had a mean temperature of 21 °C. Stomatal density has been reported as being correlated to temperature; stomatal density can be as much as 1.4 times greater in warm temperatures when compared to cooler temperatures [46]. This could help to explain the differences seen between 2019 and 2020/2021 for Xynisteri with the warmer season producing higher stomatal densities. High stomatal density has been associated with drought tolerance [47]. Studies suggest that the high stomatal density of Albarinho for example, may be responsible for its greater drought tolerance as it has an increased photosynthetic capacity [47]. However, in this study, 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 a season.

Table 7. Stomatal density measures for potted trials for seasons 2019 and 2020/2021.

Season	Variety	Stomatal Density
2019	XCV	238.6 [a]
	XK	227.5 [a]
	XM	233.2 [a]
	SBC	139.8 [b]
	Pr > F	<0.0001
2020/2021	XP	206.1 [a]
	MP	189.0 [b]
	SZ	170.5 [c]
	SBA	151.4 [d]
	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 2020/2021. Bars indicate the standard error. Means were separated by ANOVA using Tukey's test. Different letters next to the measures indicate significant differences $p < 0.05$.

4. Discussion

4.1. Vine Growth Measurements

The different vine growth results seen between the hotter 2019 and cooler 2020/2021 seasons in this study were consistent with previous studies. Research investigating Malbec grown with increased temperatures, demonstrated that shoots were longer [48]. 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 with soybean crops [49]. A warming climate can have significant effects on grapevines and other crops. It has been reported that Cabernet Sauvignon experiencing warmer spring temperatures at bud burst can lead to large differences in shoot growth, shoot architecture and leaf development [50]. These changes can be maintained or amplified during the growing season. Early season temperatures also have a persistent effect on the shoot growth rate regardless of the growing season temperature [50].

4.2. Water Potential

While the results for stem water potential in 2020/2021 were not conclusive, findings for 2019 were similar to those previously reported [39]. It has been demonstrated in vineyard trials that Xynisteri had higher stem water potential than Maratheftiko and Shiraz, while Sauvignon Blanc had the lowest stem water potential [39]. Water potential has been widely used as an indicator of plant water status for irrigation management purposes [40]. There is, however, some conjecture about the levels of stem water potential that are considered as moderate and severely stressed. A stem water potential value lower than −1.1 MPa in some cases is considered as severe stress [51], while in other cases −1.2 MPa is considered moderate stress and −1.5 MPa severe stress [29]. 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 [52]. For example, studies of high and low vigour rootstocks have previously 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 [52]. This could help to explain the root mass results seen, particularly for Xynisteri in 2020/2021. 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.

4.3. Stomatal Conductance

Previous heat and water stress studies in Cyprus involving Xynisteri and Chardonnay have demonstrated similar results for the stomatal conductance of Xynisteri as this study [23]. However, when comparing Xynisteri with Chardonnay, Chardonnay had a relatively constant stomatal conductance throughout their testing period, while Xynisteri stomatal conductance decreased with time [23]. The authors concluded that this was a possible mechanism in which Xynisteri responds to drought stress by improved stomata conductance regulation [23].

The literature, however, is not always in agreeance 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 [53]. 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. The distinction between isohydric and anisohydric plants has been described as not being so clear-cut, and that plants may be able to switch between strategies depending on drought severity and environmental conditions [54].

Some authors reject the premise of isohydric and anisohydric behaviour entirely [55]. A study of 17 different cultivars in a field experiment under three irrigation regimes measured pre-dawn and midday leaf water potential as well as midday stomatal conductance. The authors concluded that stomatal behaviour is an across-cultivar continuum and call into question the isohydric and anisohydric classification system [55]. 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 [55]. Many other authors 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 [56–58]. 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.

4.4. SPAD Readings (Indicative Chlorophyll Content)

Previous studies in Cyprus have compared Xynisteri with Chardonnay in a vineyard and found that the level of chlorophyll amongst irrigation and no irrigation groups varied [23]. 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 [23]. Xynisteri and Chardonnay grown 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 [24].

Previous Maratheftiko trials in a Cypriot vineyard 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 [21]. Similar results have been demonstrated by studying Maratheftiko in pots under heat and drought stress conditions [22]. 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 [22]. 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. Chlorophyll content has been investigated as a predictor of aboveground biomass in rice crops, with studies demonstrating that higher chlorophyll content in leaves correlated with an increase in aboveground biomass [59]. 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) grown in pots has also been studied [60]. The authors concluded that nitrogen supply altered the whole plant biomass and its distribution between annual and perennial parts of the plants. Nitrogen deficiency slowed growth and caused a higher biomass allocation to perennial parts of the plant (particularly the trunk); however, this is cultivar dependant, as Merlot grown under reduced nitrogen levels demonstrated enhanced root growth at the expense of aboveground growth [60]. This adds to the paradox of the Xynisteri results, with it demonstrating high chlorophyll content (leaf nitrogen), large aboveground biomass and large root biomass.

4.5. Biomass

Past research 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 [61]. Conversely, the study observed that stomatal density increased significantly with increased temperatures. These changes were also seen with this project, with lower stomatal density observed in the cooler 2020/2021 season compared to 2019 (Table 6). Studies involving 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 aboveground masses when compared to corresponding own rooted vines [62]. Research involving 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. The authors concluded that 110R was able to do this via drought dependent sugar and protein induction genes located in the roots [63].

Root volume has been described 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 [64]. Drought-adapted rootstocks have also demonstrated that they have deeper roots [65]. 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. The literature agrees that roots may play a role in the grapevine's response to drought stress. Previous studies of M4 rootstocks have concluded that carbon metabolism, mitochondrial function and other as yet unidentified mechanisms may be involved in the root mass, drought tolerance effect in grapevines [66].

Previous Xynisteri and Chardonnay trials have shown that non-irrigated Xynisteri had an increased level of the hormone abscisic acid (ABA) [23]. 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 [58,67]. 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 [68]. 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 [69–71], however, no definitive mechanisms have been concluded to date. It has been suggested 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 [72].

The role of Xynisteri and Maratheftiko roots could be an important factor for vineyards in Australia where 80–95% of vineyards are planted without the use of rootstocks. Only one region (Riverland) has 45% of vineyards planted with vines using rootstocks [73]. Further research into the role of these root structures is therefore warranted.

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.

5. Conclusions

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 irrigated regions, cultivation of these varieties could result in a reduction of the irrigation required, hence further investigation is warranted.

The role of the extensive biomass and root structure of Xynisteri (and to a lesser extent Maratheftiko) 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.

Therefore, in conclusion we can accept the hypothesis that Cypriot varieties and Xynisteri in particular show good agronomic and physiological behaviour under semi-arid and hot conditions.

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Chapter 7. General Discussion and Conclusions

The changing climate globally is well documented and the impacts on agriculture will continue to be a problem into the future. Many industries are seeking to find more sustainable practices and crops to assist in adapting to this challenge. This research studied the purported drought tolerant, indigenous Cypriot grape varieties Xynisteri and Maratheftiko, for their suitability to viticulture and oenology in Australia and the Australian consumer response to wines made from these varieties.

When this research project commenced, much of the knowledge regarding these Cypriot varieties was largely anecdotal. Since 2017, there has been increased interest in these varieties both from this project and with researchers in Cyprus (Grigoriou *et al.,* 2020, Litskas *et al.,* 2020, Vink *et al.,* 2021 Chrysargyris *et al.,* 2018a, 2018b, 2020, Tzortzakis *et al.,* 2020, Heymann *et al.,* 2021, Tsiakkas *et al.,* 2020). This research has provided new knowledge on several aspects; including the chemical composition, and sensory attributes of the wines made from these varieties. Consumers have demonstrated a liking for the wines and in some cases preferred these wines to wine made from more common varieties. Xynisteri and Maratheftiko growing in non-irrigated vineyards in Cyprus have been bench marked against Shiraz, Pinot Gris and Chardonnay for the first time. Irrigation trials in Australia and Cyprus compared the vine growth response to different irrigation regimes and highlighted that the Cypriot varieties were better suited to heat and drought stress and that Xynisteri in particular, was able to produce large above and below ground biomass under all irrigation conditions. This research will also guide future research in terms of how these varieties perform in Australian commercial vineyards and the mechanisms by which they achieve their drought resilience.

The sensory, chemical and consumer study of the wines made from the indigenous Cypriot grape varieties Xynisteri, Maratheftiko and Giannoudhi was the first detailed study of its kind. The results have built on previous work from authors in Cyprus and Greece (Constantinou *et al.,* 2017 and 2018 and 2019, Galanakis *et al.*, 2015, Kokkinofta *et al.,* 2017) thereby increasing the knowledge of these varieties. Xynisteri was described sensorially as citrus, herbaceous, dried fruit, savoury, apple, pear, grassy with a full length of fruit and non-fruit flavours in the after taste. (Copper *et al.,* 2019). Chemical analysis supported sensory analysis with aroma compounds of ethyl propanoate (fruity), 2-phenylethanol (honey), ethyl-3-methylbutanoate (fruity), ethyl acetate (acetone), ethyl-2-methylpropanoate (sweet), 3-methylbutanol & 2-methylbutanol (solvent), hexanoic acid (leafy, woody), ethyl octanoate (pear, pineapple), hexanoic acid (leafy, woody) and ethyl butanoate (lactate) identified in wines. Polyphenolic compounds of catechin, caftaric acid, epigallocatechin, coutaric acid B, epigallocatechin, ethyl gallate and gallic acid and have been associated with quality in Riesling wines (González-Álvarez *et al.,* 2011).

Cypriot red wines, Maratheftiko and Giannoudhi compared favourably with common European varieties and less common Greek varieties being described sensorially as dried fruit, jammy, confectionery, sweet, chocolate, herbaceous, woody, with full length of fruit flavours in the after taste. The Cypriot wines were also assessed to have aroma compounds that contributed to the above attributes, that is: strawberry, sweet, fruity, banana, cherry, pear, woody/leafy, and butter. It is also worth noting that there were only a small number of wine samples available for this preliminary study, it was therefore difficult to make in depth comparisons with the more common European varieties. However, when we consider these quality parameters and the consumer data generated in this study, we can speculate that the wines made from Cypriot varieties are comparable to common Australian wines and potentially similar to other quality European wines made from varying grape varieties and likely to meet strong consumer acceptance. These studies provided us with useful information which should be followed up with further in-depth studies to investigate specific phenolic compounds by LC-MS/MS (targeted, quantitative analysis) as well as analysis of terpenes with repeated measures, along with further quantitative analysis of specific aroma compounds by GC/MS with repeated measures. Further RATA studies of Cypriot wines should involve research wines made from different locations and standardised wine making techniques to eliminate any wine making influence on the sensory analysis.

Initial sensory analysis followed by the formal sensory and consumer trials highlighted that the Cypriot wines had characteristics similar to more common Australian wines. In particular Xynisteri had flavour and aroma characteristics reminiscent of wines such as Pinot Gris and unwooded Chardonnay. This indicated that thiol analysis of the Cypriot wines would be beneficial to determine the concentration of five thiols; 4-methyl-4-sulfanylpentan-2-one (4MSP) that has an aroma of “boxwood” and “cat urine” at high concentration, 3-sulfanylhexan-1-ol (3SH) which has been described as having a “grapefruit/tropical fruit” aroma, and 3-sulfanylhexyl acetate (3SHA) that has also been described as having an aroma of “passionfruit”. Additionally, two other potent thiols were measured including benzyl mercaptan (BM) that has an aroma of “smoke and meat” and furfuryl thiol (FFT) that has been described as having a “roasted coffee” like aroma. The thiol levels in these wines were comparable to those found in popular Australian wines such as Chardonnay and Sauvignon Blanc. These varietal thiols are important compounds in certain varieties when “fruity”, “tropical” and “citrus” aromas are desired. The thiols that were detected in all the Cypriot white wines were above threshold levels; however, the Australian Pinot Gris and Chardonnay had better correlation with the desirable thiol 3SHA and its "passionfruit/tropical" characteristics (Copper *et al.*, 2021). One explanation for this could be the age difference of the wines. Apart from one Xynisteri, which was aged in oak for six months, the remaining Xynisteri wines were at least six months older than the Australian wines, having been bottled in late 2016 or early 2017, alternatively, this may also be attributable to masking/suppression and synergistic effects as has been shown previously in Chenin Blanc (Wilson *et al.*, 2019) and in red wine (Garrido-Bañuelos *et al.,* 2020). Herbst-Johnstone *et al.,* (2011) report that thiol concentrations in Sauvignon Blanc wines were not stable and that between 62 % and 76 % of 3SHA had been lost seven months after bottling. Therefore, for the preservation of these compounds, bottle storage conditions can be an important issue. Thiol concentration in wines can also be altered pre-fermentation. For example, they can be enhanced with particular handling of grapes and must prior to fermentation. Capone *et al.,* (2012) report that storing fruit at 10 °C prior to crushing can lead to an increase in 3SH precursors. Chen *et al.,* (2019) also demonstrated that freezing grapes and musts to –20 °C prior to fermentation dramatically increased varietal thiol precursors and thiol levels in the finished Sauvignon Blanc wine. Furthermore, Maggu *et al.,* (2007) demonstrated that increasing skin contact time, less clarified juice (higher turbidity) and a greater press pressure also resulted in higher concentrations of 3SH precursors, most likely due to a greater concentration of precursors located in the skins (Roland *et al.,* 2011).

Further investigation into the role of thiols in Cypriot wines could involve analysis of a much greater number of samples and vintages to evaluate its distribution. Winemaking practices that affect thiols, such as the handling of the grapes prior to fermentation, could be applied to these varieties to be able to meet the desired wine style, whether it be to enhance or reduce these characteristics. While this study was a preliminary investigation, it highlights the importance of thiols in white wines, however, their role in red wines is not well understood and requires further research.

The vine performance study determined that the indigenous Cypriot varieties Maratheftiko and, in particular, Xynisteri were well adapted to a hot climate, continuing to perform well as the climate becomes hotter. Xynisteri and Maratheftiko achieved budburst earlier and reached harvest maturity 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 were 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. It 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 on a commercial scale should occur in the future to eliminate the possibility of these confounding influences.

The irrigation trial was composed of three parts and occurred both in Cyprus and Australia. The first part was a field trial in a Cypriot vineyard which compared the performance, yields and composition of Xynisteri under full, deficit and no irrigation regimes for 107 days. No significant differences between physiological measures and phenology data at fruit set and harvest were found when comparing the three irrigation regimes. Must composition analysis of the harvested fruit revealed fructose to be lowest in the full irrigation group compared to deficit and non-irrigated treatments. Fructose production is favoured in warmer conditions (Amerine and Thoukis 1958 cited in Trad *et al.,* 2021 and can be an indication of over ripeness and higher potential alcohol. 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 considering the long-term average growing season rainfall is 129mm. Considering that the final stem water potential for all three irrigation regimes was -1.2 MPa and only moderately stressed (according to criteria reported by Girona *et al.,* 2006), it is difficult to conclude weather the testing period rainfall had an impact on the study or not.

The second part was a potted trial in Cyprus (in 2019) of two suspected Xynisteri clones (XM and XK) and Sauvignon Blanc (SBC) under three different irrigation regimes over 67 days. The third part was a potted trial of Xynisteri (XP), Maratheftiko (MP), Sauvignon Blanc (SBA) and Shiraz (SZ) in Australia (in 2020/21) over 74 days. In 2019, phenological measurements were taken at flowering. 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. Phenological 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. This is consistent with the 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, 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 grapevine 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. 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.

The results for water potential in 2020/21 were not conclusive, yet the results for 2019 however 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). The assumption being that there is an unavoidable relationship between plant transpiration, soil water content and water potential. Computer simulations using olive tree models performed by García-Tejera *et al.,* (2021) concluded 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. Modifications of the ratio of root to leaf area can induce changes in the relationship between transpiration, water potential and soil water content. This could help to explain the inconsistencies seen with the results particularly in 2020/21. The cultivar and root structure differences for Xynisteri may be an explanation for why the water potential measurements were not significant until the end of the testing period after 74 days of water stress.

In 2019, XM and XK had higher stomatal conductance than SBC. In 2020/21 XP had higher stomatal conductance and SZ had the lowest in the early stages of testing, with SBA being the lowest in the later stages of development. Previous research has demonstrated that Xynisteri and Maratheftiko had greater stomatal conductance than Shiraz and Sauvignon Blanc in a vineyard trial (Copper *et al.,* 2020). Both trials also indicated that all the varieties had 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.

Levin *et al.,* (2020) recently 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, 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) state that 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. 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 under all irrigation regimes.

SPAD readings were used to estimate leaf chlorophyll content. Ling *et al*., (2011) report that SPAD readings are proportional to the amount of chlorophyll present in the leaf and that the 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 chlorophyll content when compared to SBC throughout the testing period in 2019. Chlorophyll content for all three varieties remained constant throughout the testing period. In 2020/21 chlorophyll content for XP and MP had the highest levels 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 four varieties increased their chlorophyll content over the testing period, XP and MP in particular increased their chlorophyll content with deficit and minimal irrigation. 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.

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 and could potential also be used as a predictor of grapevine biomass.

Chlorophyll content and the nitrogen status of Shiraz grapevines was studied by Metay *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 decreased nitrogen levels demonstrated enhanced root growth at the expense of aboveground growth (Grechi *et al.,* 2007 cited in Metay *et al.,* 2014). 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.

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.
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 minimal irrigation where it was not statistically different to that of MP and SBA had the lowest mass.
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. 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 grapevines 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. Prinsi *et al.,* (2018) investigating M4 grapevine rootstock concluded that roots may play a role in the grapevine's response to drought stress. They stated that carbon metabolism, mitochondrial function and other as yet unidentified mechanisms may be involved in the process.

In 2019, XK and XM had greater stomatal density than SBC. The Xynisteri stomatal density was also similar to the findings of Copper *et al.,* (2020). Also mirroring the results seen by Copper *et al.,* (2020), XP had the highest stomatal density, followed by MP, SZ and SBA (Table 5) in 2020/21. There was however some difference between the two seasons. 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 and found 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.

In conclusion, this research has identified the potential of the indigenous Cypriot grape varieties to tolerate drought stress. Xynisteri in particular, has shown itself to be more able to cope with drought stress than the more commonly cultivated varieties of Sauvignon Blanc, Shiraz and Chardonnay. In regions where irrigation is used to supplement rainfall, this 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 were far greater than all the other varieties investigated and with all irrigation regimes. The role of the extensive root structure of Xynisteri is one area that is an exciting outcome of this research and warrants 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.

Chapter 8. References

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Appendix. Additional published article: Identifying lower limb problems and the types of safety footwear worn in the Australian wine industry: a cross-sectional survey.

Statement of Authorship

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By signing the Statement of Authorship, each author certifies that:

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RESEARCH **Open Access**

Identifying lower limb problems and the types of safety footwear worn in the Australian wine industry: a cross-sectional survey

Alexander Willem Copper[1*], Rolf Scharfbillig[2], Thuy Phuong Nguyen[3] and Cassandra Collins[1]

Abstract

Background: The Australian wine industry is a valuable part of the wider Australian economy worth approximately A$45 billion annually and employs 163,790 people either full time or part time. Australian agricultural industries are amongst the nation's most dangerous workplaces with joint, ligament, muscle and tendon injuries being commonplace along with wounds, lacerations and musculoskeletal diseases. It is therefore important to try and minimise the risk of injuries to workers. The aims of this study were to (1) identify whether lower limb problems occur in the Australian wine industry and (2) identify the types of safety footwear worn.

Methods: Participants were recruited from the Australian wine industry. The study was a cross-sectional anonymous survey of 82 questions with $n = 207$ respondents. Questions related to job role performed, types of lower limb problems experienced, level of pain, restriction of activities, types of footwear worn, general health and physical health.

Results: The main working roles were winery (73.4%), vineyard (52.2%), laboratory (39.6%), cellar door (32.4%) and office (8.2%), with 63.3% of participants working in more than one role. Lower back pain was the most commonly reported problem at 56% followed by foot pain (36.7%), knee pain (24.6%), leg pain (21.3%), ankle pain (17.9%), hip pain (15.5%), toe pain (13%) and heel pain (11.1%). The most popular footwear used by participants were elastic sided safety boots, followed by high cut lace up safety boots with side zip. Overall, although the pain experienced was moderate, it did not impact the workers ability to perform their duties and the majority self-reported as being in very good general and physical health.

Conclusion: To date no data have been published on the types of lower limb problems or the types of safety footwear worn in the Australian wine industry. This study is the first to demonstrate that elastic sided safety boots were the most popular amongst respondents and that lower limb problems occur with workers. Therefore, further research into the safety footwear used in the Australian wine industry is needed to better support workers health while working in their varied roles and conditions.

Keywords: Safety footwear, Elastic sided safety boots, Lower limb, Occupational health, Wine industry

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Background

The Australian wine industry is a valuable part of the wider Australian economy, worth approximately A$45 billion annually and employing 163,790 people either full time or part time [1]. Therefore, the wine industry and the health and safety of its workers are an important part of Australian society and the economy. The industry is also somewhat unique, in that most businesses are small to medium in size and consist of multiple workplace environments within the one entity [2]. In 2020 there were 2361 wineries and 6251 grape growers in Australia. Approximately 64% of producers are considered small to medium and process less than 50 t of grapes per year, 20% process 50–499 t and 16% process more than 500 t [2].

Wine business owners and employees may work in various combinations of roles across the business, particularly in smaller, family run enterprises. Small wineries are comprised of a primary industry (grape growing), a secondary process (wine production) and tertiary activities such as restaurants and cellar door sales [3]. Grape growing activities can include operating heavy machinery as well as driving tractors and harvesters in the vineyard. Winery activities can include operating forklifts/pumps/crushers/conveyor belts and bottling machines, along with analysing juice and wine samples in the laboratory plus general office work. Sales and hospitality work in the cellar door can often include food service. Most of these activities involve wearing protective safety footwear and standing for long periods. This is particularly the case during the vintage/harvest season, when the weather is very hot and shifts are longer than usual due to the time constraints involved in harvesting and processing grapes at the optimal time.

To date no data have been published on the types of injuries experienced in the Australian wine industry and the cost of injury to the industry. However, the South Australian government, which is the largest wine production area in Australia and accounts for 52% of the national output, publishes data on workplace injuries across several industries [2, 4]. The majority of injuries reported by the South Australian government in 2020 were for technicians/trade workers (27.9%), labourers (26.6%) and machinery operators/drivers (16%). The injuries were predominantly upper limbs (38.5%), lower limbs (18.9%) and trunk/back (17.9%). The nature of the injuries were mainly traumatic joint/ligament/muscle/tendon injuries (37%), wounds/lacerations (29.1%) and musculoskeletal diseases (15.2%). The main mechanism for these injuries were body stresses (34.5%), being hit by an object (19.4%) and falls/trips/slips (17.3%) [4]. In 2012–13, work-related injury and disease cost the Australian economy A$61.8 billion, representing 4.1% of the gross domestic product [5].

Injuries among vineyard workers appear to be common. A study in France found that vineyard workers were likely to experience musculoskeletal pain. They reported upper limb pain (31.2%), neck/shoulder (28.9%), lower limb (25%) and back pain (55%) prevalence during grapevine pruning and grape harvesting [6]. Similar results have been seen with vineyard workers in the United States [7–9], Italy [10] and Argentina [11], but no specific data exists for Australian vineyard workers or winery workers in general.

Whilst there is limited data available for the wine industry, much can be gleaned from other agricultural industries that are reportedly some of the most dangerous workplaces [12]. For example, manual harvesting is prevalent in many agriculture industries and is the largest contributor to work-related musculoskeletal disorders [12]. The Australian aquaculture industry reports that 37.3% of injuries are body stressing events and that lower limb injuries account for 20.3% of all injuries [13]. The majority of these industries involve spending prolonged hours standing and it has been demonstrated that this can increase the risk of musculoskeletal disorders such as lower back, lower extremity and foot disorders [14].

Work environment flooring and footwear have also been demonstrated as risk factors [15], considering the variable nature of wine industry work environments, this is an area that warrants further investigation. Safety boots are compulsory in many occupations to protect the feet of workers from external stimuli, particularly in harsh environments [16]. The unique environmental conditions and tasks in different occupations necessitates a variety of boot designs to match each workers occupational requirements [17]. Unfortunately, safety boots are often designed more for safety at the expense of functionality and comfort [17].

Further risk factors for agricultural and horticulture workplace injuries include working full time, being the owner/operator, medication use, prior injury, poor health, stress/depression, poor hearing [18], heat stress [19] and inadequate sleep due to shift work [20, 21].

When these risk factors and the nature of wine industry work are taken into consideration, especially during the busy vintage/harvest period, it can be seen that wine industry work environments pose a potential risk to injury. Therefore, the aims of this study were to (1) identify whether lower limb problems occur in the Australian wine industry and (2) identify the types of safety footwear used.

Methods

Survey design and testing

The study was cross-sectional with the design based on previous validated surveys, questionnaires and studies that investigated foot health [22], musculoskeletal discomfort

[23, 24] and the footwear needs of workers [17, 25]. Content validity was considered via discussions with podiatry, physiotherapy, occupational injury and wine industry representatives and the survey questions were modified so as to be appropriate for the wine industry and capture the relevant areas of concern. Reliability was established by trialling the survey on 10 participants who completed the survey anonymously. Four weeks later the same 10 participants completed the survey a second time to test for repeatability and to ensure the questions were well understood.

The final survey consisted of 82 closed-ended questions (Likert scale and choose all that apply), that were divided in to nine sections including job role, lower limb problems at work, treatment sought, severity of pain, limitations caused by lower limb problems, types of footwear worn at work, footwear fit & comfort, general health and physical health (see supplementary information, Additional file 1 for survey).

Participants were recruited by several methods: writing to wine and grape industry bodies throughout Australia requesting surveys be distributed to members, supplying surveys to the work health and safety manager of the largest corporate wine company in Australia, emailing wine industry workers at the University of Adelaide and online via wine industry social media groups. Participants self-selected to complete the anonymous survey (n = 207) and the survey was open for 2 weeks after the Australian vintage/grape harvest period in May 2021.

For large populations, it is recommended that surveys have a sample size of 188 for 90% confidence level and 267 for 95% confidence level [26]. Therefore, for $n = 207$ and confidence level of 95%, the confidence interval was calculated as 6.8% [27].

Human Research Ethics Committee approval for the survey was given by the University of Adelaide (H-2020-267). An implied consent statement was placed at the beginning of the survey indicating that continuation with the questionnaire implied the participants consent.

Survey items

Job role

Participants were asked what job roles they performed in the Australian wine industry in the last 12 months. The question was a choose all that apply, closed-ended question with the option that workers could have several different roles within their workplace.

Lower limb problems at work

Lower limb aches, pain and injuries were assessed by asking participants if they had experienced any problems in different body areas in the last 12 months. If the participant had no lower limb problems, they were directed to the footwear section of the survey.

Treatment sought

Those participants that had experienced lower limb problems at work were asked if they had been hospitalised due to these problems or if they had sought any treatment.

Severity of pain

A Likert scale was used for participants to rate their pain (1 'low' to 5 'severe'). Likewise, a Likert scale (1 'never' to 5 'always') was used to determine; the frequency of pain, if pain limited work duties possible and any difficulties in completing work activities.

Limitations caused by lower limb problems

The final question regarding lower limb problems was related to how these problems affected daily activity in general, not only in work situations. A Likert scale (1 'not at all' to 5 'always') was used to rate any limitations.

Types of footwear worn

Participants were asked a closed-ended question relating to the style of footwear they most often used at work. A choose all that apply format was used and they were also asked if they use any additional support or cushioning in these shoes. The types of footwear were separated in to two groups: safety or non-safety.

Footwear fit and comfort

Participants were asked to rate their impression of their footwear's fit and comfort using a Likert scale (1 'strongly disagree' to 5 'strongly agree') over a series of 19 questions.

General health

Participants were asked to rate their general health with a Likert scale (1 'poor' to 5 'excellent') and answer nine questions relating to their general wellbeing using a Likert scale (1 'definitely false' to 5- 'definitely true').

Physical health

The final five questions related to general physical health and participants used a Likert scale (1 'definitely false' to 5 'definitely true') to rate their responses.

Statistical analysis

Data sorting and preparation were conducted with Microsoft Excel 2010, the closed-ended questions and Likert scale questions were counted to determine frequencies. Descriptive statistics and one-way ANOVA for the reliability trial were performed using the statistical package XLSTAT (version 2019.4.2, Addinsoft SARL, Paris, France).

Correspondence analysis and Polychoric correlation factor analysis was performed on the binary data relating to job role, area of lower limb problem and type of footwear worn using the statistical package XLSTAT (version 2019.4.2, Addinsoft SARL, Paris, France). Polychoric correlation factor analysis is the preferred method for studying the construct validity of exploratory and confirmatory data when using Likert scales and binary questionnaires [28]. Correspondence analysis is a statistical technique recommended for multivariate analysis of contingency tables. This analysis is used to graphically display the association in two-way categorical data [29].

Results

Reliability trial

Due to the anonymous nature of the survey, no respondent identification codes were used. Consequently, the use of a t-test for reliability was not possible. One-way ANOVA analysis was therefore used for the repeated trial survey to determine any differences between the response means for each question after a 4 week interval. There were no significant differences between the response means for each question, with p-values for each question ranging from 0.15 to 1.0.

Job role

The main working roles reported by the participants were winery (73.4%), vineyard (52.2%), laboratory (39.6%), cellar door (32.4%) and office (8.2%). Interestingly 63.3% of participants worked in more than one role, highlighting the multifaceted nature of wine industry work.

Lower limb problems

Lower back pain was the most commonly reported problem at 56% followed by foot pain (36.7%), knee pain (24.6%), leg pain (21.3%), ankle pain (17.9%), hip pain (15.5%), toe pain (13%) and heel pain (11.1%). Respondents who reported no problems at work were directed to the footwear, general and physical health question section.

Treatment sought

The most common practitioner survey participants sought out for treatment were physiotherapists (36.2%). Surprisingly, the same frequency of participants sought no treatment for their problems. Other practitioners consulted were general practitioners/medics (18.8%), podiatrists (18.4%), massage therapists (18.4%), chiropractors (16.4%), osteopaths (6.3%), surgeons (5.8%) and 14% had been hospitalised because of their problems.

Severity of pain

For participants that reported lower limb problems whilst working, pain was is in the 'mild' to 'moderate' range. How often this pain was experienced was in the 'occasionally' to 'very often' range. Whilst results for whether the pain limited work duties or caused difficulties performing work activities were in the 'occasionally' to 'many times' range (Table 1).

Limitations caused by lower limb problems

Overall the pain experienced by participants did not limit their ability to perform several activities (Table 2). Most activities were between 'not at all' and 'a little'. Only vigorous activities, bending and climb a hill were in the 'a little' to 'moderate' range.

Types of footwear worn

The most popular footwear used by participants were elastic sided safety boots, followed by high cut lace up safety boots with side zip (Table 3). Additional support or cushioning in shoes was reportedly used by respondents, however no details on the types of support or cushioning were recorded. That is, whether it was a custom-made foot orthosis or an off the shelf insole.

Footwear fit and comfort

The majority of participants reported that their work footwear was comfortable (Table 4), however more than a third of respondents reported that their shoes made their feet ache at work and that their shoes made their feet hurt after work. More than half of the respondents reported their boots as being hot and heavy. Some respondents reported difficulty in; finding shoes that did not hurt their feet, finding shoes to fit their feet and that

Table 1 Responses to questions relating to severity of pain at work

	Question	Mean	SD	F1%	F2%	F3%	F4%	F5%
(a)	Rate the level of pain you experienced in the last 12 months.	3.47	0.96	4.9	8.6	30.8	45.8	9.9
(b)	How often did you experience this pain?	2.94	1.01	0	43.8	20.9	27.7	7.6
	Were you limited in the duties you could do at work?	2.12	0.99	27.7	45.2	14.8	11.1	1.2
	Has it caused you to have difficulties in your work activities?	2.26	0.89	16.6	52.5	20.4	9.3	1.2

Response range and definition: (a) 1-low, 2-very mild, 3-mild, 4-moderate, 5-severe. (b) 1-never, 2-occasionally, 3-many times, 4-very often, 5-always. F1, F2, F3, F4, F5- Frequency (%) of response 1, 2, 3, 4, 5. *Mean* response mean, *SD* standard deviation

Table 2 Response to question; during a typical day how much does this pain interfere with the following activities

Question	Mean	SD	F1%	F2%	F3%	F4%	F5%
Vigorous Activities	2.65	1.19	14.8	39.5	21.6	14.2	9.9
Moderate Activities	1.98	0.91	34.4	41.3	16.2	8.1	0
Lift small objects such as shopping bags	1.59	0.86	59.4	26.9	10	2.5	1.2
Climb a hill	2.06	1.15	39.4	31.3	19.4	3.6	6.3
Walk up a flight of stairs	1.84	1.1	50.6	28.8	11.8	3.7	5.1
Bend	2.20	1.13	31.8	36.3	16.3	11.9	3.7
Walk 1 km	1.89	0.99	48.1	24.4	20	7.5	0
Walk 100 m	1.51	0.78	64.4	23.1	10	2.5	0
Shower yourself	1.36	0.68	71.9	24.4	2.5	0	1.2

Response range and definition: 1-not at all, 2-a little, 3-moderately, 4-very often, 5-always. *Mean* response mean, *SD* standard deviation. F1, F2, F3, F4, F5-Frequency (%) of response 1, 2, 3, 4, 5

their shoes were heavy. Overall, respondents thought; their shoes had good grip, their shoes were easy to take off and put on, their shoes fit well, had good ankle support, were durable, good value, felt safe and protected when wearing their footwear and they like the style.

General health

The majority of participants' general health was reported as being 'very good' to 'excellent' on the Likert scale, while very few reported their health as 'fair' to 'poor' (Table 5).

Overall, the participants agreed with statements describing themselves as healthy and happy. They were as 'healthy as anyone I know', full of life, calm and happy. However, 54.1% agreed with the statement 'I feel tired' and 21.7% agreed with the statement 'I feel depressed'.

Physical health

The participants reported on the whole that their overall physical health did not impede the types of activities they were able to achieve during work. Mean values for all questions relating to physical health ranged from 'definitely false' to 'mostly false' on the Likert scale (Table 6).

Table 3 Response to types of footwear most often worn at work

Boot Style	Frequency %
Elastic sided safety boots	46.4
High cut lace up safety boots with side zip	25.1
Sports shoe	15.0
High cut safety boots with laces	14.0
Elastic sided boots (not safety)	9.7
Rubber/Wellington/Gum boots	9.7
Dress shoe	9.7
Low-Mid cut safety shoes with laces	7.7
Use additional support or cushioning in your shoes	31.4

Correlation analysis

Fourteen correlations were identified but only two correlations relating to job role were seen (Table 7). That is, working at the cellar door and wearing sports shoes and a negative correlation for working in the office and wearing low-mid cut safety shoes as well as toe pain. The negative correlation indicates that a person working in an office is less likely to wear low-mid cut safety shoes and less likely to have toe pain. Wearing gum boots and knee pain had a moderate correlation while wearing low-mid cut safety shoes was negatively correlated with hip pain. Wearing dress shoes was negatively correlated with heel pain and ankle pain. Finally, elastic sided boots (not safety) were negatively correlated with hip, ankle and leg pain.

Correspondence analysis showed significant association between footwear worn and lower limb problems. In particular elastic sided safety boots were associated with hip, ankle, leg, lower back and foot pain (Table 8). High cut lace up safety boots with side zip were associated with heel, foot, toe and lower back pain. High cut lace up safety boots were associated with leg and ankle pain. Gum boots were associated with knee and ankle pain. These associations between footwear and site of problem, however, do not imply causation.

Discussion

To the best of our knowledge, this preliminary study is the first of its kind to identify whether lower limb problems occur in the Australian wine industry and what types of safety footwear are worn. It has also taken a snapshot of how these problems affect workers and how workers perceive their footwear. The survey was not comprehensive however, it has provided valuable information for developing further research into the future.

Table 4 Responses to questions regarding the fit and comfort of the footwear worn

Question	Mean	SD	Disagree (%)	Agree (%)
It is hard to find shoes that do not hurt my feet.	2.87	1.39	49.3	37.2
I have difficulty in finding shoes that fit my feet.	2.83	1.30	49.2	38.1
I am limited in the number of shoes that I can wear.	2.96	1.33	46.4	46.9
My shoes are comfortable.	3.59	1.00	18.4	63.8
My shoes have good arch support.	3.16	1.05	29.0	38.2
My shoes are cushioned.	3.47	0.99	18.4	58.5
My shoes make my feet ache when I am at work.	2.96	1.26	40.1	33.8
My shoes make my feet hurt after work.	3.01	1.25	41.5	41.1
My shoes have good grip.	4.10	0.74	3.4	85.5
My shoes make my feet feel hot.	3.41	1.03	23.2	56.5
My shoes are durable.	3.63	0.95	15.0	67.6
My shoes are easy to put on and take off.	4.01	0.96	10.6	79.2
My shoes fit well.	3.82	0.98	14.0	73.9
My shoes are heavy	3.55	1.02	17.9	58.9
My shoes are good value for money.	3.48	1.02	12.1	50.7
I like the style of my shoes.	3.68	1.00	12.6	63.8
I feel safe and protected when wearing my shoes.	4.02	0.76	2.9	79.7
My shoes provide good ankle support.	3.37	1.08	28.5	54.6
My shoes are waterproof.	2.70	1.14	51.2	30.9

Response range: 1-strongly disagree, 2-disagree, 3-neither agree nor disagree, 4-agree, 5-strongly agree. *Mean* response mean, *SD* standard deviation

For example, the nature of the work involved in each role was not explored in great detail, that is, how much time workers spent standing, walking or sitting in each role. Previous research has reported that an estimated 50% of the working population experience musculoskeletal disorders due to prolonged standing and that standing is implicated in lower back, lower limb and foot pain [30, 31].

Lower back pain was the most commonly reported problem at 56% followed by foot pain (36.7%), If foot, toe and heel pain are combined to total foot pain, 60.8% of respondents experienced some type of foot pain. Only 19.3% of respondents reported no lower limb problems, which is a common limitation of such self-selected surveys [18]. The results are similar to those reported for miners and their work boots, that is, lower back pain

Table 5 Response to general health and wellbeing questions

	Question	Mean	SD	% 1–2	% 4–5
(a)	How would you describe your general health?	3.81	0.67	1.5	69.1
(b)	I seem to get sick a lot easier than most people	1.68	0.85	66.7	4.3
	I am as healthy as anybody I know	3.78	0.97	12.1	73.4
	I expect my health to get worse	2.61	1.14	44.9	23.7
	My health is excellent	3.67	1.04	19.3	71.5
	I feel full of life	3.56	1.04	23.2	61.8
	I feel tired	3.24	1.25	36.7	54.1
	I feel calm	3.42	1.04	27.1	59.9
	I feel happy	3.80	0.95	15.5	74.4
	I feel depressed	2.29	1.16	65.2	21.7

Response range: (a): 1-poor, 2-fair, 3-average, 4-very good, 5-excellent. (b): 1-definitely false, 2-mostly false, 3-don't know, 4-mostly true, 5-definitely true. *Mean* response mean, *SD* standard deviation

Table 6 Responses to the questions; during the past 12 months, how much of the time have you had any of the following problems with your work or other activities as a result of your physical health?

Question	Mean	SD	False (%)	True (%)
Reduced the amount of time you spent on work or other activities.	1.68	0.80	85.9	1.9
Accomplished less than you would like.	1.85	0.85	77.3	3.3
Were limited in the kind of work or other activities.	1.87	0.89	78.2	3.8
Took extra time performing work or other activities.	1.89	0.83	79.2	2.9
Interfered with normal social activities with family and friends.	1.53	0.76	86.1	0.9

Response range and definition: 1-definitely false, 2-mostly false, 3-don't know, 4-mostly true, 5-definitely true. *Mean* response mean, *SD* standard deviation

(44.5%), foot pain (42.3%), knee pain (21.5%) and ankle pain (24.9%) [18].

The majority of lower limb pain experienced by participants was in the 'mild' to 'moderate' range (Table 1) and it was experienced 'occasionally' to 'many times', it did not however have a large impact on the ability for workers to complete their daily activities. Only vigorous activities, bending and climb a hill were in the 'a little' to 'moderate' range (Table 2). This could explain the unwillingness of participants to seek treatment for lower limb problems, as they may have felt that it was a 'normal' part of their work. Overall, physical health wasn't reported as being a concern, as was seen in Table 6, with the majority of respondents disagreeing with the statements about problems with work or other activities as a result of their physical health.

The majority of participants' general health was reported as being 'very good' to 'excellent' with very few reporting that their health was 'fair' to 'poor' (Table 5). However, 54.1% agreed with the statement 'I feel tired' and 21.7% agreed with the statement 'I feel depressed'. This figure for depression is similar to that reported by the Australian Bureau of Statistics [32] where 20.1% of Australians reported themselves as having an anxiety-related condition and depression or feelings of depression. While this survey was conducted during the SARS-CoV-2 pandemic, which may have impacted the mental health of the respondents, at the time of the survey no Australian states were under government sanctioned stay at home orders, but international travel restrictions were in place.

Table 7 Summary of polychoric correlation matrix of variables job role, footwear worn and lower limb problem (full matrix in Additional file 2)

Variables	Correlation
Foot pain x Toe pain	0.8
Heel pain x Toe pain	0.8
Cellar Door x Sports shoes	0.6
Leg pain x Ankle pain	0.5
Leg pain x Foot pain	0.5
Knee pain x Gum boots	0.5
Toe pain x Office	−0.9
Hip pain x Low-Mid cut safety shoes	−0.9
Hip pain x Elastic sided boots	−0.9
Heel pain x Dress shoes	−0.9
Ankle pain x Elastic sided boots	−0.9
Ankle pain x Dress shoe	−0.9
Leg pain x Elastic sided boots	−0.9
Office x Low-Mid cut safety shoes	−0.8

Moderate correlation 0.5 to 0.7, high correlation 0.7–1.0.
Negative correlation indicates inverse relationship of variables

Respondents reported using additional support or cushioning in their shoes, however no details on the types of support or cushioning were recorded. That is, whether it was a custom-made foot orthosis or an off the shelf insole. Dobson et al. [17] reported that in the mining cohort they studied, only 6.7% of respondents wore health professional prescribed orthoses. Flat insoles and contoured foot orthoses have been shown to increase plantar pressures at the midfoot, reduce plantar pressures at the rearfoot, and provide small reductions in tibial accelerations when used in high cut, lace up, boots [33]. However, no differences in 'boot comfort' between the no insole, flat insole and contoured foot orthosis groups were identified in this previous study [33]. Therefore, no conclusions can be made as to whether the use of additional support or cushioning had an effect on respondents' comfort in this preliminary survey.

Elastic sided safety boots were the most popular, followed by the high cut lace up safety boots with side zip. Even if high cut lace up safety boots are considered analogous to the high cut lace up safety boots with side zip, elastic sided safety boots are still the most popular. Also, workers that wear elastic sided safety boots are more likely to experience lower limb problems such as foot and lower back pain, however no link between footwear and lower limb pain can be inferred from this study. Safety footwear was also reported as being hot and heavy in this study. Heavy footwear has been associated with increased energy expenditure by workers wearing safety footwear [34]. Dobson et al. [17] report that 62.3% of miners believed that their foot and ankle pain was related to their work boots. One explanation for this

Table 8 Correspondence analysis of relationship between the footwear worn and the lower limb problem

	ElastBS	LaceZip	HSBL	LMSBL	ElasB	Sport	Gum	Dress
Lower back	32%	20%	12%	3%	6%	10%	7%	11%
Hip	45%	18%	5%	0%	0%	14%	9%	9%
Leg	39%	14%	18%	7%	0%	12%	7%	4%
Knee	31%	17%	10%	3%	4%	15%	15%	5%
Ankle	40%	7%	18%	4%	0%	16%	15%	0%
Feet	32%	24%	8%	9%	6%	9%	6%	7%
Heel	20%	31%	6%	6%	14%	6%	17%	0%
Toe	30%	23%	5%	14%	5%	9%	9%	5%

$\chi^2 = 82.9$, p-value 0.002, $\alpha = 0.05$.
ElastBS elastic sided safety boots, *LaceZip* high cut lace up safety boots with side zip, *HSBL* high cut lace up safety boots, *LMSBL* low/mid cut lace up safety shoes, *ElasB* elastic sided boots, Sport- sports shoe, *Gum* gum/wellington boots, *Dress* formal or dress shoe

is that miners may be wearing boots that are longer than their feet, possibly because boots in their correct length are too narrow [35]. Dobson et al. [36] concluded that traditional fitting methods based on foot length were insufficient when fitting miners. Grau and Barisch-Fritz [37] concur and state that foot width and girth measures are different in static and dynamic loading situations and must be considered when manufacturing and fitting safety footwear to aid in supporting workers health. Buldt and Menz [38] state that between 63 and 72% of the general population are wearing inappropriately sized footwear based on length and width measurements, and that incorrect footwear fitting is significantly associated with foot pain. In this study however, 73.9% agreed with the statement that their footwear fit well.

Many studies have explored the relationship between safety footwear and injuries with the majority focusing on; high cut lace up safety boots, military boots, gum boots, sports shoes [16] and surgical clogs [31]. These studies have identified many relationships, for example, gum boots are associated more with knee and heel pain while high cut lace up safety boots were associated with more leg and ankle pain [39]. Gum boots are associated with more force and contact area in the heel compared to the high cut lace up safety boots [40]. High cut lace up safety boots with varying sole and shaft stiffness are associated with effects on lower limb muscle activity, ankle motion [41] and plantar pressures [42]. High cut lace up safety boots also have an impact on postural control [43] and postural stability under workload [44]. High cut lace up military boots while carrying a workload also have an effect on postural stability and heel contact during slip events [45, 46].

To date no data exists on the effect of elastic sided safety boots in any industry. There is also no published data on how safety footwear is supplied to workers and protocols for when it is replaced in the wine industry. Recent research into shoe tread (sole) wear and wear measurement has highlighted the need for understanding the mechanism for shoe tread wear and individualised shoe replacement recommendations to prevent injury caused by the decline in traction of worn shoes [47–49].

The popularity of elastic sided safety boots in the Australian wine industry is a unique phenomenon and its use therefore may be more due to tradition. Elastic sided boots were developed in the early 1900s to withstand the harsh, unforgiving environment of the Australian outback by providing a boot that was comfortable, rugged and able to cope with both hot/dry and cold/wet seasons [50]. They became popular heavy-duty footwear for farming, forestry, mining, and industrial uses [50]. Another reason for the popularity of elastic sided safety boots may be the nature of the work in the Australian wine industry. As has been highlighted, many wine businesses require workers to perform varying jobs over different sites and conditions, this often involves a quick change of suitable footwear, 79.2% of respondents agreed with the statement that their shoes are easy to put on and take off, which could also help to explain the popularity of elastic sided safety boots.

Boot design features have been shown to have an influence on the lower limbs depending on the task being performed and the supporting surface [16]. Therefore, occupational specific testing of footwear effects should occur in the Australian wine industry in order to try and accommodate for individual workplace environments.

Conclusion

This study has shown that lower limb problems occur in the Australian wine industry and that even if a problem is present workers often do not seek treatment or let the problem interfere with their work activities. This may be a function of the vintage/harvest season, when harvesting and processing grapes at their optimum condition places time constraints on workers. The study also demonstrated that elastic sided safety boots were the most popular amongst respondents.

These factors highlight the need for further research into the footwear used in the Australia wine industry to better support workers health while working in varied roles and conditions. A comparison of different footwear in different environments could take place as well as exploring the time taken for footwear to deteriorate in these environments. The optimum length of efficacy of the footwear could also be assessed to ensure footwear is replaced at appropriate times and not used when worn excessively. Future research is warranted to determine any barriers and facilitators regarding boot choice in the wine industry, especially when the multi-faceted work environments and the appropriate footwear for specific roles are taken into consideration.

As is the case with all surveys, there are limitations to this study and the accuracy of self-reported measures. This may have caused some selection bias, with participants who have experienced lower limb problems more likely to self-select to participate in the survey.

Recall bias can also be a problem, an attempt to minimise this risk was to set a 12 month limit on the survey, that is, participants were asked if they had worked in the Australian wine industry in the last 12 months. If they had not, they were directed to leave the survey. No demographic data on the participants were collected in this preliminary survey. Future research involving randomised control trials of specific footwear could include the collection of demographic data for more detailed analysis. Finally, it is not possible to conclude whether specific job roles had higher risks for lower limb problems with specific footwear. Correlations of these two factors does not imply any causation and requires further research to determine any relationship.

Abbreviations

A$: Australian Dollars; ANOVA: Analysis of variance; ElastBS: Elastic sided safety boots; LaceZip: High cut lace up safety boots with side zip; HSBL: High cut lace up safety boots; LMSBL: Low/mid cut lace up safety shoes; ElasB: Elastic sided boots; Sport: Sports shoe; Gum: Gum/wellington boots; Dress: Formal or dress shoe

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13047-021-00495-3.

Additional file 1. Copy of Survey

Additional file 2. Full polychoric correlation matrix of variables; job role, footwear worn and lower limb problem.

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Authors' contributions

All authors contributed to the design, registration, conduct and reporting of this research. AC completed the quantitative analysis. All authors read and approved the final manuscript.

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Availability of data and materials

The dataset used and analysed during this study is available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Ethical permission to undertake this work was granted by the University of Adelaide (H-2020-267). All participants consented to take part in this research and for the findings to be published.

Consent for publication

Consent to publish research findings was obtained from all participants prior to their participation in this project. No personal identifiable data is included within this publication.

Competing interests

The authors declare that they have no competing interests.

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