Ph.D. Thesis in Food Science

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Relations between plant hydraulics and wine production: morpho-functional and isotopic traceability to evaluate sustainability in a climate change context

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Preface

The work presented in this thesis was mostly carried out at the Department of Agricultural Sciences of the University of Naples Federico II, over a period of three years and three months from April 2019 to July 2022 under the supervision of Prof. Veronica De Micco. The project was funded by the Campania region, in the framework of POR Campania FSE 2014-2020 ASSE III – Ob. Sp. 14 Az. 10.5.2. “Dottorati di Ricerca con Caratterizzazione Industriale” – D.D. n.155 del 17.05.2018 (CUP E66C18000900002 CML OP_7741 18062AP000000001). The industrial project partner was the farm “Società Cooperativa Agricola - La Guardiense ” where a period of nine months was spent under the supervision of Dr. Marco Giulioli (both in presence and remotely due to covid-19 pandemic). Furthermore, seven months and half were spent at the Swiss Federal Institute for Forest, Snow and Landscape Research WSL of Zurich (CH), under the supervision of Prof. Paolo Cherubini (both in presence and remotely due to covid-19 pandemic). Other collaborations included: Prof. Chiara Cirillo at the Department of Agricultural Sciences of the University of Naples Federico II, Prof. Carmen Arena at the Department of Biology of the University of Naples Federico II, Prof. Giovanna Battipaglia at the Università degli Studi della Campania "Luigi Vanvitelli" of Caserta, and Dr. Antonello Bonfante and Dr. Arturo Erbaggio at the CNR Isafom – Istituto per Sistemi Agricoli e Forestali del Mediterraneo of Portici (NA).

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Abstract

In the Mediterranean region, climate change is leading to an increase in temperature and in the frequency and severity of prolonged droughts, that are affecting grapevine growth and reproduction with consequences on grapes yield and quality. Climate change is one of the major challenges for future viticulture, especially in arid and semi-arid regions of Europe, since it has been forecasted a dramatic change in the landscape with geographical shifting of the grapevine production regions. In this thesis project (funded by the Campania region, in the framework of POR Campania FSE 2014-2020 ASSE III – Ob. Sp. 14 Az. 10.5.2. "Dottorati di Ricerca con Caratterizzazione Industriale" – D.D. n.155 del 17.05.2018), the impact of pedo- climatic variability on growth and productivity of four Falanghina vineyards (Controlled designation of origin– DOC/AOC) in southern Italy (Guardia Sanframondi, Benevento, Campania region) has been analysed over three years. The vineyards were selected and studied (SL-Santa Lucia, GR-Grottole, CA-Calvese, AC- Acquafredde) within the fields of the La Guardiense farm. The vineyards were similar for plant material and cultivation techniques, apart from water availability and pedo-climatic conditions. The vineyards were compared in terms of their eco-physiological history (through the retrospective analysis of vine-wood ring series by applying dendroanatomical and dendroisotopic approach) and in terms of growth, ecophysiology and production over the last three years. The in-vivo measurements were performed with morphological analysis (e.g. leaf area, bunch weight) and eco-physiological measurements (gas exchange and chlorophyll fluorescence emission) to analyse the plant vigor. Microscopy analyses on leaves of the vines from the four experimental vineyards were also performed to quantify stomata and vein traits to understand how they aremodulated by the environmental conditions during leaf development.

$\delta^{13}C$ was analysed on three different matrixes, namely wood, leaf and must in order evaluate the most representative matrix of the vine water status and to setup a simplified method to analyse it. The retrospective investigation with $\delta^{13}C$ and wood anatomical analyses (e.g. vessel lumen area, potential hydraulic conductivity, hydraulic diameter) showed the occurrence of differences among the four studied vineyards indicating different levels of drought stress and therefore different WUE, confirming the results obtained in the in-vivo measurements. The application of $\delta^{13}C$ analysis was useful to reconstruct vine statusfollowing the isotopic trace throughout the plant up to berries/must and results were in line with in-vivo measurements and wood anatomical parameters. The overall results of the morpho-physiological chemical and retrospective sanalysis showed differences in characteristics of the four vineyards, with the field CA (Calvese) and GR (Grottole) showing pedoclimatic conditions limiting for growth and yield compared to SL (Santa Lucia) and AC

(Acquefredde). In the latter, the application of supplemental irrigation was useful to mitigate the effects of water deficit during summer.

Abstract (ITA)

Nella regione del Mediterraneo, i cambiamenti climatici stanno portando ad un aumento delle temperature e della frequenza e severità di eventi di siccità prolungate, che stanno influenzando crescita e riproduzione della vite con conseguenze sulla produzione dal punto di vista quantitativo e qualitativo. Il cambiamento climatico è una delle maggiori sfide per la futura viticoltura, soprattutto nelle regioni aride e semi-aride d'Europa dove c'è un rischio elevato di spostamento geografico delle regioni di produzione della vite . In questo progetto di tesi (finanziato dalla Regione Campania, POR Campania FSE 2014-2020 ASSE III – Ob. Sp. 14 Az. 10.5.2. "Dottorati di Ricerca con Caratterizzazione Industriale" – D.D. n.155 del 17.05.2018) è stato analizzato l'impatto della variabilità pedoclimatica sulla crescita delle viti e produttività in quattro vigneti di Falanghina (Denominazione di Origine Controllata – DOC/AOC) nel sud Italia (Guardia Sanframondi, Benevento, regione Campania) per tre anni. I quattro vigneti sono stati selezionati (SL-Santa Lucia, GR- Grottole, CA-Calvese, AC-Acquafredde) tra quelli dell'azienda agricola La Guardiense. I vigneti sono simili per materiale vegetale e tecniche di coltivazione, ma differenti relativamente alla disponibilità idrica e condizioni pedoclimatiche. I vigneti sono stati confrontati in termini di storia ecofisiologica (analisi retrospettive attraverso l'applicazione dell'approccio dendroanatomico e dendroisotopico sul legno di vite) e in termini di crescita, ecofisiologia e produzione negli ultimi tre anni. Le misurazioni in vivo sono state eseguite con analisi morfologiche (es. area fogliare, peso del grappolo) ed ecofisiologiche (scambi gassosi ed emissione di fluorescenza di clorofilla) per analizzare il vigore delle piante. Sono state inoltre eseguite analisi al microscopio sulle foglie delle viti dei quattro vigneti sperimentali per quantificare tratti anatomici funzionali per capire come sono modulati dalle condizioni ambientali durante lo sviluppo fogliare.

Gli isotopi stabili del carbonio ($\delta^{13}C$) sono stati analizzati su tre diverse matrici, ovvero legno, foglia e mosto, al fine di valutare la matrice più rappresentativa dello stato idrico della vite e di impostare un metodo semplificato per analizzarla. L'indagine retrospettiva con $\delta^{13}C$ e analisi anatomiche del legno (es. dimensioni delle trachee, conducibilità idraulica potenziale, diametro idraulico) ha mostrato differenze tra i quattro vigneti studiati indicando diversi livelli di stress da siccità e quindi WUE diversi, confermando i risultati ottenuti nelle misurazioni in vivo. L'applicazione dell'analisi $\delta^{13}C$ è

stata utile per ricostruire lo stato idrico della vite seguendo la traccia isotopica in tutta la pianta fino agli acini/mosto e i risultati sono stati in linea con le misurazioni in vivo e i parametri anatomici del legno. I risultati complessivi della analisi morfofisiologica chimica e retrospettiva hanno mostrato differenze nelle caratteristiche dei quattro vigneti, con il campo CA (Calvese) e GR (Grottole) che mostrano condizioni pedoclimatiche limitanti rispetto a SL (Santa Lucia) e AC (Acquefredde). In quest'ultimo vigneto, l'applicazione di irrigazioni di soccorso ha mitigato gli effetti dello stress da deficit idrico nel periodo di maggiore aridità estiva.

Introduction and aim

The present thesis project titled "Relations between plant hydraulics and wine production: morpho-functional and isotopic traceability to evaluate sustainability in a climate change context" has a multidisciplinary approach and aimed towards the creation of synergies between the world of scientific research and farms. Applying the research results to technological development, this research aims to improve the innovation in agriculture and in particular viticulture sector. Indeed, the introduction of new knowledge and technologies is pivotal for future viticulture in a context of climate change scenario in the Mediterranean areas to mitigate the negative impact of water shortage on yield and quality, improving the resource use efficiency, in particular the water resource. The realization of sustainable grapevine cultivation must be based on a precise knowledge of vines morpho-anatomical development and physiological behaviour in different pedoclimatic conditions since pedoclimatic spatial and temporal variability can influence vines capacity to face environmental constraints.

In viticulture, the increasing temperature and frequency of drought stress during summer arebecoming a significant problem, which often is neglected. The frequency of extreme climatic events(e.g. severe aridity, heat waves, flooding) is likely to increase and depending on the region and the amount of change, this may have positive or negative implications on wine quality. For instance, vinephenology is driven by temperature and the forecasted rise in temperature often anticipates the phenological phases with the problem of sudden spring frosts causing serious damage to the shoots thus compromising the production. The increasing temperature shifts the ripening phase to hotter periods in the summer, and this phenomenon will affect grape composition, in particular with reference to aroma compounds. Increased water stress reduces yields and modifies fruit composition. The increasing temperature and frequency, duration and severity of drought events are also responsible for sugar accumulation increase, degradation of malic acid and secondary metabolites like anthocyanins, with the consequence to obtain wines with a too high alcohol content and an unbalanced aromatic profile. Even though there have been many studies concerning the impact of climate changes on international grapevine cultivars, the autochthonous Italian vine cultivars need to be more investigated, especially in the areas traditionally suited for viticulture that are endangered byextreme climatic changes, as the southern Italy.

In a context of climate change, given that the main viticultural areas in southern Italy are under rainfed regime and given the scarcity of the water resource accompanied by logistic difficulties to realize irrigation in specific areas, it goes without saying that the improvement of water use efficiency in vines can play a key role for the sustainability of the future viticulture. The concept of WUE reflects a balance between gains (carbon acquisition or crop yield, AN) and costs (water consumed by transpiration and water applied, E), and their balance determines the performance of the vine growth and yield. The water use efficiency depends on various factors, as: vine cultivar, rootstock, pruning system, soil properties, soil management which act alone and in interaction each-other. The management of these factors cannot be separated from the knowledge of the morpho-physiological plasticity of the vines in response to variable environmental and cultivation conditions. Knowing how vines have reacted in the past to changes in these conditions is the starting point for predicting how they will react to future changes. Important information on the response of plants to agro-environmental factors is enclosed in the wood (annual growth circles) of the shoots. The vine taken as a model in this proposal is Falanghina, widely spread throughout the Campania region. The thesis project was directed towards a characterization of the relationships between pedo-climatic variables, morpho-functional and isotopic parameters in vines and musts of four Falanghina vineyards in the area of Guardia Sanframondi (BN) at the premises of La Guardiense farm.

The present thesis is organized as follows:

CHAPTER 1 is a deep focus on morphological development, productivity and eco-physiological characterization of vines of *Vitis vinifera* L. subsp. *vinifera* 'Falanghina' (Controlled designation of origin – DOC/AOC) in four vineyards in southern Italy (Guardia Sanframondi, Benevento, Campania region) over three years. The four experimental sites were selected within the vineyards of the La Guardiense farm: SL-Santa Lucia, GR-Grottole, CA-Calvese, AC-Acquafredde. The general idea was to select vineyards similar for plant material and cultivation techniques, apart from water availability. A different coordination between biometrical vine growth parameters and eco-physiological parameters in the four vineyards was found due to the different pedoclimatic characteristics which lead to different water availability in the soil. The results were useful to better understand the vine behaviour in the continuum soil-plant-atmosphere, which is important in order to create the fundamental knowledge on which to design new cultivation strategies in viticulture to mitigate the negative effects of the future climate changes.

CHAPTER 2 presents evidence that leaf anatomical traits related to stomata and veins in Falanghina vines develop differently in a range of field pedoclimatic conditions varying in moisture availability. Microscopy analyses on leaves of the vines from the four experimental vineyards were performed to quantify stomata and vein traits, while eco-physiological analyses were conducted on vines to assess plant physiological adaptation capability. The overall findings suggested that site-specific stomata and vein traits modulation in Falanghina grapevine are an acclimation strategy that may influence photosynthetic performance. A manuscript reporting these data has been published in Plants (Damiano et al.2022b https://doi.org/10.3390/plants11111507).

CHAPTER 3 is focused on the effect of different pedoclimatic conditions on crop yield and must quality of Falanghina vines growing in the four experimental sites over three years. In this study, vine growth was monitored and yield and must quality were characterized. The overall results showed differences in yield and quality characteristics for the four vineyards, with the field CA (Calvese) and GR (Grottole) showing pedoclimatic conditions limiting growth and production. A manuscript reporting these data has been submitted in Horticulturae (MDPI).

CHAPTER 4 focuses on the possible application of proximal and remote sensing techniques in the four experimental Falanghina vineyards. Several indicators are currently used to evaluate plant growth, based on in situ data collection or remote sensing. In this study, we proposed a multiscale approach to assess and interpret plant growth indicators in vineyard systems by linking data from in vivo plant growth and eco-physiological monitoring, from UAV techniques and also reconstructing past eco-physiological behavior of vines by transferring the approach of dendro-sciences, typical of the forest science domain, to viticulture. A Manuscript reporting on these has been published in Metroagrifor proceedings (Damiano et al. 2019 https://doi.org/10.1109/MetroAgriFor.2019.8909258).

CHAPTER 5 presents the state of the art of stable isotope applications in viticulture by presenting the elements from the most to the least studied. This chapter outlines some approaches that, to date, have been sometimes considered in viticulture as the focus on a study case aiming to analyse the variability of $\delta^{13}C$ in different organs of grapevine as indicator of plant water stress. In the the study case, $\delta^{13}C$ was analysed on three different matrixes, namely wood, leaf and must in order to evaluate which is the most representative of the vine water status. In order to do this, materials from vines growing in the four experimental Falanghina vineyards were analysed since the four vineyards can be classified into two groups (wetter and dryer) based on water availability in the soil and therefore represent an ideal study model.

CHAPTER 6 report on the possible application of a simplified method of $\delta^{13}C$ extraction from musts which could enhance the application of the $\delta^{13}C$ at a larger scale to evaluate vine adaptation inthe context of climate-change. The analysis of $\delta^{13}C$ is often used in viticulture to understand vine water use. In this study, the aim was to compare the results obtained by the application of two different methodologies, using the matrix whole must, or extracted sugars as usually performed in literature. In this study published in Horticolturae (Damiano et al. 2022a https://doi.org/10.3390/horticulturae8030226) the results showed that the $\delta^{13}C$ values obtained by applying the two methodologies were comparable in all analyzed vineyards independently from the pedoclimatic conditions. Indeed, the proposed method of extraction of the $\delta^{13}C$ on the must as a whole can be both cost- and time-saving for the analysis enhancing the application of the ^{13}C at a larger scale to evaluate vine adaptation in the context of climate-change-driven increases in drought.

CHAPTER 7 focuses on how alterations in vine eco-physiological behaviour, induced by changes in environmental factors and in the cultivation management are recorded in wood anatomical and isotopic traits in grapevine trunks. In this study, we characterized the anatomical traits and carbon stable isotopes in wood rings of Falanghina vines from the four experimental vineyards, to evaluate the influence of local site conditions on wood plasticity in response to inter-annual climate variability. The overall analysis showed that the four vineyards are characterized by different wood structure with some vineyards showing wood traits targeted to favour the efficiency of water flow, while othersfavour safety against embolism. A manuscript reporting these data has been submitted in IAWA Journal (Brill).

CHAPTER 8 was dedicated on the more industrial part of the project and is a report of the microvinifications performed in the three years of plant monitoring in order to evaluate the effect of different pedoclimatic characteristics on the winemaking process and the final wine quality. Differences were observed among the four wines. The wines were different with CA and GR showing an higher alcohol level than SL and AC, which in turn showed the highest level of titratable acidity, confirming the importance of the pedoclimatic condition effect on the final quality of the musts and thus wine.

CHAPTER 1

Pedoclimatic characterization of the study sites and morpho-physiological characterization of *Falanghina* grapevine in the experimental vineyards

1. Introduction

Grapevine (*Vitis vinifera* L. subsp. *vinifera*) is the major cultivated fruit crop worldwide, with an annual European wine production accounted for about 63% of the world's total wine production in 2020 (OIV, 2021). However, climate is one of the most important factors affecting the agricultural production and the observed trends in climate changes (Junk et al., 2019; Pörtner et al., 2022) are expected to have significant impacts on viticulture. Forecasted climate changes are expected to differently influence the grapevine agroecosystems in distinct regions, altering the relations in the climate-soil-variety system (van Leeuwen et al., 2019a). In the Mediterranean Region, the grapevine cultivation is exposed to more and more extreme environmental conditions such as frequent, prolonged and severe periods of water scarcity, high temperatures, and strong winds (Pörtner et al., 2022). Usually, grapevine is cultivated in rain-fed regime in many Mediterranean cultivation areas, as requested by the production disciplinary of quality and origin labels, but the increasing temperature and drought stress in the last decade, are leading to increasing difficulties in maintaining high yields and quality levels (OIV, 2021). The Mediterranean area is indicated as a potential climate change hotspot and is highly exposed to environmental limitations, with high air temperature and soil water deficit affecting berry growth and ripening, ultimately compromising yield and grape quality (Cabral et al., 2022; Keller et al., 2016). This condition will probably result in a lower wine grape production endangering the suitability of this areas for viticulture (Cardell et al., 2019; Moriondo et al., 2013). On the contrary, the regions of central Europe with cooler seasons are becoming progressively suitable for grapevine cultivation due to the increased temperatures. Meanwhile, the increasing temperature can lead to an increase in the frequency and duration of drought episodes and also in cool-climate wine regions, viticulture is already subject to some degree of water deficit (Chaves et al., 2010; van Leeuwen et al., 2019b). In recent years, also in many German winegrowing regions, drought stress during summer has gained increasing attention (Schultz et al., 2010). The raise of drought stress largely results from the effects of (i) increased temperatures, especially in the post-flowering phenological phase, leading to a higher potential evapotranspiration rate (Molitor & Junk, 2019), and (ii) increasing of prolonged heat waves occurrence (Junk et al., 2019). Grapevine water status is one of the key factors determining grapevine yield and wine quality (Chaves et al., 2010; Gambetta et al., 2020; van Leeuwen et al., 2009, 2019b). A mild water deficit is required for the production of high-quality wines, especially for red wine, where it promotes the phenolics accumulation in grape berry skin (Gambetta et al., 2020; van Leeuwen et al., 2009). Concerning severe water deficit impacts on viticulture, there is a consensus in the literature which confirms its negative effect on vine yields due to the reduction of berry size and bud fertility (Roby et al., 2004; Triolo et al., 2019). Moreover, the effects of water deficit vary according to timing, intensity and duration during successive grapevine phenological stages.

The Mediterranean climate is characterized by mild rainy winters, and a hot and dry summer season which in viticulture typically leads to a progressive decrease in shoot growth balanced by increasing of carbon investment for berry ripening, but ultimately the sever water stress is causing physiological and metabolic disorders with negative effects on the overall plant functioning, including nutrient uptake, fruit set, and berry ripening (Pagay et al., 2016). Many adaptation mechanisms can occur under water stress conditions mostly related to an increase in water holding capacity, decreasing the water losses, reinforcement mechanical resistance to prevent any tissue damage (De Micco & Aronne, 2012). One of the first plant responses to severe water deficit is the decreasing investment in shoot growth and leaves, compared to other organs because of a change in carbon partitioning promoting the flow of assimilates towards the root (Bacelar et al., 2007). The period between flowering and veraison phenological phases represents an important moment for berry response to water deficit, which can affect berry size and composition (Gambetta et al., 2020; Intrigliolo et al., 2012; Ojeda et al., 2001, 2002; Ramos et al., 2020). The reason could be the hydraulic connection between berries and vine through xylem; therefore, berry development is strongly dependent on the vine's water status and berries in this period are prone to drought-induced shriveling. Conversely, post-veraison berries are mainly connected to the phloem, thus they become less sensitive to drought, while dependent more on availability of photosynthates (Gambetta et al., 2020; Intrigliolo et al., 2012).

The intensity and duration of the water deficit, as well as other weather conditions, can induce changes in plant behavior with responses at all levels of the complex plant organization (Shao et al., 2008). Drought stress has effects on many processes involving gas exchange, leaf water potential, etc. (Jimenez-Garcia et al., 2013). It reduces stomatal conductance, transpiration, and net photosynthesis rate. Stomatal closure is one of the first responses to water deficit that allows plants to limit respiration, but it also limits CO_2 absorption, resulting in a decrease in photosynthetic activity (Flexas

& Medrano, 2002). The expected increase in air temperature and intensity of climatic anomalies will generate also a high vapor pressure deficit (VPD) at the atmospheric level with the consequence to produce an increase in the annual rate of potential evapotranspiration (ET_o) of around 75–125 mm by the 2050 s for most of European regions (Dezsi et al., 2018). Therefore, there will be an increase in the water needs of vines and the implementation of irrigation strategies will be necessary to maintain the sustainability of vineyards and in the main time preventing severe stress in many wine-producing regions of the Mediterranean area (Iglesias & Garrote, 2015).

In the future viticulture, a sustainable development of the wine grape cultivation is of particular importance for the areas traditionally suited for viticulture in Europe. There is common agreement that monitoring the vine water status along years is needed to understand the vines' responses to year-to-year environmental variability, in order to better design cultivation strategies to mitigate the negative effects of increasing water shortage. In this direction, there are studies focused on the application of an irrigation management in viticulture. According to this, especially in the regions where rainfall is infrequent, the irrigation practice is becoming increasingly used in the vineyards, in order to homogenize yields and minimize interannual variability (Cancela et al., 2016). While the use of irrigation in viticulture for wine production is a common practice in New World countries, it is prohibited in Europe for most of the "Demarcation of controlled production areas" (DOC). However, irrigation process must be managed with attention also because many studies have shown how irrigation may modify the sensory characteristics of wines, providing herbaceous notes that vary with the amount of applied water (Mendez-Costabel et al., 2014). Therefore, to apply the irrigation in future viticulture there is need to balance vine growth and grape berries composition (Delgado et al., 2022) considering that the aroma compounds concentration in grapes change during ripening mainly depending on the temperature and water availability (Robinson et al., 2014).

In this framework, the aim of this study was to analyze the plasticity of growth and eco-physiological parameters of 'Falanghina' grapevine cultivated in four vineyards in southern Italy. We firstly characterized the four sites from pedoclimatic point of view. Then, our specific aim was to evaluate how morpho-anatomical parameters, eco-physiological parameters and grape production parameters are coordinated under different pedoclimatic conditions.

2. Material and methods

2.1 Experimental design, plant material, and soil characteristics

The study area is located in a hilly environment in southern Italy (Guardia Sanframondi, Benevento, Campania region). Four experimental sites were selected within the vineyards of the La Guardiense farm, as follows: 1) SL-Santa Lucia (lat: 41.246357; lon: 14.570825,194 m a.s.l.); GR-Grottole (lat: 41.240120; lon: 14.584056, 158 m a.s.l.); CA-Calvese (lat: 41.237675; lon: 14.587291, 163 m a.s.l.); AC-Acquafredde (lat: 41.229231 lon: 14.592362, 84 m a.s.l.). The general idea was to select vineyards as much as possible similar for plant material and cultivation techniques, apart from water availability. Therefore, in the four vineyards, the same cultivar *Vitis vinifera* L. subsp. *vinifera* 'Falanghina' (Controlled designation of origin – DOC/AOC), is grafted on the same rootstock (157-11 Couderc) and vines are characterized by similar age, training system and pruning management (double Guyot system). In all the vineyards, vines are spaced by about 2.2 × 1 m spacing (≈ 4545 vines/ha), and E-W row orientation for sites GR, CA, AC, while N-S for the SL site. The SL, GR, and CA vineyards were cultivated in a rain-fed regime, while at AC, supplemental irrigation was applied in the summer months.

2.2 Soil characteristics and meteorological data analysis

The study sites are located in two environmental systems of the soil map of the Telesina valley (1: 50,000; Terrible et al., 1996): the pre-Apennine Hill system and the Intermontana plain system. In particular, the sites of Santa Lucia (SL), Grottole (GR) and Calvese (CA) are within the hilly system in the GLA subsystem, pedological unit PEN1 (suoli della consociazione dei suoli Pennine), while the Acquefredde (AC) site is located in the intermontana plain system, subsystem TET, soil unit TAS1 (suoli della consociazione Taverna Starze) (Fig.1). In each of the four sites, a soil profile was analyzed in order to identify the soil horizons and to describe their pedological properties. Through the three year of study, meteorological data including annual mean temperature, cumulative annual precipitation and monthly data regarding the wettest and driest periods, were obtained from the Campania region meteorological whether station located in Guardia Sanframondi, (www.agricoltura.regione.campania.it/meteo/agrometeo.htm). At the end of June 2020, a weather station was installed in the CA vineyard (Netsens AgriSense IoT weather station, www.netsens.it). The positioning of the Netsens weather station was determined as representative of air temperature, air humidity, wind speed, and solar radiation of all selected vineyards, considering the distance between the experimental vineyards and the landscape form (e.g., slope, elevation). Moreover, considering that, among the weather variables, rainfall is the one characterized by the highest spatial variability, a rain gauge with three FDR probes (inserted at three different soil depths, - 15, - 35, and - 75 cm) was placed in each experimental site in order to measure soil temperature and soil water content. The FDR probes were applied to understand the soil water status during the growing season, considering that the precipitation amount does not represent available water for plants, which depends on the combination of weather conditions and soil properties (e.g., under the same climate, two soils can have a very different water availability for the plant) (Bonfante et al., 2020). The main weather information collected (e.g., temperature, solar radiation) from both weather stations in 2020 were comparable. However, the soil type and management are different at the four sites leading to different conditions of water availability.

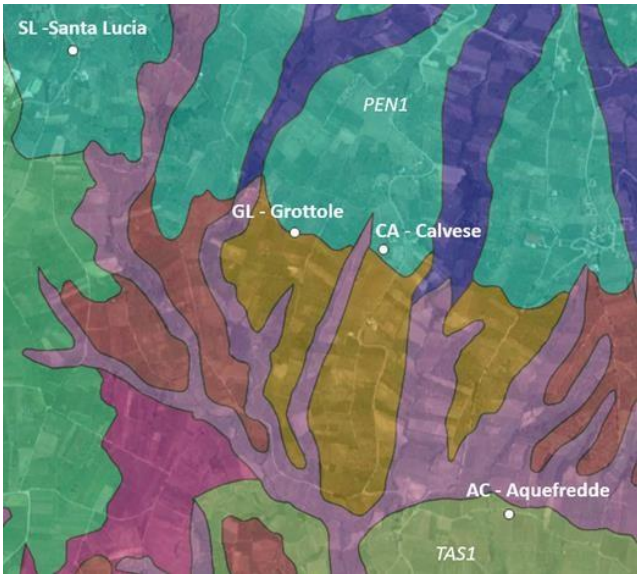

Fig.1: Study sites and soil units involved in the soil map of the Telesina valley (1: 50.000) (PEN1: Consociazione suoli Pennine; TAS1: Consociazione dei suoli Taverna Starze).

2.3 Biometrical analyses and yield

Biometrical parameters were estimated in 20 vines per vineyard on two shoots per plant at the main phenological phases (Pre-Flowering, Fruit set, Veraison and Ripening). The estimation of leaf area was performed by applying an allometric estimation model measuring the leaf lamina width in field and applying the equations calculated based on the measurement of width and area of 20 leaves per site by means of an electronic leaf area meter (LI-3100 model, LI-COR Inc., Lincoln, Nebraska, United States) (Caccavello et al., 2017; Cirillo et al., 2021). Two-one year old shoots (holding the production of the year) per plant were selected to monitor growth by recording shoot length, shoot basal diameter, single leaf area, main leaf area, anticipated leaf area and total leaf area. The fruit set was also analyzed on the same shoots. More precisely, all the bunches on the selected shoots were photographed with a digital camera and images were subjected to digital image analysis through the software Image J (Rasband, NIH) in order to count the number of visible flowers per bunch. On the same date, from other plants, 12 bunches per treatment were photographed and sampled: for them, the number of flowers per bunch was counted both through digital image analysis and manually to achieve the real flower number. The relationships between the flowers counted through image analysis and the real number of flowers were extracted and the equations used to estimate the real number of flowers per bunch also in all the other photographed bunches. In addition, at the ripening phase, the same procedure was applied for the berries in order to calculate the fruit set rate as the ratio between the number of berries and of flowers on the two selected shoots per vine. Finally, at the ripening phase, the average bunch weight (total yield per vine/bunch number per vine), and berry diameter (on 150 fruits per field) were also recorded. Vine fertility was then estimated as both real fertility (RF, the bunch number per number of buds) and potential fertility (PF, the bunch number per number of shoots.

2.4 Leaf gas exchanges and chlorophyll fluorescence emission

Leaf gas-exchange and chlorophyll "a" fluorescence emission measurements were carried out on 2 well-exposed and fully expanded leaves per 12 plants per vineyard during the four selected phenological phases over the three growing seasons (2019-2020-2021). Net CO_2 assimilation rate (Pn), stomatal conductance (gs), substomatal CO_2 concentration (Ci) and leaf transpiration rate (E) were performed by means of a portable infra-red gas-analyzer (LCA 4; ADC, BioScientific, Hoddesdon, United Kingdom equipped with a broad-leaf PLC (cuvette area 6.25 cm2). Chlorophyll "a" fluorescence emission was measured using a portable FluorPen FP100Max fluorometer with a light sensor (Photon Syste Instruments, Brno, Czech Republic). A blue LED internal light of 1–2 mmol photons m^2 s^{-1} was used to induce the ground fluorescence F_0 on 300 dark adapted leaves. A saturating light pulse of 3.000 mmol photons m^2 s^{-1} was applied to induce the maximal fluorescence level in the dark, Fm. The following parameters were considered: the maximum ΦPSII photochemical efficiency (Fv/Fm) calculated as (Fm - F0)/Fm, the quantum yield of PSII linear electron transport (ΦPSII) and the electron transmission rate (ETR) (Genty et al., 1989; Bilger and Björkman, 1990). The measurements in the light were conducted from 12:00 to 14:00 pm under environmental Photosynthetic Photon Flux Density (PPFD) ranging between 1,800 and 2,300 photons m^2 s^{-1}.

At veraison 6 well-exposed and fully expanded leaves per site, were collected and used for the extraction of chlorophylls and carotenoids. Pigments were extracted in ice-cold 100% acetone with a mortar and pestle and centrifuged at 5,000 rpm for 5 min (Labofuge GL, Heraeus Sepatech, Hanau, Germany). The absorbance of supernatants was quantified by a spectrophotometer (UV-VIS Cary 100, Agilent Technologies, Santa Clara, CA, United States) at wavelengths of 470, 645, and 662 nm. The pigment content was calculated according to (Lichtenthaler, 1987) and expressed in µg cm^{-2}.

2.5 Analysis of minerals and organic acids in leaves

During the three growing seasons, at the veraison phase, six fully expanded leaves per vineyard were sampled. Leaf dry tissues were finely ground with a mill (IKA, MF10.1, Staufen, Germany) with 0.5

mm-sieve. For the evaluation of mineral leaf composition in terms of cations (Na^+, K^+, Mg^{2+}, and Ca^{2+}), anions (SO_4^{2-}, PO_4^{3-}) and organic acids (malate, tartrate, citrate and isocitrate), 250 mg of dried material were suspended in 50 mL of ultrapure water (Milli-Q, Merk Millipore, Darmstadt, Germany), frozen and subjected to 10 min shaking in a water bath (ShakeTemp SW22, Julabo, Seelbach, Germany) at 80 °C. Anions and cations were separated and quantified by ion chromatography equipped with a conductivity detection (ICP 3000 Dionex, Thermo fisher Scientific Inc., MA, United States), according to Zhifeng and Chengguang (1994).

2.6 Statistical analysis of data

All experimental data were analyzed with the SPSS 13 statistical software (SPSS Inc., Chicago. IL, United States). The growth data were analyzed by three-way analysis of variance (ANOVA) considering the field/vineyard (F), the year (Y) and the phenological phase (PP) as main factors. A two-way ANOVA was performed on data collected at fruit set for shoot fertility and fruit set, and at harvest for yield components (average bunch weight) and for main qualitative parameters of berries (average berry diameter), considering the field (F) and the year (Y) as main factors. Whenever the interactions were significant, a one-way ANOVA was performed. To separate the fields per each measured parameter, the Duncan's multiple range test was performed. The verification of normality was performed through the Shapiro– Wilk test; the percentage data were previously subjected to arcsine transformation.

3. Results

3.1 Soil profile characterization

The soils in Fig. 1 represent variants (Phenoforms) of the reference soil of the pedological unit (PEN 1) to which they are associated, namely the Typic Calcistolls (P89), a profile characterized by a dark surface horizon and a clear depth horizon rich in carbonates. The variation of the soils consists in the effect of the reworking of the soil following the planting phases of the vineyard. In any case, these soils are characterized by the presence of a superficial Ap horizon with a strongly expressed angular polyhedral structure, an abundant skeleton with a dimension <1 cm, the presence of fine roots and common biological activity and a Bwk calcic sub-surface horizon characterized by a structure sub-angular polyhedral weakly developed, abundant fine skeleton, common presence of concretions and medium and small concentrations of calcium carbonate (Fig.2). Along the profile we find the common presence of angular clasts of variable size (from 10-25 cm), small iron-manganese nodules and a strong HCl effervescence. Biological activity is common in the first horizon in all soil profiles. The reorganization of the soil horizons and their fragmentation along the profile following the vineyard arrangement works, generate a differentiation between the sites in terms of water and nutrient management. In the case of the Santa Lucia (SL) profile, the presence of a buried surface horizon (Ab) at -70 cm and the following Bwk horizon (sequence similar to the Grottole (GR) soil profile) suggests a possible carryover of material in the implantation phase that has covered the original surface. This theory would also explain the different colouring of the surface horizon which represents a lower accumulation of organic matter over time. In the case of the Calvese (CA) profile, compared to the reference profile and the dominant theme (dark Ap followed by light Bwk horizon), the absence of a dark Ap layer is evident, probably due to its erosion during the planting organization phase. In the case of the experimental site of Acquefredde (AC), the soil profile differs greatly from the reference profile of the soil unit (TAS1) of the soil map of the Telesina Valley in which is associated. In fact, the reference soil is classified as Typic Calciustolls (P72) while the profile described does not have the characteristics of a calcic mollisol but of an inceptisol, being strongly altered with a sandy loam texture, presence of anthropic material along the profile, coals, and signs of surface compaction due to the passage of agricultural machinery. The profile is characterized by two very similar horizons, a superficial Ap horizon (0-20 cm) with a strongly developed sub-angular polyhedral structure, a common small skeleton and the presence of fine and medium roots and a sub-superficial horizon Bw (20 -70+) which differs from the surface horizon for the consistency of the aggregates which is moderately developed. Small iron-manganese concretions can be found along the soil profile.

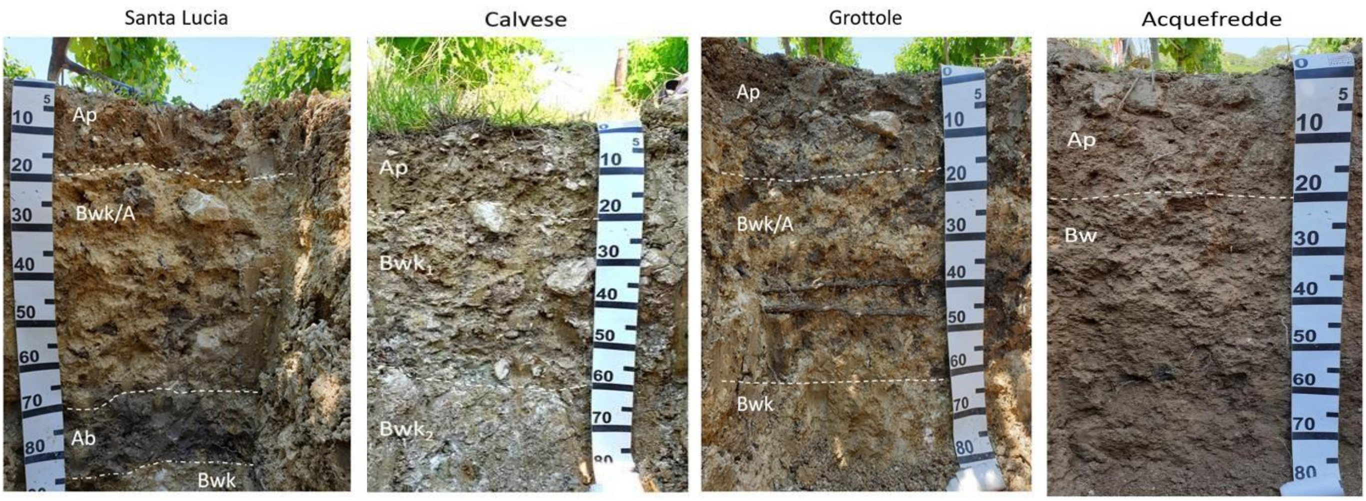

Fig. 2 Soil profiles made in the experimental sites of Santa Lucia (SL), Calvese (CA), Grottole (GR) and Acquefredde (AC).

3.2 Meteorological data analysis

The climate of Guardia Sanframondi area (BN) was characterized in the year 2019 by the annual mean temperature of 17.26 °C, with the hottest period occurring between June and August (monthly average mean temperature 27.55 °C) and the coldest month was January (monthly average mean temperature 5.95 °C). The cumulative annual precipitation was 821 mm, the wettest month was November with the cumulative monthlyprecipitation of 244 mm while the lowest value was reached in May with (cumulative monthly precipitation of 10.2 mm). In the year 2020 the annual mean temperature was 16.74°C, with the hottest period occurring between July and August (monthly average mean temperature 26.31 °C) and the coldest month was January (monthly average mean temperature 8.10°C). The cumulative annual precipitation was 950.5 mm, the wettest month was December with the cumulative monthly precipitation of 226.2 mm while the lowest value was reached in August with (cumulative monthly precipitation of 9 mm). In the year 2021 the annual mean temperature was 16.55 °C, with the hottest period occurring between July and August (monthly average meantemperature 26.60 °C) and the coldest month was January (monthly average mean temperature 7.69°C). The cumulative annual precipitation was 1206.8 mm, the wettest month was January with the cumulative monthlyprecipitation of 330.8 mm while the lowest value was reached in August (cumulative monthly precipitation of 2.8 mm). The graphs in figures 3-6 indicate that the aridity period occurred between July and August at thefour sites but with different intensity. The water availability in the soil at the four sites was different too, withSL and AC characterized by lower soil water content at least in the superficial layers compared to the other sites. In SL and GR there is low variation of SWC between 15 and 30 cm probably due to similar soil characteristic between these two different soil depths, while deeper there is a more impermeable layer of soil which would increase the SWC at 75 cm. This is an intrinsic characteristic of this vineyard observed through the measures performed in the referred period. In CA an opposite trend compared to SL soil is reported, probably due to the different soil profile. In AC vineyard, due to the steeper slope compared to the other three vineyards, there is a runoff effect of rainwater with the consequence that a lower percentage of rainwater penetrates the different layers of the soil, with the consequence of a lower SWC at the three different soil levels. Moreover, in the summer period there is less difference among the three layers likely because of the supplemental irrigation effect which mitigates the differences that are observed in SL, CA and GR.

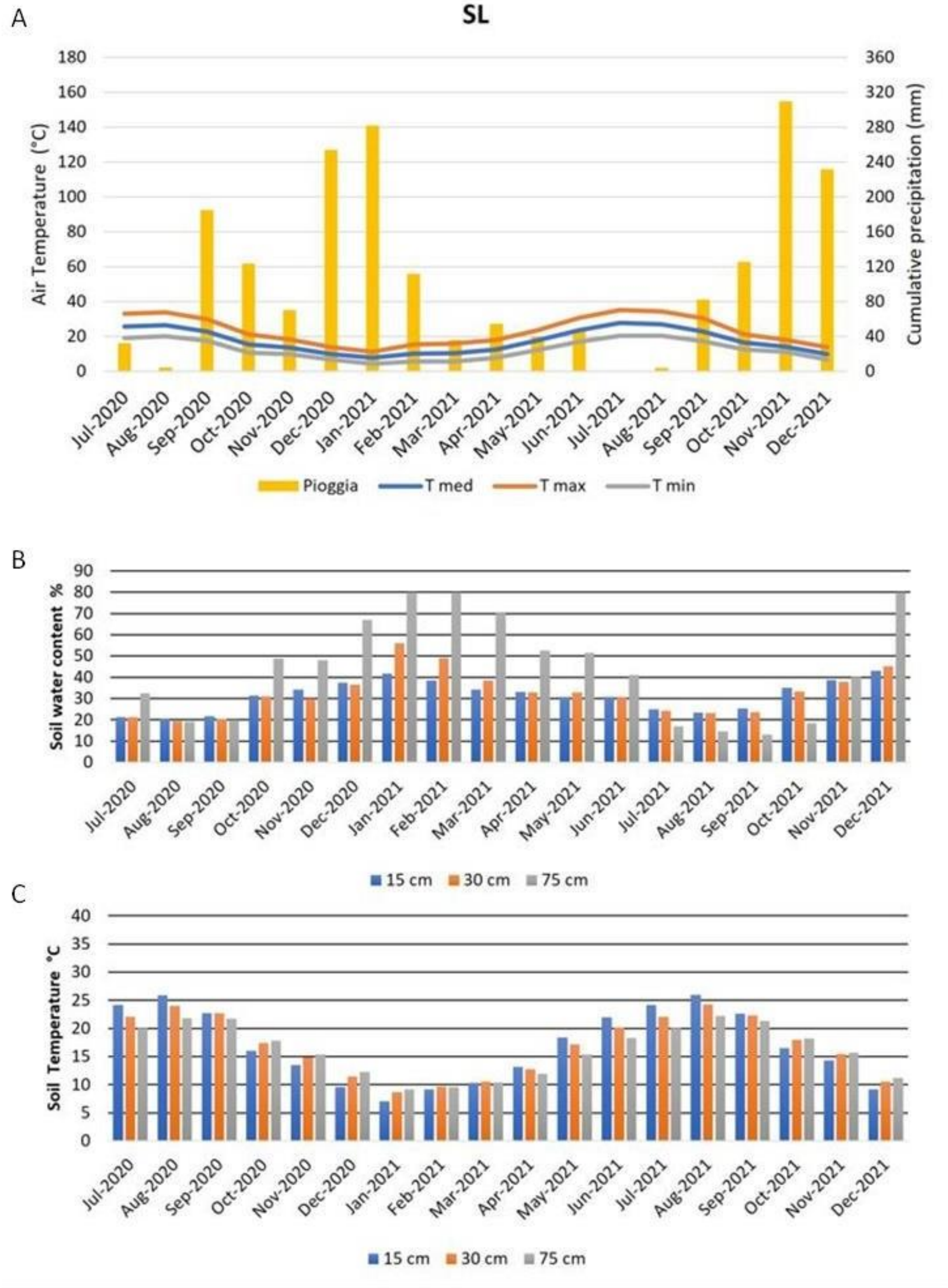

Fig.3 Climatic data of the vineyard SL for the period from 1 July 2020 until 31 December 2021 collected by the weather station Netsens . A - Walter e Lieth diagram with air temperature and cumulative precipitation; B - Soil water content; C – Soil Temperature

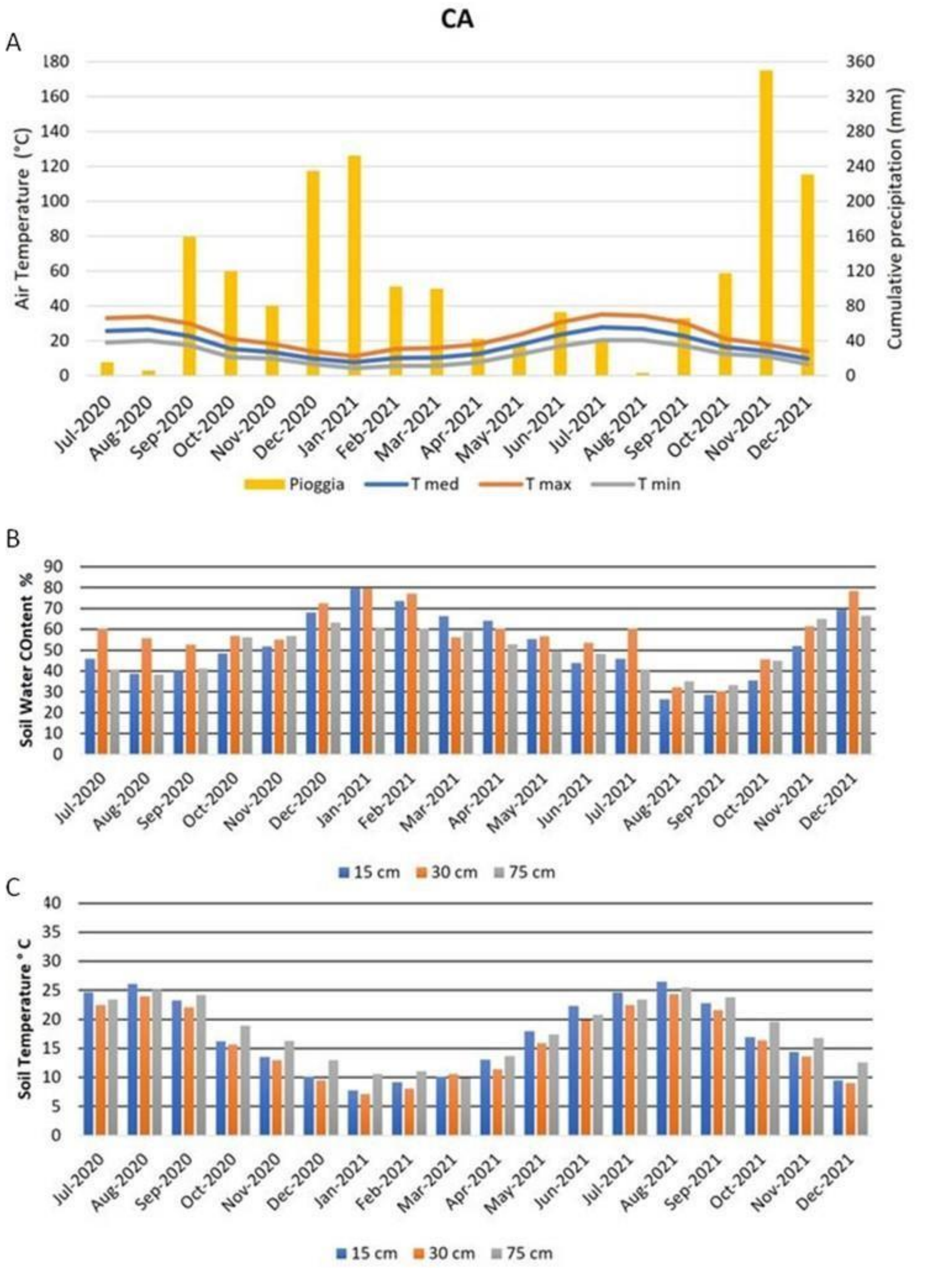

Fig.4 Climatic data of the vineyard CA for the period from 1 July 2020 until 31 December 2021, collected by the weather station Netsens. A - Walter e Lieth diagram with air temperature and cumulative precipitation; B - Soil water content; C – Soil Temperature

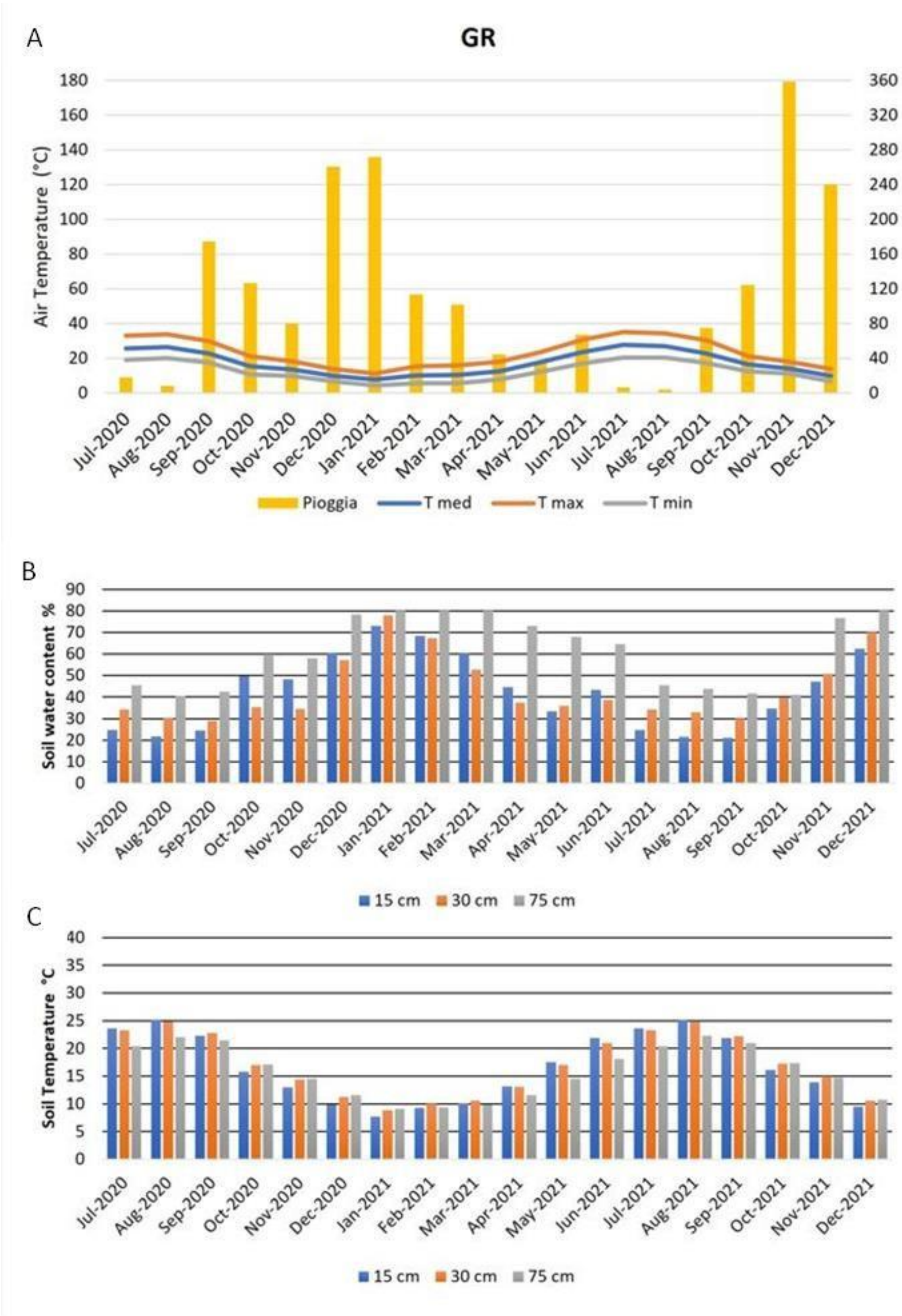

Fig.5 Climatic data of the vineyard GR for the period from 1 July 2020 until 31 December 2021, collected by the weather station Netsens. A. Walter e Lieth diagram with air temperature and cumulative precipitation; B - Soil water content; C – Soil Temperature

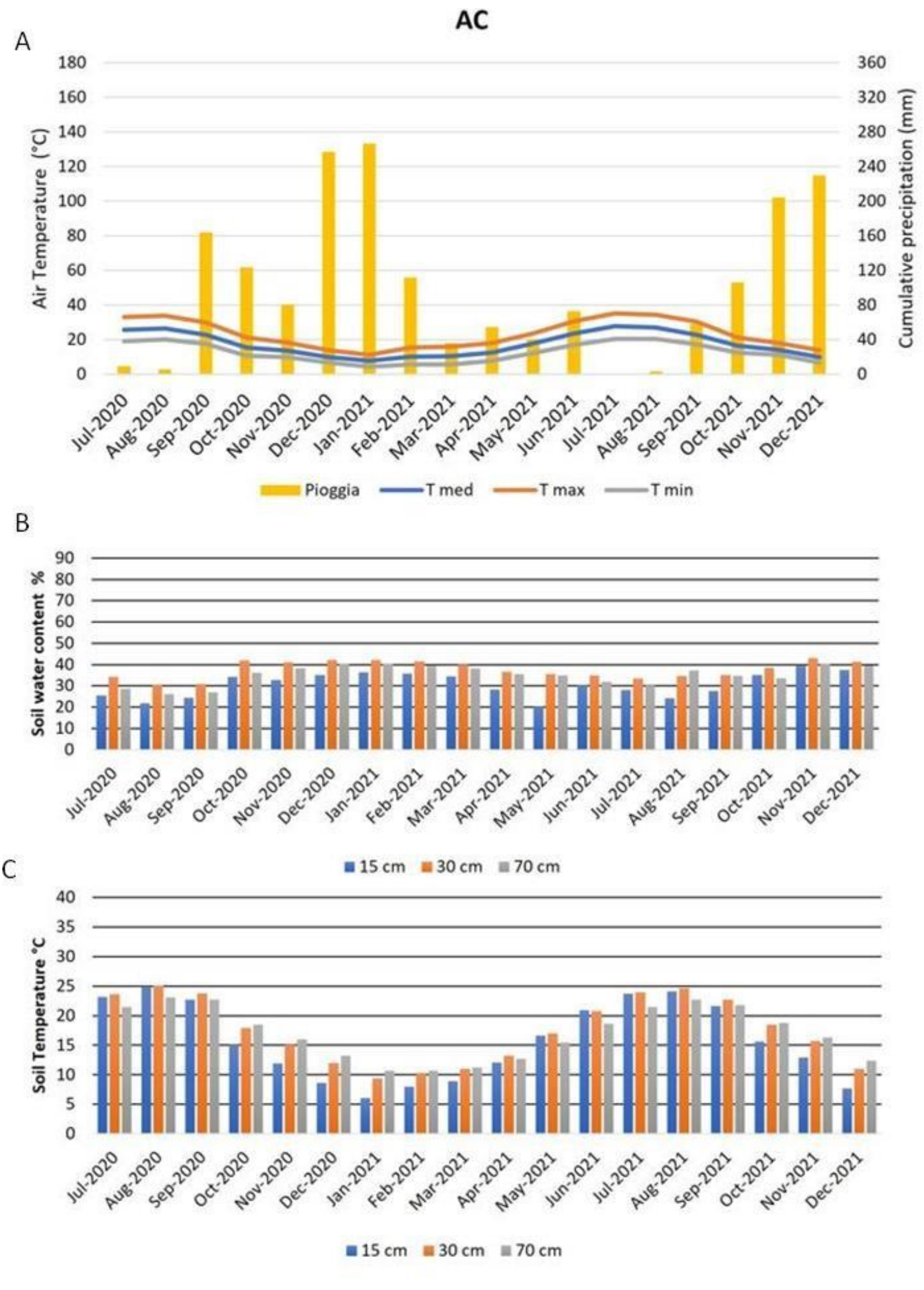

Fig.6 Climatic data of the vineyard AC for the period from 1 July 2020 until 31 December 2021, collected by the weather station Netsens. A. Walter e Lieth diagram with air temperature and cumulative precipitation; B - Soil water content; C – Soil Temperature

3.3 Growth analysis and production

Growth parameters traits (shoot leaf area, basal diameter, single leaf area, main leaves area, anticipated leaves area, total leaf area) of the four vineyards, measured during the main phenological phases through the three growing seasons (2019-2020-2021) are reported in table 1. The main effects of field (F), year (Y) and phenological phase (PP) were significant for all analyzed parameters. More specifically for shoot length, SL showed a higher value than AC, while GR showed an intermediate value and CA showed the lowest value. Basal shoot diameter showed the highest value in GR followed by SL, AC, CA. Single leaf area was significantly higher in SL than GR and CA, which in tun were significantly higher that CA. Main leaf area showed values significantly higher in SL than AC, while GR showed an intermediate value and CA showed the lowest value. Anticipated leaf area showed for GR a significant higher value than SL, which in turn was higher than CA and AC. Total leaf area was significantly higher in SL followed by GR, AC and CA. Concerning the main effect Y, for all analyzed parameters in 2019 the values were significantly higher than 2020, which in turn were significantly higher than 2021. Only for basal shoot diameter the value was significantly higher in SL than CA and GR. Concerning the main effect PP, for shoot length in the fruit set phase there was a value significant higher than in veraison and ripening which both were significantly higher than in pre-flowering. For basal shoot diameter in fruit set and veraison values were significant higher than values in pre-flowering and ripening. Single leaf area, in veraison and ripening the values were significantly higher than in fruit set, which was higher than in pre-flowering. For main leaf area, fruit set and veraison showed values significant higher than pre-flowering which in turn was higher than ripening. The anticipated leaf area in evasion was the highest, followed by ripening, pre-flowering and fruit set. The total leaf area in veraison was significantly higher than in fruit set, followed by ripening and pre-flowering. The interaction FxY was significant for Single leaf area, Main leaf area, Anticipated leaf area, Total leaf area. Single leaf area showed the significant highest value in SL 2019 and the significant lowest in CA 2021. Main leaf area showed the significant highest values in fields of SL 2019, SL 2020 and AC 2019. Anticipated leaf area showed the highest values in GR 2019 and the lowest in CA 2020. Total leaf area showed the highest values in SL 2019 and GR 2019 (table S1, Appendix). The interaction FxPP was significant for Shoot length Single leaf area, Main leaf area and Anticipated leaf area. Shoot length showed the highest values for SL Fruit Set and the lowest in both GR Pre-flowering and CA Pre-flowering. Single leaf area showed the highest values for SL Veraison and SL Ripening. Main leaf area showed the highest value in SL Fruit Set and the lowest in CA Ripening. Anticipated leaf area showed the highest value in SL Veraison, GR Veraison and GR Ripening (table S2, Appendix). The interaction YxPP was significant for Shoot length, Shoot basal diameter, Main leaf area, Anticipated leaf area and Total leaf area. Shoot length showed the highest values in 1° Year Fruit Set, 1° Year Veraison and 1° Year Ripening. Shoot basal diameter showed the highest value in the 1° Year Veraison. Main leaf area showed the highest value in 1° Year Fruit Set and 1° Year Fruit Veraison. Anticipated leaf area showed the highest value in the 1° Year Veraison. Total leaf area showed the highest value 1° Year Veraison. The interaction F*Y*PP was significant for Shoot length, Single leaf area and Anticipated leaf area (table S3, Appendix).

Table 1. Effects of field (F), year (Y), phenological phases (PP) and their interaction (F x Y, FxPP and YxPP, FxYxPP) on shoot leaf area, basal diameter, single leaf area, main leaves area, anticipated leaves area, total leaf area of *V. vinifera* subsp. *vinifera* 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

	Shoot length	Shoot basal diameter	Single leaf area	Leaf Area of main shoot	Leaf Area of anticipated shoot	Total leaf area
	cm	*mm*	*cm²*	*cm² shoot⁻¹*	*cm² shoot⁻¹*	*cm² shoot⁻¹*
Field (F)						
SL	132.0±3.195 a	8.60±0.066 b	165.4±2.95 a	2211±50.05 a	1106±42.77 b	3317±80.96 a
CA	109.7±2.921 c	7.77±0.063 d	122.6±1.96 c	1739±45.35 c	825.2±29.59 c	2565±66.31 d
GR	125.7±3.125 ab	8.78±0.068 a	134.2±1.77 b	1813±45.57 bc	1229±48.46 a	3065±84.79 b
AC	123.5±3.088 b	8.33±0.075 c	139.4±2.72 b	1908±50.03 b	877.3±33.16 c	2785±66.15 c
Year (Y)						
2019	148.5±2.689 a	8.85±0.050 a	153.5±2.07 a	2251±30.69 a	1227±26.33 a	3479±49.72 a
2020	113.6±2.065 b	8.00±0.058 b	137.8±1.92 b	1868±34.31 b	892.5±29.44 b	2773±55.59 b
2021	92.3±2.576 c	8.06±0.073 b	118.8±2.30 c	1215±43.40 c	778.6±37.24 c	1993±70.32 c
Phenological phase (PP)						
Pre-Flowering	99.1±1.966 c	8.19±0.067 b	121.14±2.12 c	1650±36.59 b	710.4±22.24 d	2381±52.83 d
Fruit set	140.1±2.764 a	8.53±0.064 a	133.71±1.78 b	2255±43.47 a	940.5±25.73 c	3195±60.68 b
Veraison	128.9±3.519 b	8.49±0.072 a	153.55±2.86 a	2153±54.45 a	1292±50.44 a	3445±92.31 a
Ripening	122.5±3.921 b	8.25±0.079 b	156.35±2.90 a	1537±47.91 c	1115±50.81 b	2652±82.39 c
Significance						
F	***	***	***	***	***	***
Y	***	***	***	***	***	***
PP	***	***	***	***	***	***
F*Y	NS	NS	***	***	***	***
F*PP	***	NS	***	**	**	NS
Y*PP	***	***	NS	**	***	**
F*Y*PP	**	NS	*	NS	***	NS

NS, *, **, and ***, Not significant or significant at p < 0.05, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests (p ≤0.05).

Growth parameters and fruit traits (potential fertility, real fertility, fruit set, bunch number, average bunch weight, berry diameter) of the four vineyards measured during the three growing seasons (2019-2020-2021) are reported in table 2. The main effects of field (F) and year (Y) were significant for all analyzed parameters. Potential fertility and real fertility showed to be both significantly higher in AC, followed by SL, GR and CA with the lowest value. Fruit set showed for GR a value significantly lower than SL, CA and AC. The bunch number was significantly different among the vineyards with the highest value in SL followed by GR, AC and CA. The average bunch weight was significantly higher in SL than AC, which in turn was significantly higher than both CA and GR. For berry diameter, SL showed the highest value followed by AC, CA and GR. Considering the main factor Y, for potential fertility and real fertility in 2019 the values were significantly higher than 2020, which in turn were significantly higher than 2021. For fruit set and average bunch weight in 2021 were found to be significantly lower than 2019 and 2020. Bunch number showed the highest value in 2021 followed by 2019 and 2020. Berry diameter was significantly higher in 2020 than both 2019 and 2021. The interaction F x Y was significant for all the analyzed parameters . The potential and real fertility was significantly high values in AC 2019 and SL 2021. Fruit set showed significant highest values in SL 2019, CA 2019 and AC 2021. Bunch weight showed the significant highest values in SL 2019, SL 2020, AC 2019 and AC 2020. Berry diameter showed the significant highest values in SL 2019, SL 2020, AC 2020 and CA 2020 (table S4, Appendix) .

Table 2. Effects of field (F), year (Y) and their interaction (F x Y) on potential fertility, real fertility, fruit set, bunch number, average bunch weight, berry diameter of *V. vinifera* subsp. *vinifera* 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

	Potential fertility	Real fertility	Fruit set	Average bunch weight	Berry diameter
			%	*g bunch⁻¹*	*cm*
Field (F)					
SL	1.39±0.029 ab	1.06±0.030 b	27.9±0.635 a	318.2±11.50 a	1.43±0.006 a
CA	1.25±0.023 c	0.92±0.037 c	29.5±0.627 a	169.7 ± 9.53 c	1.26±0.009 c
GR	1.35±0.022 b	1.06±0.034 b	25.3±0.650 b	178.2 ± 9.53 c	1.27±0.007 d
AC	1.42±0.037 a	1.23±0.053 a	29.5±0.611 a	267.8 ± 10.78 b	1.37±0.008 b
Year (Y)					
2019	1.46±0.024 a	1.22±0.034 a	28.74±0.438 a	270.1 ± 10.82 a	1.29±0.075 b
2020	1.20±0.020 b	0.80±0.031 b	29.98±0.520 a	253.1 ± 11.69 a	1.40±0.075 a
2021	1.42±0.023 a	1.18±0.029 a	26.46±0.702 b	177.3 ± 7.37 b	1.29±0.070 b
Significance					
F	***	***	***	***	***
Y	***	***	**	***	***
F*Y	***	***	***	***	***

NS, *, **, and ***, Not significant or significant at p < 0.05, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests (p ≤ 0.05).

3.4 Leaf Gas Exchange, Chlorophyll Fluorescence Emission and Photosynthetic Pigment Quantification

Eco physiological parameters (net photosynthesis (Pn), stomatal conductance (gs), substomatal CO_2 concentration (Ci), leaf transpiration rate (E), electron transmission rate (ETR), quantum yield of PSII linear electron transport (ΦPSII), maximum quantum efficiency of PSII photochemistry (Fv/Fm)) of the four vineyards analyzed in the three years (2019-2020-2021) are reported in table 3. The main effect of field (F) was significant for all the analyzed parameters, while the main factor year (Y) was significant only for net photosynthesis (Pn), substomatal CO_2 concentration (Ci) and leaf transpiration rate (E). Net photosynthesis (Pn) was significantly higher in SL than AC, which in turn was higher than GR and CA. Stomatal conductance (gs) and substomatal CO_2 concentration (Ci) were significantly higher in both SL and AC than CA and GR. Leaf transpiration rate (E) in AC showed a value significantly higher than SL, followed by GR and CA. Electron transmission rate (ETR) and quantum yield of PSII linear electron transport (ΦPSII) showed for both SL and AC values significantly higher than CA and GR. Fv/Fm showed in SL values significantly higher than GR and AC, which in turns showed values significantly higher than CA. Chl showed in GR values significantly higher than SL and AC, which in turn showed values significantly higher than CA. Concerning the main effect year (Y): net photosynthesis (Pn) showed the highest value in 2019 flowed by 2021 and 2020. Substomatal CO_2 concentration (Ci) was significantly higher in 2020 than both 2019 and 2021. Leaf transpiration rate (E) was significantly higher in 2021 than 2020 with 2019 showing an intermediate value. The interaction FxY was significant for all the analyzed parameters. Pn showed the significant highest values in SL 2019, SL 2020, SL 2021 and AC 2019. The parameters gs and Ci showed the significant highest values in AC 2020, AC2021, SL 2019, SL 2020 and SL 2021. The parameter E showed significant highest values in AC 2021, AC 2021 and SL 2019.The parameter ΦPSII shoed the significant highest value in AC 2020. The parameter Fv/Fm showed the significant highest values in SL 2019, GR 2021, AC 2019. The parameter Chl showed the significant highest values in GR 2019, GR 2020 and GR 2021 (Table S5, Appendix).

Table 3. Effects of field (F), year (Y) and their interaction (F x Y) on net photosynthesis (Pn), stomatal conductance (gs), substomatal CO_2 concentration (Ci), leaf transpiration rate (E), electron transmission rate (ETR), quantum yield of PSII linear electron transport (ΦPSII), maximum quantum efficiency of PSII photochemistry (Fv/Fm) and total chlorophyl content of *V. vinifera* subsp. *vinifera* 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

	Pn (μmol m^{-2} s^{-1})	gs (mmol m^{-2} s^{-1})	Ci μmol mol^{-1}	E (mol H_2O m^{-2} s^{-1})	ETR	ΦPSII	Fv/Fm	Chl (μg/cm^2)
Field (F)								
SL	10.9±0.33a	163±10.6 a	258±7.23 a	4.7±0.21 b	167±4.04 a	0.301 ± 0.007 a	0.777 ± 0.004 a	46.05±0.747 b
CA	4.7±0.44 c	72.2±10.6 b	224±9.64 b	2.8±0.28 d	138±3.66 b	0.253 ± 0.007 b	0.754 ± 0.005 c	36.96±1.717 c
GR	5.7±0.49 c	71.6±5.10 b	231±9.93 b	3.7±0.27 c	148±6.24 b	0.267 ± 0.011 b	0.765 ± 0.005 bc	62.54±1.603 a
AC	9.7±0.38 b	184±9.87 a	243±8.09 ab	7.0±0.18 a	175±4.50 a	0.317 ± 0.008 a	0.774 ± 0.004 ab	43.83±1.407 b
Year (Y)								
2019	8.77±0.58 a	131±14.0 a	224±13.08 b	4.6±0.27 ab	154±5.14 a	0.279 ± 0.008 a	0.772 ± 0.004 a	44.82±1.572 b
2020	6.78±0.45 c	120±8.8 a	267± 4.096 a	4.0±0.21 b	159±3.98 a	0.284 ± 0.008 a	0.766 ± 0.003 a	46.72±2.651 b
2021	7.75±0.42 b	120±8.73 a	231±3.867 b	5.1±0.35 a	160±4.52 a	0.291 ± 0.009 a	0.764 ± 0.005 a	50.52±1.836 a
Significance[1]								
F	***	***	*	*	***	***	***	***
Y	***	NS	***	***	NS	NS	NS	***
FxY	***	***	**	***	***	***	***	***

NS, *, **, and ***, Not significant or significant at $p < 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests ($p \leq 0.05$).

3.5 Leaf Mineral Composition

The leaf cations Na^+, K^+, Mg^{2+}, $Ca^{2+}$of the mineral analysis performed during the three year of study are reported in table 5 . The main effect of field (F) was significant for all the analyzed parameters, while the main factor year (Y) was significant only for Na^+, Mg^{2+}, Ca^{2+}. Concerning the main effect F, Na^+ was significantly higher in leaves of both SL and AC than GR, with leaves of CA showing an intermediate value. K^+ was significantly higher in leaves of AC than CA, with leaves of GR showing an intermediate value and SL showing the lowest value. Mg^{2+} showed significant higher value in leaves of SL than CA which in turn was significantly higher than AC. GR leaves showed an intermediate value. Ca^{2+} in SL leaves showed a significant higher value than CA, GR and AC. Concerning the main effect Y, Na^+ in the year in leaves of 2021 was significant higher than 2019 and 2020. Mg^{2+} and Ca^{2+} showed significantly higher values in leaves of both years 2019 and 2020than 2021. The interaction F x Y was not significant.

Table 4. Effects of field (F), year (Y) and their interaction (F x Y) on leaf minerals, Na^+, K^+, Mg^{2+}, Ca^{2+}, SO_4^{2-}, PO_4^{3-}, Malate, Tartrate, Citrate, Isocitrate of V. vinifera subsp. vinifera 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Different letters within column indicate significant differences according to Duncan's multiple-range test (P _ 0.05) Mean values and standard errors are shown.

Leaf cations	Na^+	K^+	Mg^{2+}	Ca^{2+}
	(g kg^{-1} DW*)*	*(g kg*$^{-1}$ DW*)*	*(g kg*$^{-1}$ DW*)*	*(g kg*$^{-1}$ DW*)*
Field (F)				
SL	2.053 ± 0.670 a	5.135 ± 0.418 c	2.098 ± 0.227 a	12.943 ± 1.134 a
CA	1.478 ± 0.581 ab	6.972 ± 0.516 b	1.689 ± 0.126 b	9.354 ± 0.311 b
GR	0.466 ± 0.114 b	6.341 ± 0.410 bc	1.512 ± 0.152 bc	8.112 ± 0.743 b
AC	1.577 ± 0.300 a	8.885 ± 0.373 a	1.380 ± 0.153 c	9.225 ± 0.872 b
Year (Y)				
2019	0.783 ± 0.142 b	7.007 ± 0.506 a	2.038 ± 0.153 a	10.090 ± 1.081 a
2020	0.849 ± 0.219 b	6.885 ± 0.483 a	1.860 ± 0.096 a	11.388 ± 0.710 a
2021	2.549 ± 0.571 a	6.608 ± 0.642 a	1.111 ± 0.074 b	8.248 ± 0.494 b
Significance[1]				
F	*	***	***	***
Y	**	NS	***	**
F*Y	NS	NS	NS	NS

NS, *, **, and ***, Not significant or significant at p < 0.05, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests (p ≤ 0.05).

The leaf anions SO_4^{2-}, PO_4^{3-}, Malate , Tartrate , Citrate, Isocitrate of the mineral analysis performed during the three year of study are reported in table 6. The main effect of field (F) was significant for PO_4^{3-}, Malate, Citrate, Isocitrate and the main factor year (Y) was significant for Malate, Tartrate, Citrate and Isocitrate. PO_4^{3-} was significantly higher in leaves of AC than SL, CA and GR. Malate showed significant lower values in AC leaves than SL, CA, and GR. Citrate in SL and GR leaves was significantly higher than CA, which in turn is significantly higher than AC. Isocitrate in SL leaves showed significant higher values than AC, with GR and CA showing an intermediate value. Regarding the main effect Y, Malate showed a significant lower value in leaves of 2019 than 2020 and 2021. Tartrate in leaves of 2020 was significantly higher than 2021 with leaves of 2020 showing an intermediate value. Citrate in leaves of 2019 showed significant higher value than 2021, with year 2020 showing an intermediate value. Isocitrate showed in 2020 leaves with higher values than in 2019, which in turn showed significant higher value than 2021. The interaction F*Y was significant only for Citrate and Isocitrate (supplemental materials). Citrate showed highest values in leaves of SL 2019, GR 2019 and GR 2020. Isocitrate showed highest values in leaves of SL 2019 (Table S6, Appendix).

Table 6. Effects of field (F), year (Y) and their interaction (F x Y) on leaf minerals SO_4^{2-}, PO_4^{3-}, Malate, Tartrate, Citrate, Isocitrate, Na^+, K^+, Mg^{2+}, Ca^{2+} of *V. vinifera* subsp. *vinifera* 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Different letters within column indicate significant differences according to Duncan's multiple-range test (P < 0.05) Mean values and standard errors are shown.

Leaf anions	SO_4^{2-}	PO_4^{3-}	Malate	Tartrate	Citrate	Isocitrate
	(g kg^{-1} DW*)*	*(g kg*$^{-1}$ DW*)*	*(g kg*$^{-1}$ DW*)*	*(g kg*$^{-1}$ DW*)*	*(g kg*$^{-1}$ DW*)*	*(g kg*$^{-1}$ DW*)*
Field (F)						
SL	0.716 ± 0.107 a	1.329 ± 0.142 b	47.830 ± 2.797 a	44.183 ± 2.976 a	2.934 ± 0.200 a	0.648 ± 0.095 a
CA	0.518 ± 0.057 a	1.716 ± 0.158 b	41.714 ± 3.404 a	43.527 ± 1.294 a	2.171 ± 0.207 b	0.503 ± 0.059 bc
GR	0.602 ± 0.117 a	1.080 ± 0.081 b	44.334 ± 3.371 a	45.682 ± 3.476 a	3.281 ± 0.276 a	0.617 ± 0.045 ab
AC	0.634 ± 0.116 a	2.821 ± 0.626 a	32.515 ± 3.340 b	47.700 ± 3.763 a	1.595 ± 0.162 c	0.455 ± 0.082 c
Year (Y)						
2019	0.630 ± 0.115 a	1.610 ± 0.117 a	36.058 ± 2.873 b	44.153 ± 2.947 ab	2.807 ± 0.298 a	0.573 ± 0.077 b
2020	0.582 ± 0.069 a	1.887 ± 0.355 a	44.213 ± 2.224 a	50.261 ± 1.951 a	2.475 ± 0.269 ab	0.715 ± 0.034 a
2021	0.639 ± 0.078 a	1.712 ± 0.468 a	44.524 ± 3.776 a	41.405 ± 2.129 b	2.204 ± 0.203 b	0.380 ± 0.034 c
Significance[1]						
F	NS	**	**	NS	***	*
Y	NS	NS	*	*	*	***
F*Y	NS	NS	NS	NS	**	**

[1]NS, *, **, and ***, Not significant or significant at $p < 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests ($p \leq 0.05$).

The relations between the different morphological and eco-physiological parameters were quite stable among the three years (Table S7, Appendix).

4. Discussion

This study highlighted how Falanghina grapevine growing under different pedoclimatic conditions develops different morphological traits and eco-physiological strategies among sites suggesting a different water use efficiency in the four vineyards in the three years of monitoring. Considering the predicted climate changes in the Mediterranean area, we expect negative effects on vines growth, yield and grape quality necessary for winemaking process. For this reason, there is need to investigate the vine plasticity in different pedoclimatic conditions in order to identify strategies to mitigate the ongoing climate changes. Vine growth, yield and grape quality in the four analyzed experimental vineyards were characterized by significant differences suggesting different regulation of the source-sink balance according to the various microclimatic and pedological conditions which can differentially influence water availability for the vines (Damiano et al., 2022). In general, during the three years, the four vineyards showed two main behaviors regarding the biomass production and photosynthetic efficiency, with SL and AC plants more performant than CA and GR ones. In general, along the three years, the vineyard SL showed the highest vegetative vigor accompanied by higher values of shoot length, single leaf area, main leaf area and total leaf area. On the contrary, in CA the lowest levels of vegetative vigor confirms how different level of biomass accumulation during the three years of growing are strictly related to the specific pedoclimatic area of the vineyards. In CA, the soil water content remains higher than the other sites at the three soil depth as if plants were not absorbing water. This was confirmed by the occurrence of the lowest values of transpiration of vines in CA, leading to low photosynthetic levels likely due to stomata closure. A strong water deficit especially at the beginning of the season negatively affects budburst since the mobilization of nutrients from the reserve structures is reduced (Vasconcelos et al., 2009). Once the number of potentially productive shoots is defined, the yield of a vineyard depends on a set of internal and external factors, and the interactions among them, all of which have an impact on the processes of floral induction and differentiation and the growth of the berries. These factors include the genotype of the vine, environmental conditions (climate and soil) and cultivation practices (Baeza et al., 2019). GR as vegetative vigor showed a shift in biomass allocation towards anticipated leaf area, indicating an high investment of the vines in vegetative growth also in the phenological phases of veraison (Table 1). CA and AC showed the lowest value of the anticipated shoot leaf area indicating probably a drought stress event as described in literature (Pellegrino et al., 2005). Pellegrino et al. 2005, analyzed the effects of water deficit on shoot vegetative growth (i.e the number of leaves to emerge on the first- and second-order laterals), in cv. Shiraz and showed that the rate of emergence of second-order laterals decreases in response to water deficit. For plants, the allocation of carbon in somatic traits, like shoots and leaves, is an investment to intercept more light to assimilate more carbon but this can either decrease the amount of carbon allocated to reproduction (because of competition with vegetative growth) (Sadras & Denison, 2009) in particular when this tendency is assessed in the veraison phenological phase as the case of GR for anticipated leaf area. Despite the lowest level of total leaf area, in AC, the values of the potential fertility and real fertility were the highest. Water deficit usually reduces bud fertility due to the decreasing in the number and size of inflorescences (Ferrara & Mazzeo, 2021). The bud fertility is particularly sensitive to water stress, with shortages during flowering normally leading to important reductions in bud fertility (Jackson, 2008). Vasconcelos et al. 2009 reviewed the different means by which water status can affect floral induction and differentiation, and therefore bud fertility is reported to be influenced (1) directly, via the amount of water available to processes determining cell division and expansion, and (2) indirectly, via its effect on photosynthetic activity, nutrition, the microclimate of the renewal zone and hormonal balance. Induction and floral differentiation are processes intermediated by the interaction between two hormones with opposed effects: cytokinins and gibberellins. Gibberellins synthesized at leaf level are responsible for the initiation of the anlagen, but later inhibit its development as an inflorescence, promoting the formation of tendrils. The xylem sap of the grapevine contains high cytokinin levels during budbreak and flowering which promote fruit set in grape. Considering that the hormonal balance is strongly dependent on the genetic traits of the cultivar, the analysis of the same cultivar and the same rootstock at the four vineyards has likely reduced the variability of the hormonal balance in this study (Monteiro et al. 2021, Williams et al. 2000). In SL and AC vines, the occurrence of an higher average bunch weight suggest a more balanced regulation of the source-sink distribution of the carbon resource between vegetative and reproductive parameters, compared to GR with high value of vegetative parameters but the lowest values of production parameters. The leaf area is crucial for the production of carbohydrates and consequently the allocation in reproductive organs of grapevine but an excess of vigor not managed with pruning practices could lead to the opposite problem with unbalanced resource distribution between source-sink organs. In the experiment was found the single leaf area (SLA) positively correlated with the average bunch weight (ABW) and berry diameter (BD). In the case of CA concerning grape production parameters, the reduced bunch weight was in accordance with the significant lower values of total leaf area, Pn and gs. Concerning the fruit set which determines the number of flowers developing into berries, in GR it was found the lowest value probably due to a water deficit occurring especially early in the season (table 2) (Bondada & Shutthanandan, 2012). The different growth and production performances showed to be related with the values of leaf gas exchange and fluorescence emission where the Pn and gs were significantly higher in SL and AC than CA and GR, supporting the highest level of average bunch weight and berry diameter of the twofields SL and AC due to larger availability of photosynthates. Berry size is widely acknowledged as a factor determined by level of Pn and constrained by water deficits which generally lead to smaller berries and changes in berry skins and seeds, where anthocyanins and tannins respectively are located, with final effects on wine composition (Baeza et al., 2019). In fact, the berry diameter (BD) and ABW showed a positive correlation with Pn in all the three year and a positive correlationwith gs for the years 2019 and 2020 (table 7). The higher photosynthetic rate observed in SL and ACplants may be due to a higher gs and a better PSII photochemical efficiency (i.e., elevated values of ETR, FPSII, Fv/Fm) also influenced by the leaf structure, in terms of stomata size and frequency, which may have contributed to the different grapevines' capability to perform photosynthesis in the different vineyards (Damiano et al., 2022; Vitale et al., 2012). In vines during the three years of experiment, Pn showed a high positive correlation with potential fertility (PF), real fertility (RF) andABW showing of the importance of Pn to obtain high yields. The low level of Fv/Fm values in CA and GR vineyards, where gs was lower, compared to SL and AC, confirm that CA and GR were morewater stressed than SL and AC (Arena et al., 2008). As reported in the chapter 2 this is due not only to stomatal but also to not-stomatal limitations. Indeed, even if the stomatal closure reduced stomatal conductance (gs) and transpiration (E) in vines growing at CA and GR, substomatal CO_2 concentration remained comparable among plants of different sites (Damiano et al.2022). The occurrence of supplemental irrigation in ACmay have mitigated the water stress while, high photosynthetic rates in SL likely relies on a strategyof the plant behaving as a water spender thanks to its structural modifications (Please ref. to chapters2 and chapter 7). This hypothesis is supported by the high E, ETR and low soil water content, suggesting that vines keep stomata open to maintain active photosynthesis.

About the mineral nutrition, the vines need an adequate supply of macro- and micro-nutrients in order to achieve their normal physiological and biochemical function. Basic mineral nutrients are considered to be essential for plant metabolic processes seeing that are cofactors and/or activators of many metabolic enzymes (Pandey 2015). The nutrients are required for vine life cycle from budburst to leaf senescence, and generally they limit grape production (Brataševec et al. 2013). Nutrient excessive supply and deficiencies can both lead to physiological disorders. In the leaves of the site SL, a concentration of Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) higher than those of the other vineyards was observed. Ca^{2+} determines a greater resistance of the tissues, fungal and bacterial infections and is also important for fruit ripening; furthermore being involved as a reaction intermediate in the Krebs cycle it contributes to the production of sugars which are used for photosynthesis. Mg^{2+}, on the other hand, is responsible for activating numerous enzymes involved in the metabolism of the plant, and then participates in the formation of pigments, such as carotene and xanthophylls; it facilitates the translocation of phosphorus in the vegetative apexes and seeds, and also enters the processes of synthesis of starch and sugars. Potassium (K^+) was significantly higher in AC compared to the other three vineyards, indicating a better capacity to maintain turgor and activating an array of enzymes in metabolic reactions. The turgor pressure-driven solute transport causes cell extension, stomatal movements. Moreover, the loading and unloading of sucrose in phloem are also dependent on K^+ concentration in the sieve tubes (Villette et al., 2020).

In general, considering the overall results of both growth and photosynthetic parameters performed along the three years of experiment, the four vineyards showed two main patterns of behavior with SL and AC showing higher growth and productive performance compared to CA and GR. This is confirmed also by the analyses of the wood and leaf anatomical traits (chapter 2 and chapter 7) which suggest a different ability of the vines at the four vineyards to control water use. Morphological and eco-physiological tendencies were confirmed also by the values of WUEi, calculated in different part compartments up to berries, thus musts, which clearly indicate a higher level of water stress for fields CA and GR, compared to SL and AC (chapter 6). The WUEi that affects the photosynthetic and reproductive organs is dependent on the water available coming from rain or irrigation water supply, but another variable not much considered in literature is the soil characteristics which may influence the amount of water truly available for plants and affect the absorption of water by the vine roots (Roig-Puscama et al. 2021; van Leuween et al. 2018). It is known the soils under the influence of the Mediterranean climate have low organic carbon content(0.5-1.0 % organic carbon) and climatic conditions are not suitable for the accumulation of organic matter in soils (Rodeghiero et al., 2011). This theory would also explain the different coloring of the surface horizon which represents a lower accumulation of organic matter over time. Although the surface horizon of SL, CA and GR are similar, showing a low accumulation of organic matter over time, in the case of CA a soil unstructured with low presence of organic matter was found which may have negative effect in water retention of the small amount of raining water during the driest periods of the summer, compared to the other vineyards. To underline the importance of the soil properties in agriculture and therefore in viticulture, it is possible to observe the cumulative precipitations in the four study sites that although show differences, they do not change so much to justify such difference in growth and productive parameters analyzed. For this reason, it was decided to consider also the soil characteristics as a variable which may affect the vine water absorption and therefore the WUEi.

In particular, the probes FDR installed, showed a different distribution of soil water content (SWC) at the three different deep levels. SL and GR showed during the winter and spring a high SWC in the lowest level (-75 cm) instead of CA where the SWC was highly concentrated in the superficial layers of the soil (- 15 and – 35). AC showed a lower SWC in all the three level. This data may suggest that in SL and GR there is a good water storage in the lowest layers of the soil which may help to maintain a wet soil environment during the summer, instead of CA where although there is a good storage of water resources in soil, this is stored on the upper soil layer determining the easy evaporation with the increasing of temperature leading to a soil more drayed, than those of SL and GR. AC also suffers this condition with a lower SWC in all three different soil layer compared to the other three vineyards, but here of the application of supplemental irrigation provided by drip irrigation system may have played a mitigation role.

In conclusion the morpho-functional characterization of the vines interpreted together with fine pedo-climatic information proved to be useful to achieve a comprehensive understanding of the vine behavior, especially with reference to photosynthetic behavior and plant hydraulics (please also ref. to following chapters) in the continuum soil-plant-atmosphere, thus providing information as valuable inputs to manage terroirs in the sight of the ongoing climate change. More precisely at the sites with relatively low soil moisture (CA and GR), the photosynthetic rate was lower, as was stomatal conductance, photosystem electron transfer rate, and quantum yield of PSII linear electron transport. In AC, the relatively low soil moisture was likely compensated by the application of the supplemental irrigation during the hottest periods of the year, allowing the maintenance of high vegetative-productive performances. In Falanghina grapevine our findings support the hypothesis, that both microclimatic and pedological conditions during growing seasons may influence growth vine performances and yield. Therefore, the need to apply a site-specific approach to manage the agricultural parameters as pruning, soil management, water supply etc. is becoming more and more recognized to achieve sustainable vineyard management. Finally, for the design of cultivation strategies, especially regarding water management, there is increasing awareness that the deep knowledge on soil properties as well as on plant hydraulics, and especially the coordination between xylem traits in stem and leaf functional anatomical traits, are pivotal and cannot be disregarded as detailed in the following chapters.

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Appendix

Table S1. Data of interaction analysis F x Y for Shoot length, Main leaf area, Anticipated leaf area and Total leaf area. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

	Single leaf area	Main leaf area	Anticipated leaf area	Total leaf area
	cm² shoot⁻¹	*cm² shoot⁻¹*	*cm² shoot⁻¹*	*cm² shoot⁻¹*
FxY				
SL 2019	180±5.7 a	2422±83.97 a	1418±81.59	3841±147.75 a
SL 2020	157.5±3.48 b	2307±69.09 a	967±44.06	3274±96.58 b
SL 2021	149.9±4.43 bc	1618±90.67 d	722±64.49	2340±117.06 d
CA 2019	139.6±3.01 cd	2242±69.84 ab	996±47.25	3239±99.22 b
CA 2020	118.2±2.18 ef	1546±55.44 d	757±45.77	2304±93.01 d
CA 2021	95.8±4.41 g	1068±68.68 ef	591±54.43	1659±101.64 e
GR 2019	136.9±2.86 d	2054±76.74 bc	1620±91.9	3675±147.07 a
GR 2020	138.7±2.86 cd	1887±65.61 c	1038±55.22	2986±121.78 bc
GR 2021	120.9±3.27 e	1208±65.4 e	793±54.57	2001±86.26 de
AC 2019	157.4±3.36 b	2283±65.95 a	875±44.07	3159±84.73 bc
AC 2020	136.4±5.3 d	2017±84.56 bc	804±58.17	2822±125.4 c
AC 2021	108.3±3.8 f	964±49.02 f	1007±80.59	1971±102.11 de
Significance[1]				
F*Y	***	***		***

[1]NS,*, **, ***: Non-significant or significant at P ≤ 0.05, 0.01, 0.005, respectively.

Table S2. Data of interaction analysis F x PP for Shoot length, Single leaf area, Main leaf area, Aticipated leaf area. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

	Shoot length	Single leaf area	Main leaf area	Anticipated leaf area
	cm	cm^2	cm^2 $shoot^{-1}$	cm^2 $shoot^{-1}$
F x PP				
SL Flowering	118.1±3.815 efg	137.9±3.036 cd	1966±64.85 c	772±39.7 efg
SL Fruit set	161.4±5.412 a	151.6±2.867 bc	2717±83.5 a	1059±47.6 bcd
SL Veraison	124.2±6.29 cdef	186.4±8.506 a	2376±120.89 b	1451±114.7 a
SL Ripening	115.9±11.19 efg	190.7±5.216 a	1676±87.35 de	1147±106.58 b
CA Flowering	84.2±3.071 l	111.7±2.205 f	1483±50.85 def	585±37.14 g
CA Fruit set	120.6±5.919 defg	123.7±4.286 def	2044±88.08 c	835±48.15 def
CA Veraison	123.9±6.483 cdef	131.1±2.824 de	2014±95.66 c	1105±69.95 bc
CA Ripening	110.4±6.672 fg	124±5.989 def	1335±102.77 f	762±65.52 efg
GR Flowering	91.2±3.406 il	113.3±2.788 f	1475±58.85 def	829±44.47 def
GR Fruit set	140.1±4.66 bc	128.2±2.515 de	2135±64.91 bc	980±50.16 bcde
GR Veraison	145.8±7.902 ab	148.8±3.699 bc	2142±107.55 bc	1660±124.21 a
GR Ripening	130.1±7.549 bcde	149.3±3.791 bc	1421±100.34 ef	1502±122.12 a
AC Flowering	103.1±4.475 hi	121.4±6.783 ef	1676±98.65 de	654±51.72 fg
AC Fruit set	138.1±5.356 bcd	131.2±3.682 de	2124±92.76 bc	887±57.23 cdef
AC Veraison	123.7±7.602 cdef	147.8±4.594 bc	2076±108.15 c	953±66.33 bcde
AC Ripening	130.3±7.093 bcde	161.3±5.414 b	1714±86.49 d	1048±87.2 bcd
Significance[1]				
FxPP	***	***	***	***

[1]NS,*, **, ***: Non-significant or significant at P ≤ 0.05, 0.01, 0.005, respectively

Table S3. Data of interaction analysis Y x PP for Shoot length, shoot basal diameter, Main leaf area, Anticipated leaf area, Total leaf area. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

	Shoot lenght	shoot basal diam	Main leaf area	Anticipated leaf area	Total leaf area
	cm	*mm*	*cm² shoot⁻¹*	*cm² shoot⁻¹*	cm² shoot⁻¹
Y x PP					
2019 Flowering	114.9±2.765 bc	8.66±0.1 b	1939±46.12 cd	792.4±32.3 def	2731±69.8 c
2019 Fruit set	163.4±4.053 a	8.77±0.08 ab	2504±67.11 a	994.7±35.65 cd	3498±90.78 b
2019 Veraison	165.4±7.562 a	9.12±0.1 a	2618±95.24 a	1689.7±100.5 a	4307±169.38 a
2019 Ripening	156.2±6.345 a	8.81±0.1 ab	1942±66.65 cd	1434.5±77.58 b	3377±113.36 b
2020 Flowering	101.7±3.077 cd	8.05±0.1 de	1718±62.08 d	586.1±29.25 g	2357±95.27 d
2020 Fruit set	128.9±4.354 b	8.24±0.1 cde	2272±67.48 b	968.1±43.32 cde	3240±100.9 b
2020 Veraison	118.1±3.822 b	7.96±0.11 de	2111±65.78 bc	1116.4±53.1 c	3228±107 b
2020 Ripening	97.5±4.962 d	7.48±0.14 f	1367±76.26 e	899.3±84.98 def	2266±136.72 d
2021 Flowering	62.5±2.268 e	7.49±0.11 f	936±32.64 f	794.9±64.82 def	1731±76.49 e
2021 Fruit set	115.8±5.106 bc	8.59±0.15 bc	1723±78.25 d	776.9±60.16 efg	2500±101.65 cd
2021Veraison	96.1±4.916 d	8.27±0.15 cd	1303±79.15 e	849.9±70.22 def	2153±111.43 d
2021 Ripening	94.6±5.767 d	7.88±0.13 e	895±60.33 f	692.5±69.47 fg	1587±98.54 e
Significance[1]					
YxPP	***	***	***	***	***

[1]NS,*, **, ***: Non-significant or significant at P ≤ 0.05, 0.01, 0.005, respectively

Table S4. Data of interaction analysis F x Y for Potencial Fertility, Real fertility, Fruit set, Berry diameter, Average bunch weight. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

	Potencial Fertility	Real fertility	Fruit set	Berry diameter	Average bunch weight
			%	*cm*	*kg bunch⁻¹*
Field (F)					
SL 2019	1.49±0.035 bc	1.13±0.036 cd	28.1±0.673 bc	1.4±0.011 bc	342.8±16.48 a
SL 2020	1.14±0.028 i	0.73±0.032 fg	34.6±1.34 a	1.48±0.011 a	377.7±16.17 a
SL 2021	1.55±0.033 b	1.33±0.043 b	22.1±0.907 de	1.41±0.009 bc	234.3±10.51 bc
CA 2019	1.33±0.038 fg	1.09±0.053 d	35.3±0.998 a	1.17±0.012 f	177.7±11.83 e
CA 2020	1.14±0.032 i	0.65±0.043 g	27.2±0.723 cd	1.42±0.012 b	238.3±10.78 bc
CA 2021	1.28±0.038 gh	1.02±0.048 d	26.2±1.25 cd	1.18±0.008 f	93.15±5.51 g
GR 2019	1.35±0.036 efg	1.09±0.045 d	26.6±1.38 cd	1.23±0.012 e	215.91±11.99 cd
GR 2020	1.31±0.041 fgh	0.98±0.074 de	26.9±0.695 cd	1.25±0.013 e	133.18±11.96 f
GR 2021	1.42±0.038 def	1.12±0.049 cd	24.4±1.01 de	1.32±0.011 d	185.5±8.47 de
AC 2019	1.66±0.044 a	1.62±0.049 a	26.7±0.77 cd	1.38±0.01 c	343.82±14.92 a
AC 2020	1.19±0.048 hi	0.85±0.063 ef	30.4±0.957 b	1.43±0.011b	263.32±12.61 b
AC 2021	1.45±0.054 cde	1.26±0.062 bc	36±1.83 a	1.25±0.014 e	196.12±11.13 de
Significance[1]					
F*Y	***	***	***	***	***

[1]NS,*, **, ***: Non-significant or significant at P ≤ 0.05, 0.01, 0.005, respectively.

Table S5. Data of interaction analysis F x Y Pn, gs, Ci, E, ETR, ΦPSII, Fv/Fm, Chl. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

	Pn	gs	Ci	E	ETR	ΦPSII	Fv/Fm	Chl
	(μmol m^{-2} s^{-1})	(mol m^{-2} s^{-1})	μmol mol^{-1}	(mol H_2O m^{-2} s^{-1})				(μg/cm^2)
F*Y								
SL 2019	11±1.03 a	0.174±0.042 ab	274±24.62 a	5.1±0.6 bc	172±6.85 ab	0.310±0.010 ab	0.786±0.005 a	44.68±1.26 cd
SL 2020	10.8±0.43 a	0.19±0.006 ab	268±5.44 a	4.8±0.13 bcd	171±8.46 ab	0.308±0.010 ab	0.781±0.003 ab	45.53±1.78 cd
SL 2021	10.9±0.3 a	0.142±0.005 abc	242±3.82 abc	4.4±0.25 bcde	157±4.85 bcd	0.284±0.013 bc	0.763±0.006 bc	47.86±0.54 cd
CA 2019	6.6±0.7 b	0.103±0.02 bcd	231±21.64 abcd	3.9±0.43 cde	134±5.22 de	0.258±0.010 cd	0.753±0.005 c	42.79±2.48 de
CA 2020	5.7±0.68 b	0.102±0.019 bcd	258±12.97 ab	3.4±0.54 ef	140±4.17 cde	0.240±0.005 d	0.75±0.003 c	29.32±2.16 g
CA 2021	2.2±0.27 c	0.021±0.003 d	192±9.46 de	1.3±0.18 h	141±9.3 cde	0.258±0.016 cd	0.759±0.013 c	38.77±2.12 ef
GR 2019	6.2±1.36 b	0.168±0.098 ab	212±29.26 cde	3.8±0.62 def	125±8.52 e	0.233±0.015 d	0.755±0.005 c	56.38±1.53 b
GR 2020	3.9±0.56 c	0.062±0.007 cd	268±7.05 a	2.7±0.26 f	147±7.4 cde	0.253±0.011 cd	0.751±0.006 c	65.78±3.64 a
GR 2021	6.3±0.44 b	0.078±0.007 cd	220±5.05 bcde	4.2±0.37 cde	176±9.86 ab	0.321±0.018 ab	0.787±0.007 a	65.44±1.25 a
AC 2019	11.2±0.73 a	0.182±0.026 ab	178±21.82 e	5.4±0.36 b	185±7.6 a	0.320±0.012 ab	0.794±0.004 a	35.42±1.16 f
AC 2020	6.8±0.34 b	0.131±0.009 abc	273±5.63 a	5.1±0.28 bc	175±5.27 ab	0.330±0.009 a	0.78±0.004 ab	46.08±1.09 cd
AC 2021	10.2±0.32 a	0.214±0.006 a	259±3.52 ab	9.2±0.23 a	162±8.93 abc	0.300±0.018 ab	0.744±0.002 c	49.98±0.87 c
Significance[1]								
F*Y	***	***	***	***				

[1]NS,*, **, ***: Non-significant or significant at P ≤ 0.05, 0.01, 0.005, respectively.

Table S6. Data of interaction analysis F x Y Citrate, isocitrate. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

	Citrate (*g kg*$^{-1}$ DW)	isocitrate (*g kg*$^{-1}$ DW)
Field		
SL 2019	3.644±0.185 a	0.914±0.167 a
SL 2020	2.592±0.228 b	0.653±0.083 bcd
SL 2021	2.566±0.124 b	0.376±0.023 gh
CA 2019	2.519±0.282 b	0.44±0.036 fgh
CA 2020	1.535±0.241 c	0.712±0.07 abc
CA 2021	2.458±0.271 b	0.356±0.035 h
GR 2019	3.592±0.485 a	0.61±0.019 cde
GR 2020	3.714±0.415 b	0.734±0.088 abc
GR 2021	2.535±0.289	0.507±0.053 efgh
AC 2019	1.47±0.11 c	0.326±0.034 h
AC 2020	2.058±0.037 bc	0.758±0.054 ab
AC 2021	1.256±0.36 c	0.279±0.085 h
Significance[1]		
FxY	***	***

[1]NS,*, **, ***: Non-significant or significant at P ≤ 0.05, 0.01, 0.005, respectively

Table S7. Interactions among the main analyzed parameters during the three year study.

Year 2019				Year 2020				Year 2021			
Param.1	*Param.2*	**r**	**sign.**	*Param.1*	*Param.2*	**r**	**sign.**	*Param.1*	*Param.2*	**r**	**sign.**
SL	*SBD*	0.142	NS	*SL*	*SBD*	0.553	****	*SL*	*SBD*	0.278	***
SL	*SLA*	0.166	NS	*SL*	*SLA*	0.451	****	*SL*	*SLA*	0.189	*
SL	*MLA*	0.760	****	*SL*	*MLA*	0.848	****	*SL*	*MLA*	0.754	****
SL	*ALA*	0.486	****	*SL*	*ALA*	0.595	****	*SL*	*ALA*	0.548	****
SL	*TLA*	0.750	****	*SL*	*TLA*	0.803	****	*SL*	*TLA*	0.800	****
SL	*PF*	0.116	NS	*SL*	*PF*	0.080	NS	*SL*	*PF*	0.179	NS
SL	*RF*	0.064	NS	*SL*	*RF*	0.105	NS	*SL*	*RF*	0.240	**
SL	*FS*	0.076	NS	*SL*	*FS*	-0.025	NS	*SL*	*FS*	-0.036	NS
SL	*ABW*	-0.044	NS	*SL*	*ABW*	0.218	**	*SL*	*ABW*	-0.005	NS
SL	*BD*	-0.100	NS	*SL*	*BD*	-0.115	NS	*SL*	*BD*	0.179	NS
SL	*Pn*	-0.187	NS	*SL*	*Pn*	-0.118	NS	*SL*	*Pn*	0.008	NS
SL	*gs*	0.020	NS	*SL*	*gs*	-0.200	*	*SL*	*Gs*	0.023	NS
SL	*Ci*	-0.373	NS	*SL*	*Ci*	-0.040	NS	*SL*	*Ci*	0.079	NS
SL	E	0.046	NS	*SL*	E	-0.136	NS	*SL*	E	0.046	NS
SL	ETR	-0.067	NS	*SL*	ETR	0.012	NS	*SL*	ETR	0.067	NS
SL	*ΦPSII*	-0.093	NS	*SL*	*ΦPSII*	-0.021	NS	*SL*	*ΦPSII*	0.000	NS
SL	*Fv/Fm*	-0.136	NS	*SL*	*Fv/Fm*	-0.005	NS	*SL*	*Fv/Fm*	0.064	NS
SBD	*SLA*	0.279	***	*SBD*	*SLA*	0.612	****	*SBD*	*SLA*	0.501	****
SBD	*MLA*	0.077	NS	*SBD*	*MLA*	0.599	****	*SBD*	*MLA*	0.174	NS
SBD	*ALA*	0.321	****	*SBD*	*ALA*	0.603	****	*SBD*	*ALA*	0.251	NS
SBD	*TLA*	0.208	*	*SBD*	*TLA*	0.656	****	*SBD*	*TLA*	0.267	***
SBD	*PF*	0.213	*	*SBD*	*PF*	0.029	NS	*SBD*	*PF*	0.130	NS
SBD	*RF*	0.060	NS	*SBD*	*RF*	0.050	NS	*SBD*	*RF*	0.110	NS
SBD	*FS*	-0.211	*	*SBD*	*FS*	-0.033	NS	*SBD*	*FS*	-0.210	*
SBD	*ABW*	0.265	***	*SBD*	*ABW*	0.069	NS	*SBD*	*ABW*	0.220	**
SBD	*BD*	0.421	****	*SBD*	*BD*	-0.122	NS	*SBD*	*BD*	0.194	*
SBD	*Pn*	-0.037	NS	*SBD*	*Pn*	0.045	NS	*SBD*	*Pn*	0.102	NS
SBD	*gs*	0.072	NS	*SBD*	*gs*	0.020	NS	*SBD*	*Gs*	0.090	NS
SBD	*Ci*	0.166	NS	*SBD*	*Ci*	-0.050	NS	*SBD*	*Ci*	0.202	*
SBD	E	0.141	NS	*SBD*	E	0.069	NS	*SBD*	E	0.114	NS
SBD	ETR	0.200	NS	*SBD*	ETR	0.234	NS	*SBD*	ETR	0.154	NS
SBD	*ΦPSII*	0.143	NS	*SBD*	*ΦPSII*	0.140	NS	*SBD*	*ΦPSII*	0.162	NS
SBD	*Fv/Fm*	0.285	**	*SBD*	*Fv/Fm*	0.152	NS	*SBD*	*Fv/Fm*	-0.029	NS
SLA	*MLA*	0.429	****	*SLA*	*MLA*	0.627	****	*SLA*	*MLA*	0.324	****
SLA	*ALA*	0.345	****	*SLA*	*ALA*	0.591	****	*SLA*	*ALA*	0.241	**
SLA	*TLA*	0.458	****	*SLA*	*TLA*	0.671	****	*SLA*	*TLA*	0.347	****
SLA	*PF*	0.220	**	*SLA*	*PF*	0.159	NS	*SLA*	*PF*	0.196	*
SLA	*RF*	0.170	NS	*SLA*	*RF*	0.133	NS	*SLA*	*RF*	0.197	*
SLA	*FS*	-0.011	NS	*SLA*	*FS*	-0.013	NS	*SLA*	*FS*	-0.197	*
SLA	*ABW*	0.341	****	*SLA*	*ABW*	0.351	****	*SLA*	*ABW*	0.289	****
SLA	*BD*	0.214	**	*SLA*	*BD*	0.003	NS	*SLA*	*BD*	0.416	****
SLA	*Pn*	0.255	**	*SLA*	*Pn*	0.152	NS	*SLA*	*Pn*	0.046	NS
SLA	*gs*	0.061	NS	*SLA*	*gs*	0.067	NS	*SLA*	*Gs*	-0.039	NS

SLA	*Ci*	-0.121	NS	*SLA*	*Ci*	-0.042	NS	*SLA*	*Ci*	0.016	NS
SLA	E	-0.037	NS	*SLA*	E	-0.011	NS	*SLA*	E	-0.094	NS
SLA	ETR	0.128	NS	*SLA*	ETR	0.027	NS	*SLA*	ETR	0.067	NS
SLA	*ΦPSII*	0.083	NS	*SLA*	*ΦPSII*	0.004	NS	*SLA*	*ΦPSII*	0.071	NS
SLA	*Fv/Fm*	0.199	NS	*SLA*	*Fv/Fm*	0.270	*	*SLA*	*Fv/Fm*	0.053	NS
MLA	*ALA*	0.460	****	*MLA*	*ALA*	0.654	****	*MLA*	*ALA*	0.314	****
MLA	*TLA*	0.898	****	*MLA*	*TLA*	0.922	****	*MLA*	*TLA*	0.799	****
MLA	*PF*	0.081	NS	*MLA*	*PF*	0.146	NS	*MLA*	*PF*	0.103	NS
MLA	*RF*	0.134	NS	*MLA*	*RF*	0.150	NS	*MLA*	*RF*	0.205	*
MLA	*FS*	0.123	NS	*MLA*	*FS*	-0.033	NS	*MLA*	*FS*	-0.277	***
MLA	*ABW*	0.009	NS	*MLA*	*ABW*	0.325	****	*MLA*	*ABW*	0.083	NS
MLA	*BD*	-0.082	NS	*MLA*	*BD*	-0.102	NS	*MLA*	*BD*	0.310	****
MLA	*Pn*	0.009	NS	*MLA*	*Pn*	0.034	NS	*MLA*	*Pn*	0.013	NS
MLA	*gs*	-0.022	NS	*MLA*	*gs*	-0.029	NS	*MLA*	*Gs*	-0.100	NS
MLA	*Ci*	-0.308	**	*MLA*	*Ci*	0.039	NS	*MLA*	*Ci*	-0.011	NS
MLA	E	-0.104	NS	*MLA*	E	-0.001	NS	*MLA*	E	-0.154	NS
MLA	ETR	-0.105	NS	*MLA*	ETR	0.021	NS	*MLA*	ETR	0.019	NS
MLA	*ΦPSII*	-0.148	NS	*MLA*	*ΦPSII*	0.044	NS	*MLA*	*ΦPSII*	-0.087	NS
MLA	*Fv/Fm*	-0.121	NS	*MLA*	*Fv/Fm*	0.176	NS	*MLA*	*Fv/Fm*	0.096	NS
ALA	*TLA*	0.803	****	*ALA*	*TLA*	0.895	****	*ALA*	*TLA*	0.822	****
ALA	*PF*	-0.162	NS	*ALA*	*PF*	0.095	NS	*ALA*	*PF*	0.496	****
ALA	*RF*	-0.208	*	*ALA*	*RF*	0.142	NS	*ALA*	*RF*	0.427	****
ALA	*FS*	0.235	**	*ALA*	*FS*	-0.001	NS	*ALA*	*FS*	0.039	NS
ALA	*ABW*	-0.107	NS	*ALA*	*ABW*	0.090	NS	*ALA*	*ABW*	0.246	**
ALA	*BD*	-0.024	NS	*ALA*	*BD*	-0.062	NS	*ALA*	*BD*	0.207	*
ALA	*Pn*	-0.238	*	*ALA*	*Pn*	-0.226	**	*ALA*	*Pn*	0.263	*
ALA	*gs*	-0.121	NS	*ALA*	*gs*	-0.235	**	*ALA*	*Gs*	0.294	**
ALA	*Ci*	-0.223	NS	*ALA*	*Ci*	0.071	NS	*ALA*	*Ci*	0.185	NS
ALA	E	0.012	NS	*ALA*	E	-0.256	**	*ALA*	E	0.226	NS
ALA	ETR	-0.332	**	*ALA*	ETR	-0.184	NS	*ALA*	ETR	0.229	NS
ALA	*ΦPSII*	-0.304	**	*ALA*	*ΦPSII*	-0.248	*	*ALA*	*ΦPSII*	0.186	NS
ALA	*Fv/Fm*	-0.303	**	*ALA*	*Fv/Fm*	-0.020	NS	*ALA*	*Fv/Fm*	-0.069	NS
TLA	*PF*	-0.026	NS	*TLA*	*PF*	0.135	NS	*TLA*	*PF*	0.376	****
TLA	*RF*	-0.013	NS	*TLA*	*RF*	0.161	NS	*TLA*	*RF*	0.394	****
TLA	*FS*	0.199	*	*TLA*	*FS*	-0.020	NS	*TLA*	*FS*	-0.142	NS
TLA	*ABW*	-0.047	NS	*TLA*	*ABW*	0.237	**	*TLA*	*ABW*	0.206	*
TLA	*BD*	-0.066	NS	*TLA*	*BD*	-0.092	NS	*TLA*	*BD*	0.318	****
TLA	*Pn*	-0.115	NS	*TLA*	*Pn*	-0.091	NS	*TLA*	*Pn*	0.171	NS
TLA	*gs*	-0.076	NS	*TLA*	*gs*	-0.136	NS	*TLA*	*Gs*	0.122	NS
TLA	*Ci*	-0.331	***	*TLA*	*Ci*	0.060	NS	*TLA*	*Ci*	0.108	NS
TLA	E	-0.067	NS	*TLA*	E	-0.128	NS	*TLA*	E	0.047	NS
TLA	ETR	-0.249	*	*TLA*	ETR	-0.077	NS	*TLA*	ETR	0.156	NS
TLA	*ΦPSII*	-0.268	*	*TLA*	*ΦPSII*	-0.094	NS	*TLA*	*ΦPSII*	0.066	NS
TLA	*Fv/Fm*	-0.246	*	*TLA*	*Fv/Fm*	0.102	NS	*TLA*	*Fv/Fm*	0.014	NS
PF	*RF*	0.814	****	*PF*	*RF*	0.835	****	*PF*	*RF*	0.844	****
PF	*FS*	-0.342	****	*PF*	*FS*	-0.221	**	*PF*	*FS*	-0.181	NS
PF	*ABW*	0.466	****	*PF*	*ABW*	0.211	*	*PF*	*ABW*	0.432	****
PF	*BD*	0.364	****	*PF*	*BD*	-0.153	NS	*PF*	*BD*	0.325	****
PF	*Pn*	0.295	**	*PF*	*Pn*	-0.468	****	*PF*	*Pn*	0.503	****
PF	*gs*	0.149	NS	*PF*	*gs*	-0.346	***	*PF*	*Gs*	0.372	***

PF	*Ci*	-0.021	NS	*PF*	*Ci*	0.262	*	*PF*	*Ci*	0.263	*
PF	E	0.178	NS	*PF*	E	-0.186	NS	*PF*	E	0.212	NS
PF	ETR	0.397	****	*PF*	ETR	0.136	NS	*PF*	ETR	0.023	NS
PF	*ΦPSII*	0.302	**	*PF*	*ΦPSII*	0.164	NS	*PF*	*ΦPSII*	0.075	NS
PF	*Fv/Fm*	0.572	****	*PF*	*Fv/Fm*	0.075	NS	*PF*	*Fv/Fm*	-0.135	NS
RF	*FS*	-0.428	****	*RF*	*FS*	-0.176	NS	*RF*	*FS*	-0.110	NS
RF	*ABW*	0.277	***	*RF*	*ABW*	0.236	**	*RF*	*ABW*	0.413	****
RF	*BD*	0.246	**	*RF*	*BD*	-0.134	NS	*RF*	*BD*	0.310	****
RF	*Pn*	0.240	**	*RF*	*Pn*	-0.356	****	*RF*	*Pn*	0.512	****
RF	*gs*	0.149	NS	*RF*	*gs*	-0.346	***	*RF*	*Gs*	0.372	***
RF	*Ci*	-0.131	NS	*RF*	*Ci*	0.223	NS	*RF*	*Ci*	0.333	***
RF	E	0.198	*	*RF*	E	-0.083	NS	*RF*	E	0.249	*
RF	ETR	0.332	****	*RF*	ETR	0.207	NS	*RF*	ETR	-0.053	NS
RF	*ΦPSII*	0.264	***	*RF*	*ΦPSII*	0.208	NS	*RF*	*ΦPSII*	-0.058	NS
RF	*Fv/Fm*	0.410	****	*RF*	*Fv/Fm*	0.047	NS	*RF*	*Fv/Fm*	-0.096	NS
FS	*ABW*	-0.263	***	*FS*	*ABW*	0.036	NS	*FS*	*ABW*	-0.268	***
FS	*BD*	-0.132	NS	*FS*	*BD*	0.121	NS	*FS*	*BD*	-0.309	****
FS	*Pn*	-0.199	NS	*FS*	*Pn*	0.024	NS	*FS*	*Pn*	0.035	NS
FS	*gs*	-0.184	NS	*FS*	*gs*	0.043	NS	*FS*	*Gs*	0.292	**
FS	*Ci*	0.006	NS	*FS*	*Ci*	0.062	NS	*FS*	*Ci*	0.082	NS
FS	E	-0.070	NS	*FS*	E	-0.037	NS	*FS*	E	0.424	****
FS	ETR	-0.283	**	*FS*	ETR	-0.126	NS	*FS*	ETR	-0.202	NS
FS	*ΦPSII*	-0.119	NS	*FS*	*ΦPSII*	-0.139	NS	*FS*	*ΦPSII*	-0.146	NS
FS	*Fv/Fm*	-0.388	****	*FS*	*Fv/Fm*	-0.274	*	*FS*	*Fv/Fm*	-0.250	*
ABW	*BD*	0.530	****	*ABW*	*BD*	0.378	****	*ABW*	*BD*	0.680	****
ABW	*Pn*	0.442	****	*ABW*	*Pn*	0.524	****	*ABW*	*Pn*	0.591	****
ABW	*gs*	0.269	*	*ABW*	*gs*	0.529	****	*ABW*	*Gs*	0.341	****
ABW	*Ci*	0.027	NS	*ABW*	*Ci*	0.135	NS	*ABW*	*Ci*	0.396	****
ABW	E	0.131	NS	*ABW*	E	0.397	****	*ABW*	E	0.157	NS
ABW	ETR	0.434	****	*ABW*	ETR	0.431	****	*ABW*	ETR	0.203	NS
ABW	*ΦPSII*	0.356	***	*ABW*	*ΦPSII*	0.430	****	*ABW*	*ΦPSII*	0.165	NS
ABW	*Fv/Fm*	0.565	****	*ABW*	*Fv/Fm*	0.456	****	*ABW*	*Fv/Fm*	0.153	NS
BD	*Pn*	0.439	****	*BD*	*Pn*	0.545	****	*BD*	*Pn*	0.495	****
BD	*gs*	0.250	*	*BD*	*gs*	0.537	****	*BD*	*gs*	0.212	NS
BD	*Ci*	0.056	NS	*BD*	*Ci*	-0.044	NS	*BD*	*Ci*	0.291	**
BD	E	0.244	*	*BD*	E	0.377	****	*BD*	E	0.073	NS
BD	ETR	0.524	****	*BD*	ETR	0.227	NS	*BD*	ETR	0.173	NS
BD	*ΦPSII*	0.516	****	*BD*	*ΦPSII*	0.244	*	*BD*	*ΦPSII*	0.134	NS
BD	*Fv/Fm*	0.574	****	*BD*	*Fv/Fm*	0.187	NS	*BD*	*Fv/Fm*	0.227	NS
Pn	*gs*	0.272	*	*Pn*	*gs*	0.858	****	*Pn*	*gs*	0.856	****
Pn	*Ci*	-0.347	***	*Pn*	*Ci*	0.094	NS	*Pn*	*Ci*	0.245	*
Pn	E	0.063	NS	*Pn*	E	0.009	NS	*Pn*	E	0.631	****
Pn	ETR	0.139	NS	*Pn*	ETR	0.092	NS	*Pn*	ETR	0.199	NS
Pn	*ΦPSII*	0.045	NS	*Pn*	*ΦPSII*	0.203	NS	*Pn*	*ΦPSII*	0.225	NS
Pn	*Fv/Fm*	0.160	NS	*Pn*	*Fv/Fm*	-0.014	NS	*Pn*	*Fv/Fm*	-0.111	NS
gs	*Ci*	-0.055	NS	*gs*	*Ci*	0.344	***	*gs*	*Ci*	0.710	****
gs	E	0.736	****	*gs*	E	0.886	****	*gs*	E	0.933	****
gs	ETR	0.322	**	*gs*	ETR	0.250	*	*gs*	ETR	0.131	NS
gs	*ΦPSII*	0.346	***	*gs*	*ΦPSII*	0.313	**	*gs*	*ΦPSII*	0.141	NS
gs	*Fv/Fm*	0.056	NS	*gs*	*Fv/Fm*	0.410	****	*gs*	*Fv/Fm*	-0.341	***

Ci	E	-0.079	NS	*Ci*	E	0.457	****	*Ci*	E	0.665	****
Ci	ETR	0.063	NS	*Ci*	ETR	0.020	NS	*Ci*	ETR	0.035	NS
Ci	*ΦPSII*	-0.054	NS	*Ci*	*ΦPSII*	0.129	NS	*Ci*	*ΦPSII*	0.034	NS
Ci	*Fv/Fm*	-0.037	NS	*Ci*	*Fv/Fm*	0.016	NS	*Ci*	*Fv/Fm*	-0.371	***
E	ETR	0.415	****	E	ETR	0.178	NS	E	ETR	0.179	NS
E	*ΦPSII*	0.446	****	E	*ΦPSII*	0.331	**	E	*ΦPSII*	0.198	NS
E	*Fv/Fm*	0.134	NS	E	*Fv/Fm*	0.312	**	E	*Fv/Fm*	-0.333	**
ETR	*ΦPSII*	0.931	****	ETR	*ΦPSII*	0.906	****	ETR	*ΦPSII*	0.902	****
ETR	*Fv/Fm*	0.509	****	ETR	*Fv/Fm*	0.517	****	ETR	*Fv/Fm*	-0.071	NS
ΦPSII	*Fv/Fm*	0.389	****	*ΦPSII*	*Fv/Fm*	0.598	****	*ΦPSII*	*Fv/Fm*	-0.057	NS

[1]NS,*, **, ***,****: Non-significant or significant at $P \leq 0.1$, 0.05, 0.02, 0.01, respectively.

Legend: Shoot length: SL; Shoot basal diameter: SBD; Single leaf area: SLA; Main leaf area: MLA; Anticipated leaf area: ALA; Total leaf area: TLA; Potential fertility: PF; Real fertility: RF; Fruit set: FS; Average bunch weight: ABW: Berry diameter: BD; Net CO2 assimilation rate: Pn, stomatal conductance: gs; substomatal CO2 concentration: Ci; leaf transpiration rate: E; the maximum ΦPSII photochemical efficiency: Fv/Fm; the quantum yield of PSII linear electron transport: ΦPSII; and the electron transmission rate: ETR.

CHAPTER 2

How Leaf Vein and Stomata Traits Are Related with Photosynthetic Efficiency in Falanghina Grapevine in Different Pedoclimatic conditions

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Article

How Leaf Vein and Stomata Traits Are Related with Photosynthetic Efficiency in Falanghina Grapevine in Different Pedoclimatic Conditions

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Abstract: The increase in severe drought events due to climate change in the areas traditionally suitable for viticulture is enhancing the need to understand how grapevines regulate their photosynthetic metabolism in order to forecast specific cultivar adaptive responses to the changing environment. This study aims at evaluating the association between leaf anatomical traits and eco-physiological adjustments of the 'Falanghina' grapevine under different microclimatic conditions at four sites in southern Italy. Sites were characterized by different pedoclimatic conditions but, as much as possible, were similar for plant material and cultivation management. Microscopy analyses on leaves were performed to quantify stomata and vein traits, while eco-physiological analyses were conducted on vines to assess plant physiological adaptation capability. At the two sites with relatively low moisture, photosynthetic rate, stomatal conductance, photosystem electron transfer rate, and quantum yield of PSII, linear electron transport was lower compared to the other two sites. Stomata size was higher at the site characterized by the highest precipitation. However, stomatal density and most vein traits tended to be relatively stable among sites. The number of free vein endings per unit leaf area was lower in the two vineyards with low precipitation. We suggest that site-specific stomata and vein traits modulation in Falanghina grapevine are an acclimation strategy that may influence photosynthetic performance. Overall in-depth knowledge of the structure/function relations in Falanghina vines might be useful to evaluate the plasticity of this cultivar towards site-specific management of vineyards in the direction of precision viticulture.

Keywords: climate changes; leaf traits; photosynthesis; vein and stomata traits; *Vitis vinifera*

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1. Introduction

Nowadays, climate change is challenging agriculture since it can drastically modify plant growth, with possible negative effects, especially in arid and semi-arid regions of Europe. In the Mediterranean area, climate models often show irregularities in precipitation patterns and significantly rising temperatures leading to the increase in frequency, severity, and duration of drought events [1–3]. The interest in understanding how Mediterranean crops face drought is currently increasing due to the severe limitations expected in plant growth and productive yield in the future [4,5].

Grapevine (*Vitis vinifera* L. subsp. *vinifera*) is a high-income crop, rain-fed cultivated in many Mediterranean areas, according to the specific requirements of quality and origin labels. The productivity of the 'Falanghina' cultivar, which is important in southern Italy, is expected to be severely impacted by environmental changes. The typical temperature and precipitation regimes during summer lead to a decrease in leaf area and photosynthesis in the water-stressed vines, ultimately causing physiological and metabolic disorders with negative effects on the overall plant functioning, including nutrient uptake, fruit set, and berry ripening [6,7]. Under water stress conditions, many adaptation mechanisms can occur mainly related to an increase in water holding capacity, decrease in water losses, and mechanical reinforcement to prevent any tissue damage [8]. For instance, one of the first plant responses to water deficit is a decrease in the investment in leaves compared to other organs due to a change in carbon partitioning favoring the flow of assimilates towards the root [9]. In the current climate change scenario, the occurrence of osmotic stress due to soil and water salinization can also affect plants' gas exchange and lead to leaf anatomy adjustments similar to those observed in response to water stress [10]. All these morpho-physiological alterations affect both yield and berry composition (e.g., soluble solids, organic acids, polyphenols), often associated with decreasing must quality [11]. In many areas of southern Italy, grapevines are subjected to water stress when high evapotranspiration is accompanied by low precipitation [12], and it has been emphasized that strategies engaged by plants to mitigate the environmental stress must be based on a deep knowledge of plant plasticity in terms of structure/functions relationships [13,14].

An important question in many crops, including grapevine, is how plants efficiently produce leaves capable of supplying enough water to balance the transpiration losses. In the regulation of this mechanism, it is critical that plants have a satisfactory equilibrium between the stomata density/size, which controls maximum stomatal conductance and the transpiration rate [15], and leaf vein density, which regulates water supply throughout the leaf tissues [16,17]. Generally, the balance between the investment in vapor and liquid conductance in the leaf is well conserved in plant groups along evolutionary trends [18].

In the open field, under saturating light conditions, the most efficient combination of stomata and vein investment is reached when the soil water supply is enough to maintain stomata fully open [19]. However, if the vascular system is not sufficiently developed to support the maximum evaporative capacity of the leaves, when the water supply is limited, stomata closure occurs to maintain leaf water status [20]. The harmonization between stomata and vein traits is a delicate question, as an excessive stomatal density may determine high costs for the construction and regulation of guard cells that are not necessary for greater photosynthetic yield when stomata are closed. Similarly, the venation excess may not be efficient when photosynthesis declines (due to increased leaf volume occupied by the vascular system to the detriment of photosynthetic parenchyma) and the cost of synthesizing lignin increases [21]. The coordination between water transport and stomatal systems allows leaves to maintain an efficient balance between water use and carbon gain while accommodating the different rates of photosynthesis and transpiration experienced under high and low irradiance [22,23].

Based on the stomatal regulation, grapevine cultivars have been classified as isohydric or anisohydric, with isohydric vines being able to promptly regulate stomatal responses to maintain constant water potential, and anisohydric vines close their stomata only when water potential is very low [24]. Efforts to relate such behavior with leaf and stem anatomical traits are reported for a few cultivars [24–26]. The isohydric behavior is associated with higher stomata frequency and larger vessels in the stem, thus to a higher hydraulic conductance (corresponding to higher vulnerability to embolism) compared to anisohydric models, whose anatomical traits allow delaying stomatal closure and reaching lower water potentials without xylem cavitation [24]. Therefore, anisohydric behavior would allow a more efficient carbon fixation under short-term mild stress [27]. However, the classification of grapevine cultivars as isohydric or anisohydric is still under debate, and it seems that the same cultivar can show either behavior depending on environmental conditions, such as the severity and duration of the stress event [26,28].

Apart from the stomatal behavior, the photosynthetic rate in plants is also influenced by the transfer resistance for CO_2 diffusion throughout the mesophyll, which contains two main components. The first regards the pathway of CO_2 diffusion from the sub-stomatal cavity to the outer surface of mesophyll cells and is related to the 3D pattern of intercellular spaces; the second involves the path to reach the carboxylation sites in the chloroplasts and is influenced by the permeability of cell walls of the photosynthetic cells [26]. Both components have been found to contribute to a higher photosynthetic rate in *V. vinifera* 'Ribier' compared to *Vitis labrusca* 'Isabella', while differences in photosynthesis among *V. vinifera* 'Athiri', 'Asyrtiko', and 'Syrah' have been mainly ascribed to the resistance across cell walls [29,30]. However, more recently, the variability in mesophyll conductance in seven grapevine cultivars has been shown to be independent of mesophyll anatomical parameters [31].

In the last decade, grapevine morpho-anatomy has been claimed as an understudied topic with a possible important impact on functional responses of vines to environmental stress factors. Leaf epidermal, stomata, and mesophyll traits have been studied in relation to physiological traits only in a few cultivars. Therefore, there is an increasing need to expand knowledge of vine structural and eco-physiological plasticity to finely design precision viticulture strategies for the implementation of irrigation management plans [28,32]. To the best of our knowledge, the strict relations between stomata traits and leaf venation have not been analyzed yet to infer their role in the physiological adjustment of vines growing under limiting environmental conditions.

In this framework, the aim of this study is to better assess the coordination between leaf vein and stomata traits and eco-physiological parameters in *Vitis vinifera* L. subsp. v*inifera* 'Falanghina' grown at four sites in southern Italy. More specifically, we aimed to evaluate how anatomical and eco-physiological parameters are coordinated under different pedoclimatic conditions. We focused on the veraison phenological phase, which corresponds to maximum vegetative growth when water availability is limited in semi-arid Mediterranean environments.

2. Results

2.1. Environmental Data Characterization

The weather information collected from the Guardia Sanframondi station during the phenological phase of veraison showed that temperature was similar in 2019 and 2020, with a July average temperature of 26.9 °C (SD 2.6) and 26.0 °C (SD 2.0), with a maximum temperature of 34.0 °C and 34.3 °C, and with a minimum of 20.4 °C and 19.1 °C,respectively, in 2019 and 2020. July 2020 tended to have less rainfall (14 mm) and lower ET_0 (120 mm) than 2019 (29 mm rainfall, 176 mm ET_0). In 2020, it was possible to measure the cumulative precipitation separately in each experimental vineyard: 32mm SL, 15mm CA,18 mm GR, and 9 mm AC. Supplemental irrigation was provided at the AC site; therefore, the vineyards could be grouped as SL and AC receiving relatively abundant moisture, whereas CA and GR had relatively limited moisture.

Soil water content (SWC) at 15 and 30 cm depth was higher in CA compared to the other sites. At 75 cm depth, GR had the highest SWC, followed by CA. SWC values of SL and GR tended to increase with increasing depth (Table S1). The soil temperature decreased with increasing depth at SL, GR, and AC (Table S1).

2.2. Growth and Production Parameters

Growth and production parameters (total shoot leaf area, single leaf area, shoot basal diameter, bunch weight, and number) are reported in Table 1. The main effect of field (F) was significant for all analyzed parameters; the year (Y) as the main factor showed a significant effect on all factors but bunch weight (Table 1).

Table 1. Effects of field (F), year (Y), and their interaction (F x Y) on total shoot leaf area, single leaf area, shoot diameter, bunch weight, and bunch number per shoot of V. vinifera subsp. vinifera 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Mean values and standard errors are shown.

	Total Shoot Leaf Area	Single Leaf Area	Shoot Basal Diameter	Average Bunch Weight	Bunch Number
	(cm^2 $shoot^{-1}$)	(cm^2)	(mm)	(g $bunch^{-1}$)	(n° $shoot^{-1}$)
Field (F)					
SL	4142 ± 250 ^{a}	192 ± 10.4 ^{a}	8.79 ± 0.16 ^{ab}	360 ± 11.7 ^{a}	1.3 ± 0.04 ^{b}
CA	3416 ± 171 ^{b}	135 ± 3.24 ^{c}	7.89 ± 0.13 ^{c}	208 ± 9.27 ^{c}	1.2 ± 0.03 ^{c}
GR	4194 ± 228 ^{a}	151 ± 4.22 ^{b}	9.02 ± 0.16 ^{a}	175 ± 10.7 ^{d}	1.3 ± 0.03 ^{b}
AC	3320 ± 156 ^{b}	156 ± 5.21 ^{b}	8.49 ± 0.17 ^{b}	304 ± 11.6 ^{b}	1.4 ± 0.05 ^{a}
Year (Y)					
2019	4308 ± 169 ^{a}	168 ± 5.75 a	9.13 ± 0.10 ^{a}	270 ± 10.8 ^{a}	1.5 ± 0.02 ^{a}
2020	3228 ± 107 ^{b}	149 ± 3.42 b	7.97 ± 0.11 ^{b}	253 ± 11.7 ^{a}	1.2 ± 0.02 ^{b}
Significance					
Field (F)	***	***	***	***	***
Year (Y)	***	**	***	NS	***
F x Y	NS	NS	NS	***	***

NS, **, and ***, Not significant or significant at $p < 0.01$, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests ($p \leq 0.05$).

In particular, the average total shoot leaf area was significantly higher in SL and GR compared to CA and AC. Single leaf area was significantly higher in SL than in AC and GR, which, in turn, showed significantly higher values than CA. Shoot basal diameter was higher in GR than AC which resulted in being significantly higher than CA, whileSL showed intermediate values between GR and AC. For average bunch weight, SL was higher than AC, which was significantly higher than CA. The lowest yield was found in GR. For bunch number, AC was higher than SL and GR, which in turn showed significantly higher values than CA. All parameters except bunch weights were higher in 2019 than in 2020.

The interaction between field and year (F x Y) was significant only in the case of bunch weight and bunch number (Table 1). For bunch weight, SL always showed the highest value in 2020, while GR in 2020 was the lowest. CA showed a significant increase in bunch weight from 2019 to 2020, while the opposite significant trend occurred in GR and AC (Figure 1a). For bunch numbers in 2019, there were significant highest values for all the fields compared to 2020 (Figure 1b).

2.3. Gas-Exchange and Chlorophyll a Fluorescence

As regards eco-physiological parameters, the main effect of field (F) was significant for all analyzed parameters except for substomatal CO_2 concentration (Ci) and $_{in}$WUE. The main effect of year (Y) was significant for net photosynthetic rate (Pn), Ci, and $_{in}$WUE; more specifically, Pn and $_{in}$WUE showed significantly lower values in 2020 compared to 2019, while the opposite was found for Ci (Table 2). In particular, in SL leaves, Pn was significantly higher than in AC ones, which, in turn, showed higher Pn than CA and GR. The stomatal conductance (gs), transpiration rate (E), electron transport rate (ETR), quantum yield of PSII electron transport rate (ΦPSII), and maximum PSII photochemical efficiency (Fv/Fm) showed similar values in SL and AC plants, which were also significantly higher than values measured in both CA and GR plants (Table 2).

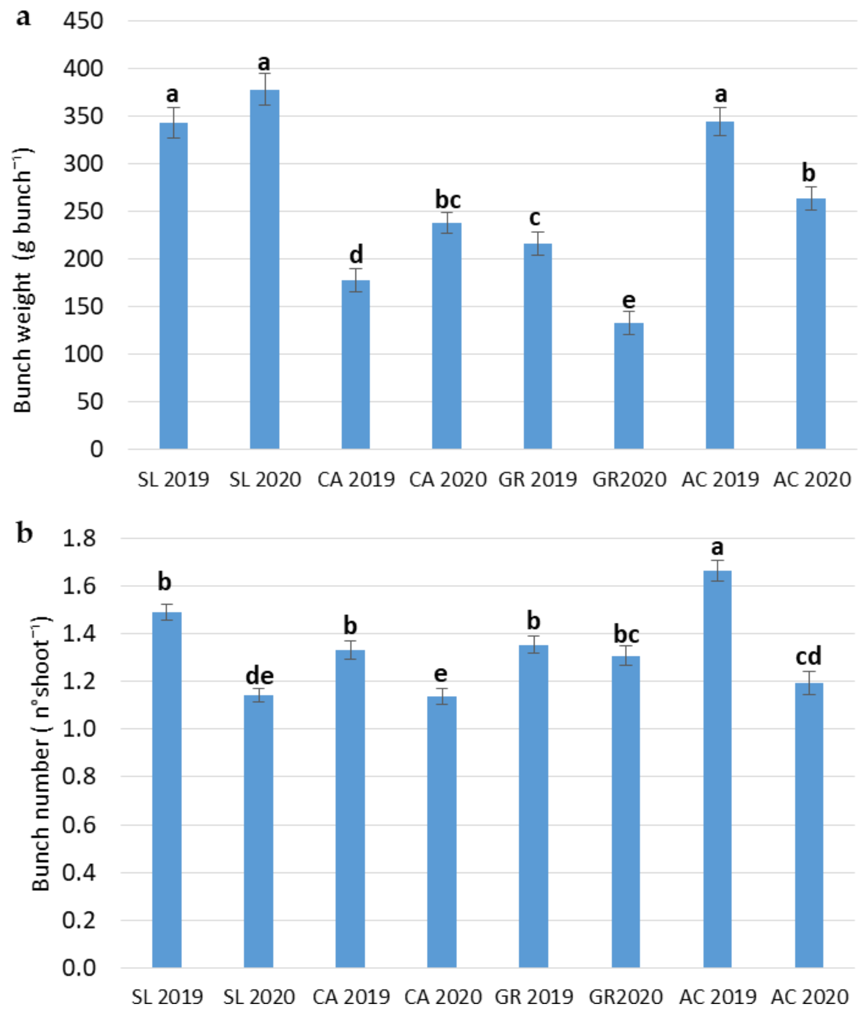

Figure 1. Combined effect of field and year (F x Y) on bunch weight (**a**) and bunch number (**b**) of V. vinifera subsp. vinifera 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Mean values and standard errors are shown. Different letters indicate significant differences according to Duncan's multiple range test ($p \leq 0.05$).

Table 2. Effects of field (F), year (Y) and their interaction (F x Y) on net photosynthetic rate (Pn), stomatal conductance (gs), substomatal CO_2 concentration (Ci), leaf transpiration rate (E), instantaneous water use efficiency ($_{in}$WUE), electron transport rate (ETR), quantum yield of PSII linear electron transport (ΦPSII), and maximum quantum efficiency of PSII photochemistry (Fv/Fm) of V. vinifera subsp. vinifera 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Mean values and standard errors are shown.

	Pn	**gs**	**Ci**	**E**	**inWUE**	**ETR**	**ΦPSII**	**Fv/Fm**
	(μmol CO_2 $m^{-2}s^{-1}$)	**(mmol H_2O m^{-2} s^{-1})**	**(μmol CO_2 mol^{-1})**	**(mol H_2O m^{-2} s^{-1})**	**(μmol CO_2/ mol H_2O)**			
Field (F)								
SL	10.9 ± 0.66 ^{a}	183.5 ± 19.1 ^{a}	267.6 ± 12.5 ^{a}	5.00 ± 0.31 ^{a}	2.55 ± 0.26 ^{a}	172.1 ± 5.30 ^{a}	0.309 ± 0.007 ^{a}	0.784 ± 0.003 ^{a}
CA	6.20 ± 0.49 ^{c}	103.6 ± 12.1 ^{b}	228.3 ± 16.0 ^{a}	3.37 ± 0.34 ^{b}	1.97 ± 0.20 ^{a}	137.3 ± 3.40 ^{b}	0.250 ± 0.006 ^{b}	0.752 ± 0.003 ^{b}
GR	5.15 ± 0.78 ^{c}	66.7 ± 10.9 ^{b}	237.9 ± 17.8 ^{a}	3.33 ± 0.37 ^{b}	1.83 ± 0.32 ^{a}	135.1 ± 6.19 ^{b}	0.242 ± 0.010 ^{b}	0.754 ± 0.004 ^{b}
AC	9.31 ± 0.61 ^{b}	159.4 ± 15.5 ^{a}	225.2 ± 15.3 ^{a}	5.31 ± 0.24 ^{a}	1.98 ± 0.17 ^{a}	181.2 ± 4.81 ^{a}	0.325 ± 0.008 ^{a}	0.788 ± 0.003 ^{a}
Year (Y)								
2019	8.77 ± 0.58 ^{a}	131.5 ± 14.0 ^{a}	214.6 ± 13.6 ^{b}	4.60 ± 0.27 ^{a}	2.26 ± 0.21 ^{a}	153.8 ± 5.14 ^{a}	0.279 ± 0.008 ^{a}	0.772 ± 0.004 ^{a}
2020	6.78 ± 0.45 ^{b}	120.4 ± 8.8 ^{a}	267.4 ± 4.10 ^{a}	4.00 ± 0.22 ^{a}	1.74 ± 0.09 ^{b}	159.3 ± 3.98 ^{a}	0.284 ± 0.008 ^{a}	0.766 ± 0.002 ^{a}
Significance								
Field (F)	***	***	NS	***	NS	***	***	***
Year (Y)	**	NS	***	NS	*	NS	NS	NS
F x Y	*	NS	NS	NS	NS	NS	NS	NS

NS, *, **, and ***, Not significant or significant at $p < 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests ($p \leq 0.05$).

The interaction between field and year (F x Y) was significant only for Pn (Table 2). Vines at the SL site in both years and at the AC site in 2019 showed significantly higher values than all the other conditions (Figure 2), with the lowest value recorded at GR in 2020. At all the sites, Pn decreased in 2020 compared to 2019 but significantly only in AC (Figure 2).

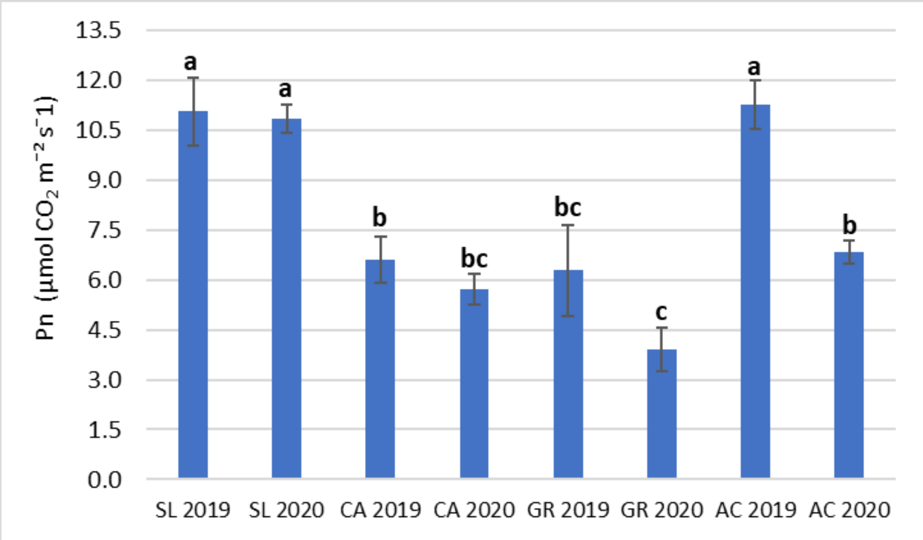

Figure 2. Combined effect of field and year (F x Y) on net photosynthetic rate (Pn) of V. vinifera subsp. vinifera 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Mean values and standard errors are shown. Different letters indicate significant differences according to Duncan's multiple range test ($p \leq 0.05$).

2.4. Stomata and Vein Traits

Microscopy analysis of the abaxial epidermis showed that stomata tended to be larger in leaves collected from vines at SL and CA sites, while they seemed smaller at the other sites (Figure 3). The quantification of stomata traits confirmed that there was a significant main effect of field (F) on guard cell length and width, while the main effect of year (Y) was significant for guard cell width and stomata frequency (Table 3). In particular, stomata were significantly larger at SL compared to the CA field, which in turn showed higher values than GR, whose values were significantly higher than AC (Table 3). As regards stomata frequency, only Y had a significant effect as the main factor, with values measured in 2019 leaves higher than in 2020 (Table 3).

In particular, for both stomata length and width, the fields are significantly different in the decreasing order SL, CA, GR, and AC. Considering the main effect Y, the stomata frequency was significantly higher in 2019 than in 2020, and the stomata width was significantly higher in 2020 than in 2019. The interaction (F x Y) was significant only for stomata frequency (Table 3) which was quite steady among fields in 2019. Significant differences among fields were recorded in 2020, with the lowest values in leaves collected at SL and GR sites (Figure 4).

Microscopy analysis of abaxial epidermis and quantification of vein traits evidenced that the field as the main factor had a significant effect only on Total VLA, Minor VAA, Total VAA, and FVEA (Table 4). Minor VAA was significantly higher in leaves collected at AC compared to CA and GR sites which, in turn, showed significantly higher values than SL leaves. Total VAA was significantly lower values in SL leaves compared to all the other fields (Table 4). On the contrary, SL showed significantly higher values than CA and AC leaves which in turn exhibited significantly higher values than GR.

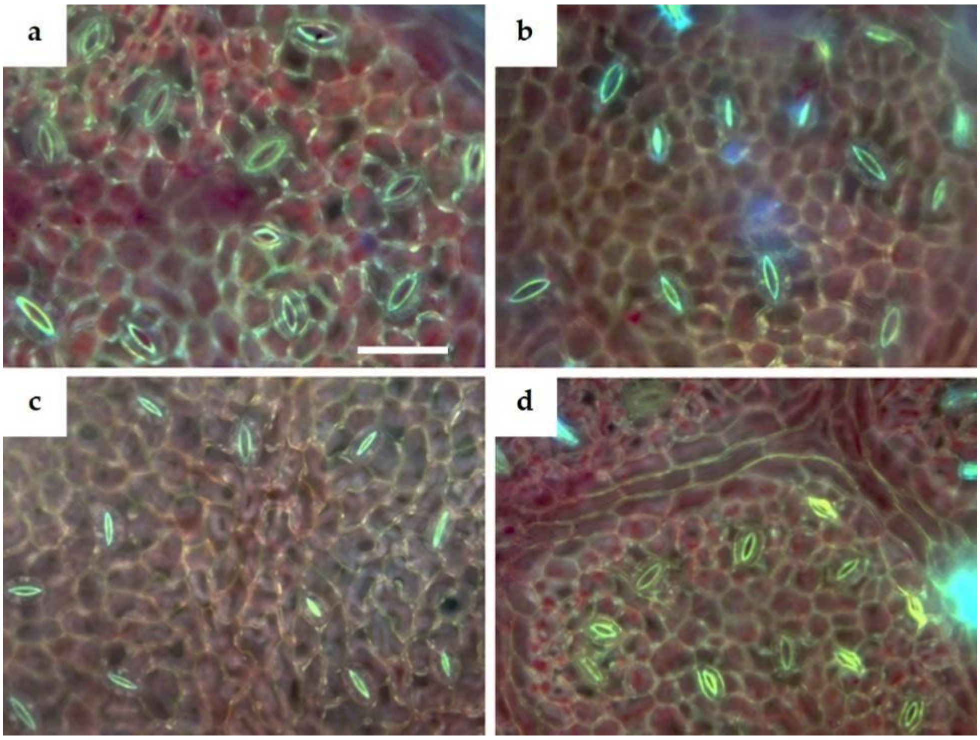

Figure 3. Epi-fluorescence microscopy views of abaxial leaf epidermis of V. vinifera 'Falanghina' vines at the four study sites: SL-Santa Lucia (**a**), CA-Calvese (**b**), GR-Grottole (**c**), AC-Acquafredde (**d**). Images are all at the same magnification. Bar = 50 μm.

Table 3. Effects of field (F), year (Y), and their interaction (F x Y) on stomata traits of V. vinifera subsp. vinifera 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Mean values and standard errors are shown.

	Stomata Length (μm)	**Stomata Width (μm)**	**Stomata Frequency (n/mm^2)**
Field (F)			
SL	33.2 ± 0.43 ^{a}	19.2 ± 0.31 ^{a}	140.3 ± 3.74 ^{a}
CA	29.6 ± 0.34 ^{b}	16.9 ± 0.23 ^{b}	149.2 ± 3.54 ^{a}
GR	27.2 ± 0.55 ^{c}	15.5 ± 0.36 ^{c}	138.6 ± 4.04 ^{a}
AC	24.8 ± 0.38 ^{d}	14.3 ± 0.21 ^{d}	139.9 ± 2.50 ^{a}
Year (Y)			
2019	28.8 ± 0.37 ^{a}	16.1 ± 0.22 ^{b}	151.4 ± 2.56 ^{a}
2020	28.6 ± 0.36 ^{a}	16.8 ± 0.24 ^{a}	132.6 ± 1.88 ^{b}
Significance			
Field (F)	***	***	NS
Year (Y)	NS	*	***
F x Y	NS	NS	*

NS, * and ***, Not significant or significant at $p < 0.05$ and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests ($p \leq 0.05$).

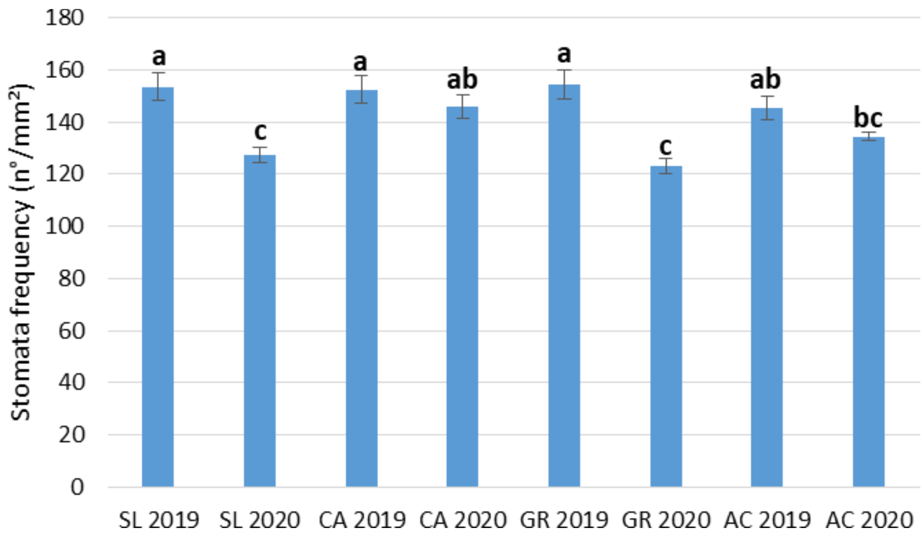

Figure 4. Combined effect of field and year (F x Y) on stomatal frequency of V. vinifera subsp. vinifera 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Mean values and standard errors are shown. Different letters indicate significant differences according to Duncan's multiple range test ($p \leq 0.05$).

Table 4. Effects of field (F), year (Y), and their interaction (F x Y) on vein traits in leaves of V. vinifera 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Mean values and standard errors are shown.

	Minor VLA (mm/mm^2)	Major VLA (mm/mm^2)	Total VLA (mm/mm^2)	Minor VAA (mm^2/mm^2)	Major VAA (mm^2/mm^2)	Total VAA (mm^2/mm^2)	FVEA (n/mm^2)
Field (F)							
SL	2.31 ± 0.06 a	0.729 ± 0.036 a	2.89 ± 0.06 c	0.118 ± 0.004 c	0.065 ± 0.004 a	0.175 ± 0.005 b	2.93 ± 0.11 a
CA	2.59 ± 0.01 a	0.865 ± 0.049 a	3.27 ± 0.08 a	0.132 ± 0.004 b	0.072 ± 0.004 a	0.194 ± 0.005 a	2.47 ± 0.05 c
GR	2.47 ± 0.09 a	0.751 ± 0.054 a	3.04 ± 0.07 bc	0.134 ± 0.006 b	0.072 ± 0.004 a	0.197 ± 0.005 a	2.37 ± 0.10 c
AC	2.58 ± 0.14 a	0.817 ± 0.042 a	3.22 ± 0.12 ab	0.145 ± 0.004 a	0.069 ± 0.003 a	0.194 ± 0.007 a	2.68 ± 0.03 b
Year (Y)							
2019	2.45 ± 0.06 a	0.799 ± 0.028 a	3.07 ± 0.05 a	0.137 ± 0.003 a	0.075 ± 0.002 a	0.202 ± 0.003 a	2.48 ± 0.04 b
2020	2.52 ± 0.09 a	0.781 ± 0.039 a	3.14 ± 0.07 a	0.127 ± 0.004 b	0.064 ± 0.003 b	0.178 ± 0.004 b	2.74 ± 0.06 a
Significance							
Field (F)	NS	NS	*	**	NS	**	***
Year (Y)	NS	NS	NS	*	*	***	**
F x Y	NS	NS	NS	NS	NS	**	**

NS, *, **, and ***, Not significant or significant at $p < 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests ($p \leq 0.05$).

The factor year showed a significant effect on all VAA parameters and FVEA, with values significantly lower and higher in 2020 compared to 2019 for VAA and FVEA, respectively (Table 4). The interaction (F x Y) was significant only for Total VAA and FVEA (Figure 5a,b), with values of Total VAA decreasing from 2019 to 2020 for all the fields but GR. Instead, FVEA showed increasing values from 2019 to 2020 for SL and GR, while no tendency was found in CA and AC.

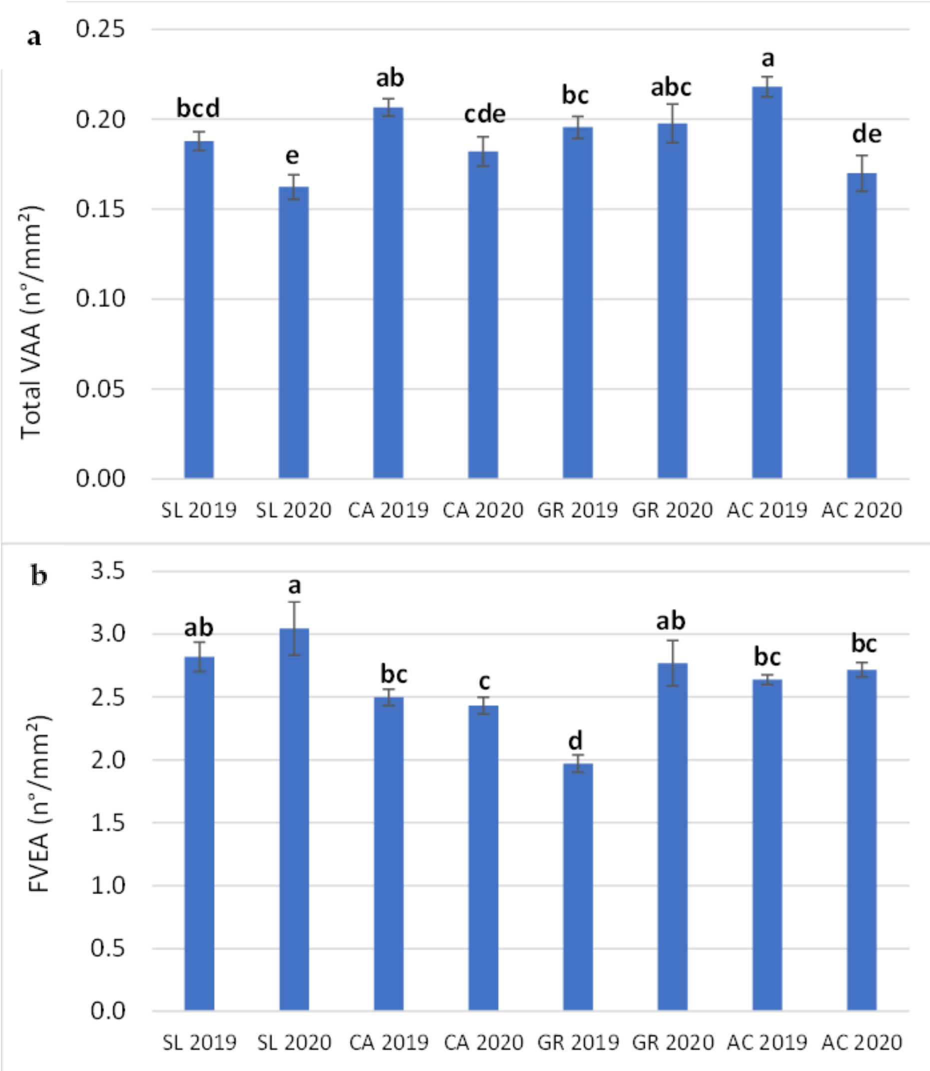

Figure 5. Combined effect of field and year (F x Y) on Total VAA (**a**) and FVEA (**b**) of *V. vinifera* subsp. *vinifera* 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Mean values and standard errors are shown. Different letters indicate significant differences according to Duncan's multiple range test ($p \leq 0.05$).

3. Discussion

This study highlighted how Falanghina grapevine growing under different pedoclimatic conditions develops stomata and vein traits in line with different photosynthetic behavior and productivity. Anatomical and physiological traits varied among sites suggesting a different water use efficiency in the four vineyards in the two analyzed years, likely triggered by different precipitation amounts. In general, in both years, the four vineyards showed two main behaviors regarding the photosynthetic efficiency and biomass production, with SL and AC plants more performant than CA and GR ones. This agrees with a previous study in which $\delta^{13}C$ values of musts were significantly higher in CA and GR vineyards, indicating they were drought-stressed compared to SL and AC ones [33]. The different growth and production performances are likely related to increased water availability due to higher precipitation levels in the case of the SL site and to the application of supplemental irrigation in the AC site, which would have compensated for the scarce amount of precipitation registered in July 2020 compared to the other study sites. The measurement of soil water content at the three soil depths also suggests that vines at SL adopted a strategy to maintain stomata open to sustain high photosynthetic rates, notwithstanding the increasing water losses through transpiration. Such a mechanism suggests that the Falanghina at the SL site would respond to water shortage conditions with slower stomata closure, also in line with the occurrence of larger stomata [24]. The risk for leaf vein embolism deriving from the delayed stomatal closure would also be prevented in vines at the SL site by virtue of the narrower veins (e.g., lower minor VAA at the same minor VLA), which are less prone to drought-induced cavitation [34,35]. Leaf embolism thresholds have been recently explored in grapevine, suggesting an important role of leaf hydraulic traits in coordination with other physiological traits to contribute to vine drought tolerance [36]. In SL vines, the occurrence of higher FVEA, compared to the vines of the other sites, also suggests a more balanced distribution of the hydraulic system across the leaf lamina, which would favor water conductivity across the mesophyll cells despite the lower VLA. High values of FVEA are associated with higher K_{leaf} (leaf hydraulic conductance) and better sugar loading in the cases when they do not correlate positively to VLA and contain phloem [37,38]. On the other hand, the Falanghina vines at the CA and GR sites were characterized by a lower net CO_2 assimilation rate, accompanied by lower stomatal conductance and transpiration rate likely ascribed to a more efficient stomatal control due to prompt stomatal closure allowed by smaller guard cells typical of isohydric behavior. An intermediate behavior would have been assumed by AC vines which, although having anatomical traits expected for an isohydric model, were likely able to maintain stomata open due to supplemental irrigation. The higher FVEA accompanied by high total VLA, in this case, would be associated with high K_{leaf} and would support high photosynthetic efficiency. General trends to increasing VLA are reported according to growing aridity as a strategy to favor more photosynthesis during the moments of high water availability [39–41]. Therefore, vines at the AC site, being characterized by high VLA, high FVEA, and smallest stomata, show traits designed to benefit from supplemental irrigation leading to high photosynthetic activity and yield.

The Falanghina grapevine has been classified as a near-isohydric model, but there is evidence that cultivars classified as near-isohydric are able to change their behavior towards anisohydric status, as in Syrah [42,43]. Our findings in Falanghina suggest that this cultivar is able to acclimate eco-physiological traits by assuming different quantitative leaf stomata and vein traits under different cultivation environments. Indeed, there is evidence that vine eco-physiological behavior is dependent on the water availability in soil and the duration of water shortage [44]. In the four analyzed vineyards, stomata frequency was quite stable and within the range reported for grapevine (50–400 stomata/mm^2) [45]. Furthermore, the observed stability of stomatal frequency across several environments agrees with the general principle by which stomata frequency is considered more an evolutionary adaptation rather than a short-term acclimation mechanism. This statement is also supported by studies reporting that limiting environmental conditions determine a more substantial effect on stomata size than on their frequency [46,47]. The environmental conditions at the early stages of growth in the Pinotage grapevine have been suggested as major determinants in modulating stomata frequency and size with implications on stomatal conductance, which, in turn, affects the whole plant water balance [48]. Indeed, this assumption has also been suggested in other species in which the environmental conditions, especially water availability, during organogenesis have been demonstrated to play a major role in the development of specific quantitative traits (e.g., stomata size and frequency, vein and xylem features) which pose the limits of physiological acclimation [49].

The leaf structure, in terms of stomata size and frequency, may have contributed to the different grapevines' capability to perform photosynthesis in the different vineyards. The higher photosynthetic rate found in SL and AC plants may be due to a higher stomatal conductance and a better PSII photochemical efficiency (i.e., elevated values of ETR, ΦPSII, Fv/Fm). The higher stomatal conductance may allow a better CO_2 supply within substomatal chambers, thus enhancing carbon fixation [50]. Conversely, the photosynthetic activity declined significantly in CA and GR plants due not only to stomatal but also to not-stomatal limitations. Indeed, even if the stomatal closure reduced stomatal conductance (gs) and transpiration (E) in vines growing at CA and GR, substomatal CO_2 concentration remained comparable among plants of different sites suggesting that the utilization of CO_2 at carboxylation sites was somewhat limited [51]. The lower photochemical efficiency of CA and GR plants (i.e., reduced ΦPSII and ETR) may limit the synthesis of ATP and NADPH through the electron transport chain and could explain why the carbon fixation was lower in these plants. Under the particular environmental conditions of the samplingseason, the partial closure of stomata in CA and GR plants may be interpreted as a safetystrategy to avoid an excessive water loss by transpiration, thus preserving the photosyn- thetic apparatus from permanent damages. The low Fv/Fm values of CA and GR, which were the sites with limited precipitation and where gs was lower, compared to SL and AC,where more moisture was available, suggest that Fv/Fm may have responded to a stress condition, supporting the hypothesis that efficient avoiding strategies are needed by plants to overcome the stress [52].

Our data also indicate that the photosynthetic performance significantly depends on the year. The lower precipitation in July 2020 compared to 2019 likely caused the recorded reduction in photosynthetic levels and overall biomass production. The co-occurring decrease in Pn and $_{in}$WUE and increase in Ci indicated that during the second season, in response to more severe stress, non-stomatal limitations occurred that contributed significantly to the reduction in carbon fixation at the carboxylation sites [53].

The overall analysis supports the idea that stomata and vein traits are likely modulated by environmental conditions during leaf development and may severely influence the physiological responses of a grapevine cultivar to short-term changes in water availability. Therefore, such traits should be considered, together with other hydraulic structural and physiological characteristics, in evaluating the drought tolerance of grapevine as also suggested by other authors, who highlighted the importance of integrating multiple traits in grapevine as already accepted for hydraulic traits in other models as forest species [36,54,55]. We suggest that only with a multi-trait approach, including the analysis of structural traits, will it be possible to have a comprehensive understanding of the single cultivar strategies adopted to cope with specific environmental constraining conditions in order to allow site-designed cultivation plans addressing the needs of precision viticulture.

4. Materials and Methods

4.1. Study Area and Vineyard Characteristics

The study area was in southern Italy in the Campania region, Guardia Sanframondi (Benevento, Figure 6), in a hilly environment characterized by a typically Mediterranean climate (cold winters and hot summers). The selected four experimental sites were placed within the vineyards of the La Guardiense farm: 1) SL-Santa Lucia, 41°14′45” N, 14°34′16”, 194 m a.s.l.; 2) CA-Calvese, 41°14′19” N, 14°35′11” E, 163 m a.s.l.; 3) GR-Grottole, 41°14′21” N, 14°34′56” E, 158 m a.s.l.; 4) AC-Acquafredde, 41°13′44” N, 14°35′33” E, 84 m a.s.l. The vine cultivar studied was *Vitis vinifera* L. subsp. v*inifera* ‘Falanghina’ (Controlled designation of origin—DOC/AOC), and the four sites were selected with the criterion to identify four vineyards similar for plant material and cultivation management but different in plant water use due to pedological and microclimatic spatial variability as reported in a previous study [33].

In the four vineyards, the vines, grafted onto 157-11 Couderc rootstock, were 8–13 years old (depending on the vineyard), were spaced 1–1.25 m between plants with 2.1–2.2 m between the rows, and were trained at double Guyot. One shoot trimming was performed after the fruit set phenological phase. The vine rows of GR, CA, and AC sites were oriented E–W, while the SL site is oriented N–S. The SL, GR, and CA vineyards were cultivated in a rain-fed regime, while at AC, supplemental irrigation was applied [33].

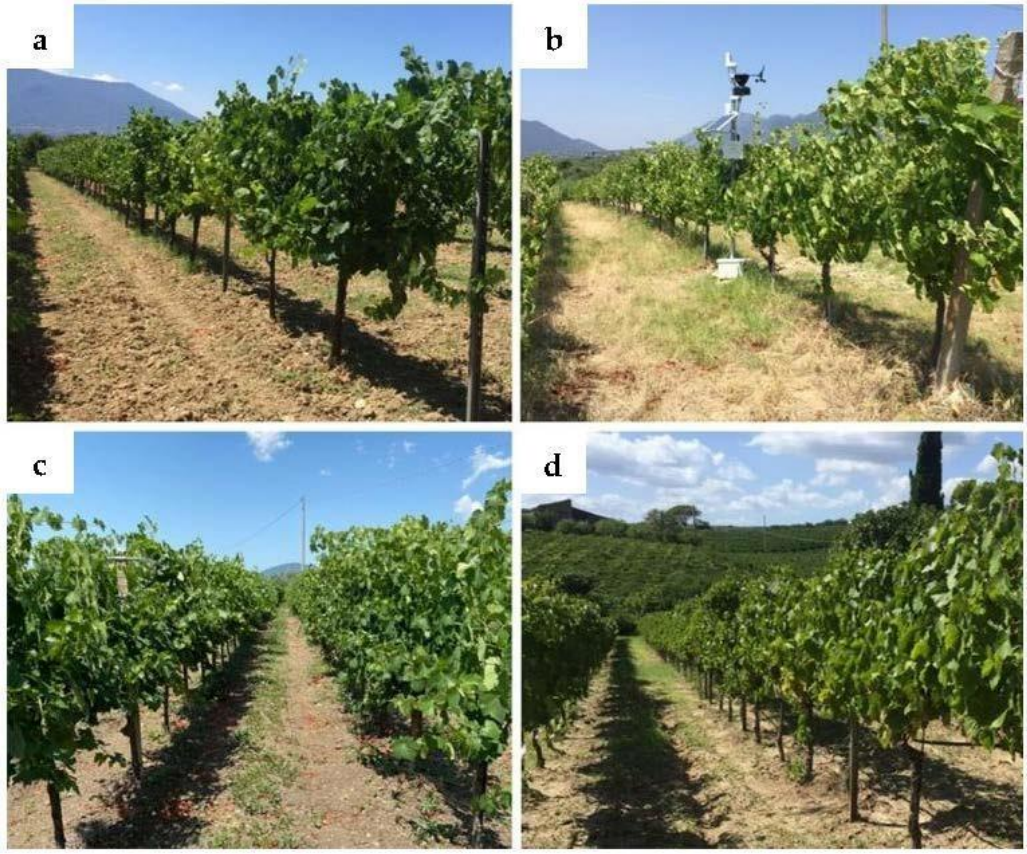

Figure 6. The four experimental sites Santa Lucia (**a**), Calvese (**b**), Grottole (**c**), Acquefredde (**d**) vineyards.

Daily weather information (temperature, rainfall, wind, solar radiation, etc.) was collected during the experiment, in 2019 from the Guardia Sanframondi (BN) weather station (41°14′17.2″ N; 14°35′49.8″ E) of the Campania region weather network, while in 2020 from a weather station dedicated to the experiment, placed in the CA vineyard (Netsens AgriSense IoT weather station, www.netsens.it). The positioning of the Netsens weather station was determined as representative of air temperature, air humidity, wind speed, and solar radiation of all selected vineyards, considering the distance between the experimental vineyards and the landscape form (e.g., slope, aspect, elevation). Moreover, considering that, among the weather variables, rainfall is the one characterized by the highest spatial variability, a rain gauge with three FDR probes (inserted at three different soil depths, 15, 35, and 75 cm) was placed in each experimental site able to measure soil temperature and water content. The FDR probes were applied to better understand the soil water status during the growing season, given that the precipitation amount doesnot represent available water for plants, which depends on the combination of weather conditions and soil properties (e.g., under the same climate, two soils can have a very different water availability for the plant) [56]. The main weather information collected (e.g., temperature, solar radiation) from both weather stations in 2020 were comparable.

The soils present in the experimental sites were Mollisols, classified as Typic Calciustolls and referring to two principal soil series of the soil map of the Valle Telesina area (1:50.000) [57]: Consociazione dei suoli Pennine (SL, CA, and GR sites) and Consociazione dei suoli Taverna Starze (CA site). The soil profile was characterized by Ap and Bw horizons, and the differences between the experimental sites were principally due tothe variability of the percent of stones along the soil profile and by the effect of vineyard planting, which has modified the soil horizons thickness and depth between the sites.

The relations between anatomical and functional leaf traits were analyzed by performing eco-physiological and microscopy analyses on fully expanded leaves at plant maturity over two growing seasons.

4.2. Biometry and Yield

The canopy of 20 vines per vineyard was characterized by performing biometrical and production measurements on 2 annual shoots per plant at the veraison phenological phase corresponding to 81 BBCH (Biologische Bundesantalt, Bundessortenamt, and Chemische Industrie). More specifically, per each shoot, the following parameters were quantified: shoot length, shoot basal diameter, number of leaves, and leaf area. At harvest (89 BBCH), the number of bunches per shoot and bunch weight were determined (weighing all bunches from the same shoots). The estimation of leaf area was performed by applying an allometric estimation model measuring the leaf lamina width in the field and applying the equations calculated based on the measurement of width and area of 20 leaves per site by means of an electronic leaf area meter (LI-3100 model, LI-COR Inc., Lincoln, NE, USA) [13,58,59].

4.3. Gas-Exchange and Chlorophyll a Fluorescence Emission Measurements

Leaf gas exchange and chlorophyll "a" fluorescence emission measurements were carried out on well-exposed and fully expanded leaves, characterized by similar position and exposition within the canopy per 15 plants in each site. The analyses were performed during the veraison phase of the two growing seasons, 2019 and 2020, between 10.00 and 14.00. Net CO_2 assimilation rate (Pn), stomatal conductance (gs), substomatal CO_2 concentration (Ci), and transpiration rate (E) were measured using an airflow rate set to 200 μmol s^{-1}, at ambient CO_2 concentration (about 400 μmol mol^{-1}) and ambient temperature, with a portable infra-red gas-analyzer (LCA 4; ADC, BioScientific, Hoddesdon, UK) equipped with a broad-leaf PLC (cuvette area 6.25 cm^2). The instantaneous water use efficiency ($_{in}$WUE) was calculated as the ratio between Pn and E. The average VPD (vapor pressure deficit) in the leaf chamber, Tch (chamber air temperature), and RH% (relative humidity) were 5.45 kpa, 37.43 °C, and 33.33% for 2019, and 4.97 kPa, 38.76 °C, and 27.38% for 2020. Chlorophyll "a" fluorescence emission was measured using a pulse amplitude modulated portable fluorometer (Plant stress kit ADC Bioscientific Ltd., Hoddesdon, UK). Fluorescence measurements were performed on the same day as gas exchanges on the same leaves. A weak measuring of 3.4 μmol photons m^2 s^{-1} light was used to induce the ground fluorescence signal, F_0, on 30′ dark-adapted leaves. A saturating light pulse of 7.000 μmol photons m^2 s^{-1} was applied to induce the maximal fluorescence level in the dark, Fm, and in the light, Fm'. The maximum PSII photochemical efficiency (Fv/Fm) was calculated as (Fm-F_0)/Fm, and the quantum yield of PSII electron transport rate (ΦPSII) and the electron transport rate (ETR) were estimated following Genty et al. (1989) [60] and Bilger and Björkman (1990) [61]. The measurements in the light were conducted from 12:00 to 14:00 pm under environmental Photosynthetic Photon Flux Density (PPFD) ranging between 1800 and 2300 μmol photons m^2 s^{-1}.

4.4. Microscopy and Digital Image Analysis

At the beginning of veraison, one fully expanded leaf characterized by similar position and exposition within the canopy was collected from the same plants analyzed for eco-physiological measurements in the four vineyards. Directly in the field, the leaf samples, including the main vein, were cropped and chemically fixed in FAA (40% formaldehyde, glacial acetic acid, 50% ethanol, 5:5:90 by volume). To observe the vine leaf traits, the samples were bleached in acetone for 48 h and, when completely clear, the acetone was removed, and leaf samples were rinsed several times with distillate water. For stomatal analysis, a part of each sample was peeled off and mounted on a slide with distilled water. The remaining part of the sample was immersed in ethanol dilutions in water (30%, 50%, 70%, 100%) for 5 min each. Afterward, samples were stained in safranin for 1 min and fast Green for 15 s, rinsed in the decrescent ethanol dilutions until 100% distilled water, and mounted on a slide with distilled water [48]. The samples for stomatal analysis were observed under a BX51 epi-fluorescence microscope (Olympus, Germany) equipped with a Mercury lamp, a 330–385 nm band-pass filter, dichromatic mirror of 400 nm and above, and a barrier filter of 420 nm and above in order to detect the different auto-fluorescence emissions of stomata over the other epidermal structures [62]. For each sample, three fields were observed at 20x magnification (field area 0.237 mm^2), and the stomatal frequency was expressed as the number of stomata per mm^2. Images of the lamina surface from three separate regions were collected by means of a digital camera (EP50, Olympus), taking care to avoid the main veins. The digital images were analyzed with the image analysis software program CellSens 3.2 (Olympus). The size of 10 stomata per field was measured, considering both the guard cell major (pole to pole) and minor axes to calculate the area of an imaginary ellipse. The samples for vein traits analysis were mounted with distilled water on microscope slides that were observed under the BX51 light microscope (Figure 7), and for each sample, three images were collected at 5x magnification and analyzed for digital image analysis, as reported above. For leaf venation analysis, we followed Sack and Scoffoni (2013) [38] but considered the third order veins together with the higher orders vein to avoid bias in measuring because they were often looping and not easily distinguishable from higher orders.

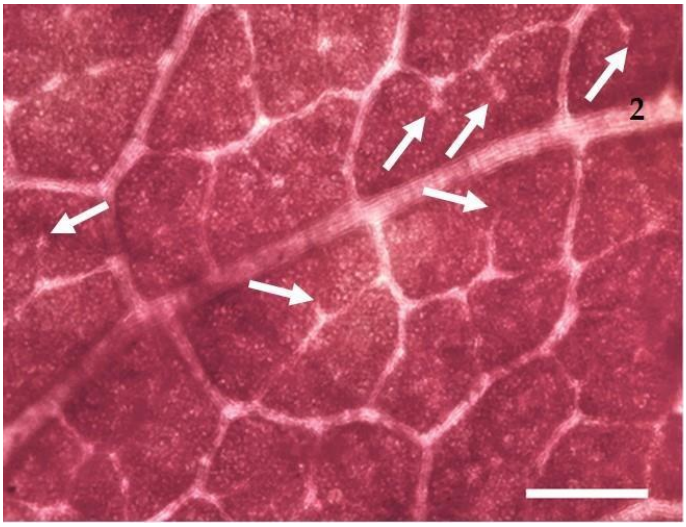

Figure 7. Light microscopy views of V. vinifera 'Falanghina' leaf lamina sample with arrows pointing to the FVEA (2, second-order vein). Bar = 300 μm.

Therefore, the analyzed parameters are as follows:

- minor vein length per unit area (Minor VLA) = sum of vein lengths of third or higher orders of veins divided by the difference between the area imaged and the area occupied by the second-order veins (mm/mm^2);
- major vein length per unit area (Major VLA) = sum of vein lengths of second-order veins divided by the area imaged (mm/mm^2);
- minor vein area per unit area (Minor VAA) = sum of vein areas of third or higher orders of veins divided by the difference between the area imaged and the area occupied by the second-order veins (mm/mm^2);
- major vein area per unit area (Major VAA) = sum of vein areas of second-order veins divided by the area imaged (mm/mm^2);
- total vein length per area (Total VLA) = sum of vein lengths of all order veins divided by the area imaged (mm/mm^2);
- total vein area per unit area (Total VAA) = sum of vein areas of all order veins divided by the area imaged (mm^2/mm^2);

- free vein endings per unit area (FVEA) = number of vein endings divided by the area imaged (n°/mm^2).

4.5. Statistical Analysis of Data

The experimental data were analyzed with the SPSS 27 statistical software (SPSS Inc., Chicago, IL, USA). The data were analyzed by two-way analysis of variance (ANOVA), considering the field (F) and year (Y) as the main factors. Whenever the interactions were significant, a one-way ANOVA was performed. Multiple comparison tests were performed with Duncan's coefficient using $p \leq 0.05$ as the level of probability. The Shapiro–Wilk test was performed to check for normality.

5. Conclusions

The research question we aimed to address was whether leaf anatomical traits related to stomata and veins in Falanghina vines develop differently in a range of field pedoclimatic conditions varying in moisture availability. We further explored the relationship between leaf anatomical traits and leaf gas exchange and photosystem attributes in these environ- ments. At the two sites with relatively low moisture, the photosynthetic rate was lower, as was stomatal conductance, photosystem electron transfer rate, and quantum yield of PSII linear electron transport. Stomata length and width were higher at the site characterized by the highest precipitation. However, stomatal density and most vein traits tended to be relatively stable among sites. Free vein endings per unit leaf area were fewer in the two vineyards with low precipitation. We suggest that the site-specific leaf traits adjustment in Falanghina grapevine, at stomata and veins level, may represent an acclimation strategy that may influence photosynthetic performance. The findings support the hypothesis that stomata and vein traits are likely modulated by environmental, both microclimatic and pedological, conditions during leaf development and may influence the physiological responses of Falanghina grapevine to short-term changes in water availability, supporting the idea that this cultivar may behave as an isohydric or anisohydric model, as previously reported [42].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants11111507/s1, Table S1: Soil water content (SWC) and soil temperature at the four sites.

Author Contributions: Conceptualization, N.D., V.D.M. and C.C.; methodology, C.A., A.B., C.C., V.D.M.; formal analysis, N.D., R.C. and A.E.; investigation, N.D., C.A., C.C. and V.D.M.; resources, C.A., C.C. and V.D.M.; data curation, N.D., C.A., A.B., R.C., A.E., C.C. and V.D.M.; writing—original draft preparation, N.D., C.A. and V.D.M.; writing—review and editing, N.D., C.A., A.B., R.C., A.E., C.C. and V.D.M.; supervision, V.D.M.; project administration, V.D.M.; funding acquisition, V.D.M. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data supporting the findings of this study are available from the corresponding authors (V.D.M. and C.C.) upon reasonable request.

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Appendix

Table S1. Soil water content (SWC) and soil temperature at three different depths (-15, -30 and -75 cm) of four experimental sites (SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde) during the period1 - 31 July 2020. Mean values and standard deviation are reported.

Field	Soil depth (cm)	Soil water content (SWC) (%)	Temperature (T) (°C)
SL	15	21.4±0.47	24.2±1.44
CA	15	45.8±1.99	24.7±0.93
GR	15	24.7±2.63	23.6±1.10
AC	15	25.4±1.84	23.2±0.91
SL	30	21.3±0.57	22.1±0.52
CA	30	60.5±0.96	22.5±0.68
GR	30	34.2±0.99	23.3±0.59
AC	30	34.3±0.79	23.6±0.56
SL	75	32.5±5.48	20.0±0.44
CA	75	40.7±0.22	23.4±0.62
GR	75	45.6±1.34	20.5±0.54
AC	75	28.7±0.83	21.5±0.67

CHAPTER 3

Vine yield and must quality of Falanghina grapevine under different pedoclimatic conditions of southern Italy

This article has been submitted to Horticulturae MDPI

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Falanghina Grapevine (Vitis vinifera) yield and berry quality under different pedoclimatic conditions in southern Italy

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Abstract: Climate is a determinant driver for grapevine geographical distribution influencing yield and berry quality. The current environmental changes are intensifying the need to improve the knowledge of the soil-plant-atmosphere system in the vineyard to properly manage cultivation factors to increase berry yield and quality. Since most of the berry growth and ripening phases occur during the driest period in the Mediterranean area, the increasing environmental constraints are expected to impose more and more limitations to grapevine productivity and finally to wine quality. The aim of this study was to evaluate whether different pedoclimatic conditions in four close vineyards of the Campania Region in Southern Italy determine differences in crop yield and must quality of *Vitis vinifera* L. subsp. *vinifera* 'Falanghina'. This study was conducted over three growing seasons, by monitoring vine growth and characterizing yield and must quality. The overall results showed differences in yield and berry quality characteristics for the four vineyards, with the field CA (Calvese) and GR (Grottole) showing pedoclimatic conditions limiting growth and yield compared to SL (Santa Lucia) and AC (Acquefredde).

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1. Introduction

Climate is a determinant driver for grapevine geographical distribution, berry characteristics, must and wine quality around the world [1]. The Mediterranean region is threatened by climate change, where climate models consistently project significant increase in temperature and high irregularities in precipitation patterns [2]. Since it has been forecasted a dramatic change in the landscape with geographical shifting of the grapevine production regions , climate change is one of the major challenges for future viticulture, especially in arid and semi-arid regions of Europe [3] Often, the combination of heat and severe water-deficit stress may compromise photosynthesis causing source–sink imbalance and incomplete berry maturation, with the consequence of obtaining reduced yield and low quality grapes and musts for vinification. Increase in the frequency, duration and severity of drought events as well as a shift in time of their occurrence will likely induce plastic adaptive responses in plants, expecting a negative impact on plant growth, as the case of grapevine, which is one of the most widespread crops worldwide with about 38% of vineyards areas located in Europe [4,5]. Mild water stress can positively impact berry composition, flavors, and color, while high drought stress affects negatively grapevine production, acting on vine vigor, yield, and berry quality [6]. Water-deficit stress can lead to a reduction in yield also through metabolic pathways modifications, shifting the relative abundance of transcripts and metabolites involved in phenylpropanoid, isoprenoid, carotenoid, amino acid, and fatty acid metabolism [7,8,9]. The temperature variability strongly influences the developmental cycle of plants affecting both carbon assimilation in source organs as well as the activity of carbon sinks. The optimum temperature for photosynthesis is between 25 and 35 °C, while below 10 °C and above 40 °C physiological processes decline **[10]**. Therefore, changes in temperature can promote or inhibit the development and growth rate of flowers, fruits, and shoots according to vine sensitivity, risking to impairing the balance between vegetative growth, reproductive activity and then grapevine quality and wine production [10,11]. The first effect of climate changes is an alteration of the normal course of the phenological phases (e.g. flowering, fruit set, veraison, ripening) which are reached earlier [12,13,14]. As consequence of increasing temperature, grape ripening occurs earlier in the season and in warmer conditions than in the past,. Concerning the grape quality, a common problem in Mediterranean vine- yards is related to the high temperatures influence on the dynamics of soluble solids accumulation in the berries during ripening, with subsequent changes in berry chemical composition resulting in higher sugar content, lower organic acid concentration and higher pH [15,16]. High temperature is known also to decrease the accumulation of anthocyanins in berry skins with effect also on color and aromas [17,18,19, 14]. Moreover, the high temperatures in summer combined to drought stress, create optimal conditions for sunburn damages in sun-exposed grapes, inducing an imbalance between light energy absorption and usage that compromise the electron transport activity. As consequence, fruit respiratory mechanisms are altered and the higher level of anaerobic respiration caused by raising temperatures induces the accumulation of reactive oxygen species. [20]. The level of temperature that berries reach during the day is a function of radiative heat transfer and air temperature [21]. In particular, a direct exposure to the sun increases fruit surface temperature by 12–15°C above air temperature on the berry's sun-exposed side [17].

Like in any agricultural crop, increased water deficit is likely to impact yield and economic sustainability of wine producing estates. In the last 15 years, a decrease in yield has been recorded in most winegrowing regions in France [14]. For this reason, in semi-arid areas, irrigation management is becoming a quite compelling solution to better control grape ripening, mitigating the negative effects of climatic changes. In order to adapt the viticulture to this changing situation, there is a rising interest in designing proper management techniques suitable either to improve the way the vines use water or to introduce irrigation techniques, such as deficit irrigation (DI) at different percentage of the estimated crop evapotranspiration (ETc). However, there are still difficulties in establishing precisely the irrigation strategies (e.g. volumes, timing, etc), in order to stabilize seasonal yield and improve must and wine quality. Indeed, there is still a lack of systematic knowledge about if, how and to what extent the same cultivar in different pedoclimatic contexts can develop different morpho-physiological traits affecting quantity and quality of berries and related musts [22].

Within this general framework, the aim of this work was to analyze the variability in terms of growth, yield and berry/must quality, as well as their relations, in four vineyards of Falanghina grapevine growing in Southern Italy under four pedoclimatic conditions, two more mesic the others more xeric, over three years. The Falanghina grapevine is an autochthonous cultivar of Campania region in southern Italy [23], characterized by middle trunk conical bunch, medium sized grapes, waxy peel and crispy pulp [24,25]. In this cultivar, climate changes are expected to influence quality of musts more than yield, therefore knowing the relations between vegetative growth and berry quality can furnish valuable information for the vineyard management targeted to specific oenological objectives.

2. Materials and Methods

2.1Study site

The selected study area was located at Guardia Sanframondi (Benevento), in a hilly environment in the Campania region (southern Italy) characterized by a Mediterranean climate (cold wet winters and hot dry summers). The selected four vineyards are managed by members of La Guardiense winery are: 1) SL-Santa Lucia, 41° 14' 45" N, 14°34' 16" ,194m a.s.l.); 2) CA-Calvese, 41° 14’ 19 N, 14° 35' 11"E, 163 m a.s.l.); 3) GR-Grottole, 41°14' 21" N, 14°34' 56" E, 158 m a.s.l.); 4) AC-Acquafredde, 41° 13 ' 44"N, 14° 35' 33"E, 84 m a.s.l.). The vine cultivar studied is *Vitis vinifera* L. subsp. vinifera ‘Falanghina’ (Controlled origin designation – DOC/AOC) and the vineyards were selected with the idea to identify four sites similar for plant material and cultivation management, but different in water availability and plant water use [26]. For the three years of study, in each vineyard were closed 20 vines, 8 - 13 years old, grafted onto 157-11 Couderc rootstock, spaced 1-1.25 m on the-row and 2.1-2.2 m between the rows, and trained at double Guyot. The SL, GR and CA vineyards are cultivated in rain-fed regime, while at AC a supplemental irrigation is applied [27]. The soils in the experimental sites are Mollisols type, classified as Typic Calciustolls and assigned to two principal soil series of the Valle Telesina soil map 1:50.000, [28] which are: Consociazione dei suoli Pennine (SL, CA and GR sites) and Consociazione dei suoli Taverna Starze (AC site). The differences between the four sites are principally due to the variability of the percent of stones along the soil profile (Damiano et al. 2022b.) and to the modification in thickness and depth of the soil horizons in the sites, induced by vineyard planting. Moreover, previous studies demonstrated that the four vineyards can be grouped into two groups with SL and AC characterized by higher water availability compared to CA end GR [26]. For the details on climatic data of the four vineyards, refer Damiano et al. [26,27]. Growth and development analyses and analytical determinations on berries and musts were performed in the three growing seasons 2019, 2020 and 2021.

2.1 Vegetative growth and yield components, at harvest

The biometrical parameters total leaf area, bunch weight per vine (yield) and number of bunches per vine (n° bunches) were recorded on 20 vines per vineyard. The total shoot leaf area was estimated during the maximum vegetative vigour corresponding to the veraison phenological phase 81 BBCH (Biologische Bundesantalt, Bundessortenamt and Chemische Industrie). The estimation of leaf area was performed applying an allometric estimation model measuring the leaf lamina width in field and applying the equations calculated based on the measurement of width and area of 20 leaves per site by means of an electronic leaf area meter (LI-3100 model, LI-COR Inc., Lincoln, Nebraska, United States) [29,30]. The yield and n° bunches were analyzed at ripening stage (89 BBCH), weighing and counting the entire grape production for each of the 20 selected vines in the four studied vineyards. The rate yield/total leaf area (Y/LA) was also calculated for each vineyard. ..

2.2 Berry and must quality traits

At harvest, for each vineyard analytical determinations of standard chemical parameters were performed. For pH and titratable acidity (TA), the analyzes were performed on must samples obtained from 6 vines squeezing 60 berries (chosen among the 20 selected vines) and soluble solids content (SSC) was analyzed on 10 berries for each of the 20 selected vines. Berries were sampled picking from the internal to the external part and from the top to the bottom of the bunch harvested to ensure a representative sample. The SSC expressed in °Brix, was determined by a digital refractometer (HI96801, HANNA Instruments Italia Srl, Padua) by squeezing individually the 10 berries on the instruments [31]. For pH and TAthe 6 must samples per vineyards were filtered,20 ml of must were kept and diluted 1:1 in distilled water. Afterwords,pH values were measured using a digital pH meter (CLB22, Crison Instruments, Alella, Barcelona, Spain); whereas for TA determination, samples were titrated with a solution of NaOH 0.1 N up to pH 8.2 and expressed as g/L of tartaric acid equivalents.

Then, 500 ml unfiltered must for 3 vines per vineyard was obtained to perform the analyzes through WineScan™ with a Foss Integrator software (Padova, Italy) and Dyonisos 150 SinaTech (Grottazzolina FM - Italy) to quantify the following parameters: Reducing sugars (RSU), pH, Yeast Assimilable Nitrogen (YAN), Calcium (Ca), Catechins(CAT), Anthocyanin (ANT), Total polyphenols (TPO), Titratable acidity (TA), Volatile acidity (VOL), Malic acid (MAL), Gluconic acid (GLU), Citric acid (CIT), Tartaric acid (TAR).

2.3 Berry Mineral Composition

The analyzes of mineral must composition was performed on 3 samples of must for each vineyard. For the evaluation of mineral must composition in terms of cations (Na^+, K^+, Mg^{2+}, and Ca^{2+}), anions (SO_4^{2-}, PO_4^{3-}) and organic acids (malate, tartrate, citrate, and isocitrate), 1 g of must was suspended in 20 mL of ultrapure water (Milli-Q, Merk Millipore, Darmstadt, Germany), freezed and subjected to 10 min shaking in a water bath (ShakeTemp SW22, Julabo, Seelbach, Germany) at 80 °C. Subsequently, the extracts were centrifuged, and the supernatant was collected and stored in vials. Anions and cations were separated and quantified by ion chromatography equipped with a conductivity detection (ICP 3000 Dionex, Thermo fisher Scientific Inc., MA, United States), according to Zhifeng and Chengguang [32].

2.4 Data elaboration

All experimental data were analyzed with the SPSS 13 statistical software (SPSS Inc., Chicago. IL, United States). A two - way ANOVA) was performed considering the main effects field (F) and year (Y) on data collected for growth parameters, yield, must quality and mineral composition. Whenever the interactions were significant, a one-way ANOVA was performed. To separate means per each measured parameter, the Duncan's multiple range test was performed. The verification of normality was performed through the Shapiro–Wilk test; the percentage data were previously subjected to arcsine transformation. Pearson correlation was performed among biometrical parameters, yield and must quality traits. Asterisks indicate the significance of the Pearson correlation coefficient (*, **, ***,**** correspond to $p < 0.1$, 0.05, 0.02, and 0.01, respectively).

3. Results

3.1 Growth and Berry Quality Traits

Growth parameters (total leaf areayield (bunch weight), bunch number, Y/LA) and berry quality traits (SSC, pH, TA) of the four vineyards, measured during the three growing seasons (2019-2020-2021) are reported in table 1. The main effect of field (F) was significant for all analyzed parameters except for pH and TA; the year (Y) as main factor showed significant effects on all parameters (table 1). In particular, the total shoot leaf area was significantly different among the vineyards with SL showing the highest value followedndby GR, CA and AC.

Yield was significantly different among vineyards with the highest value in SL followed by AC, GR, and CA. Bunch number showed the highest value in SL, followed by GR, AC and CA. The rate Y/LA showed in AC a significant higher value than SL, which in turn was significantly higher than both CA and GR. For SSC, CA grapes showed higher value than GR, while AC grapes showed intermediate values; SL grapes showed the lowest value. Concerning the main factor Y, its effect was significant for all analyzed parameters: for total shoot leaf area, SSC and TA the values in 2019 were significantly higher than 2020, which in turn were significantly higher than 2021. For yield, in 2019 and 2021 the values were similar and were higher than 2020. Bunch number showed the highest value in year 2021, followed by 2019 and 2020. The rate Y/LA showed significant higher values for the year 2021 than both 2019 and 2020. The pH in 2021 was higher than in 2020, while 2019 showed intermediate values. The interaction F x Y was significant for bunch weight, bunch number, Y/LA and SSC for which is showed a table in supplemental materials. Forbunch weight significant highest values were found in SL among the three years and for SSC significant highest values were found in the years 2019 and 2020 for all the filed (TableS1).

Table 1. Effects of field (F), year (Y) and their interaction (F x Y) on total Total leaf area, yield ,Y/LA, bunch number, SSC, pH, TA of *V. vinifera* subsp. *vinifera* 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Ac- quafredde. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

	Total leaf area	Yield	Bunch number	Y/LA	SSC	pH	TA
	m^2 vine^{-1}	*kg vine^{-1}*	*n° vine^{-1}*	*Kg m^{-2}*	*°Brix*		*g l^{-1} tartaric acid equivalent*
Field (F)							
SL	7.87±0.60 a	6.92±0.28 a	23.2±1.20 a	1.12±0.12 b	16.3±0.32 c	3.16±0.076 a	6.26±0.615 a
CA	5.04±0.30 c	2.25±0.15 d	13.8±0.65 d	0.53±0.03 c	19.3±0.45 a	3.30±0.082 a	5.47±0.600 a
GR	6.30±0.46 b	3.65±0.23 c	20.4±0.99 b	0.67±0.09 c	17.8±0.47 b	3.39±0.079 a	5.61±0.572 a
AC	3.79±0.22 d	4.37±0.20 b	17.1±0.81 c	1.40±0.10 a	18.4±0.57 ab	3.22±0.042 a	5.76±0.443 a
Year (Y)							
2019	7.14±0.48 a	4.85±0.27 a	17.7±0.60 b	0.89±0.07 b	20.0±0.40 a	3.26±0.070 ab	7.62±0.603 a
2020	5.49±0.22 b	3.56±0.23 b	14.2±0.59 c	0.66±0.03 b	17.8±0.44 b	3.16±0.033 b	5.63±0.195 b
2021	3.50±0.24 c	4.48±0.29 a	23.9±1.04 a	1.56±0.16 a	16.0±0.32 c	3.38±0.073 a	4.07±0.186 c
Significance							
F	**	***	***	***	***	NS	NS
Y	***	***	***	***	***	*	*
FxY	NS	***	***	***	***	NS	NS

[1]NS, *, **, and ***, Not significant or significant at $p < 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests (p≤0.05).

3.2 Must mineral and organic acids composition

Must mineral and organic acids composition of the four vineyards in the three years (2019-2020-2021) are reported in tables 2 and 3. The main effect of field (F) was significant for all analyzed parameters but Na^+. Considering the main factor F, the values for SO_4^{2-} and Isocitrate concentrations were significantly higher for both must CA and GR, than SL and AC must. For PO_4^{3-} in must of CA, the value was higher than SL, with the musts of AC and GR having intermediate values. For Malate, SL and AC must showed higher values than CA and GR must. For Tartrate and Ca^{2+}, CA grapes showed values significantly higher than GR which in turn showed significantly higher values than SL and AC. For Citrate, GR must showed significantly higher values than all the other fields. For Mg^{2+}, CA must showed significantly higher values than all the other fields. For $K^{+,}$, CA grapes had a higher value than GR and AC, which in turn showed higher values than SL. The main factor year (Y) was significant for all parameters but Na^+ and K^+. For SO_4^{2-} and $Ca^{2+}$the values in 2019 were significantly lower than 2020 and 2021. For PO_4^{3-} and Mg^{2+}, in must of 2020 there was a significant higher value than 2019 and 2021. For Malate in mustof 2019, there was a value significantly higher than 2020 and 2021. For Tartrate in must of2021 there was a value significantly higher in 2021 than the previous years. For Citrate inmust of 2019 and 2020, there were values significantly lower than 2021. For Isocitrate in must of 2020, there is a value higher than 2019, which in turn was higher than in 2021. The interaction F x Y was significant for Tartrate, Citrate, Isocitrate, K^+, Mg^{2+} and Ca^{2+}, with the differences showed in the table of the supplemental materials. For K^+ the significant highest values were found in CA must and the lowest in SL must. Mg^{2+} was significantly higher in must of 2020 than the other two years for all the filed. Ca^{2+} showed the significant highest values in must of CA 2021, CA 2020 and GR 2020. Tartrate showed significant highest values for must CA 2021, CA 2019 and GR 2021. Citrate showed significant highest values in must of GR 2019, GR 2020 and CA 2019. Isocitrate showed the significant highest values for musts of GR 2020 and AC 2020 (Table S2).

Table 2. Effects of field (F), year (Y) and their interaction (F x Y) on must minerals (Na^+, K^+, Mg^{2+}, Ca^{2+}, SO_4^{2-}, PO_4^{3-}) content in *V. vinifera* subsp. *vinifera* 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Different letters within column indicate significant differences according to Duncan's multiple-range test (P _ 0.05) Mean values and standard errors are shown.

	Na^+ (*g kg*$^{-1}$ DW)	K^+ (*g kg*$^{-1}$ DW)	Mg^{2+} (*g kg*$^{-1}$ DW)	Ca^{2+} (*g kg*$^{-1}$ DW)	SO_4^{2-} (*g kg*$^{-1}$ DW)	PO_4^{3-} (*g kg*$^{-1}$ DW)
Field (F)						
SL	0.150 ± 0.024 a	0.97 ± 0.04 c	0.066 ± 0.004 b	0.081 ± 0.003 bc	0.034 ± 0.002 b	0.101 ± 0.007 c
CA	0.091 ± 0.030 a	1.60 ± 0.10 a	0.089 ± 0.005 a	0.128 ± 0.016 a	0.045 ± 0.004 a	0.174 ± 0.012 a
GR	0.106 ± 0.021 a	1.34 ± 0.07 b	0.065 ± 0.006 b	0.086 ± 0.007 b	0.045 ± 0.003 a	0.128 ± 0.009 bc
AC	0.097 ± 0.016 a	1.38 ± 0.04 b	0.068 ± 0.008 b	0.068 ± 0.006 c	0.035 ± 0.003 b	0.156 ± 0.014 ab
Year (Y)						
2019	0.091 ± 0.023 a	1.31 ± 0.09 a	0.064 ± 0.002 b	0.069 ± 0.004 b	0.033 ± 0.002 b	0.140 ± 0.012 ab
2020	0.129 ± 0.019 a	1.31 ± 0.08 a	0.093 ± 0.003 a	0.101 ± 0.005 a	0.042 ± 0.002 a	0.155 ± 0.012 a
2021	0.113 ± 0.020 a	1.35 ± 0.09 a	0.059 ± 0.006 b	0.103 ± 0.015 a	0.044 ± 0.004 a	0.123 ± 0.011 b
Significance[1]						
F	NS	***	***	***	**	***
Y	NS	NS	***	***	**	*
F*Y	NS	**	**	***	NS	NS

[1]NS, *, **, and ***, Not significant or significant at $p < 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests ($p \leq 0.05$).

Table 3. Effects of field (F), year (Y) and their interaction (F x Y) on must organic acids content in *V. vinifera* subsp. *vinifera* 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Different letters within column indicate significant differences according to Duncan's multiple-range test (P _ 0.05) Mean values and standard errors are shown.

	Malate	Tartrate	Citrate	Isocitrate
	($g\ kg^{-1}$ DW)	($g\ kg^{-1}$ DW)	($g\ kg^{-1}$ DW)	($g\ kg^{-1}$ DW)
Field (F)				
SL	2.46 ± 0.37 a	6.64 ± 0.27 c	0.358 ± 0.011 b	0.079 ± 0.011 b
CA	1.59 ± 0.33 b	10.0 ± 1.18 a	0.371 ± 0.028 b	0.098 ± 0.011 a
GR	1.86 ± 0.25 b	7.63 ± 0.43 b	0.458 ± 0.028 a	0.100 ± 0.013 a
AC	2.76 ± 0.22 a	6.48 ± 0.26 c	0.373 ± 0.018 b	0.083 ± 0.012 b
Year (Y)				
2019	3.04 ± 0.24 a	7.18 ± 0.42 b	0.430 ± 0.024 a	0.101 ± 0.007 b
2020	1.83 ± 0.12 b	6.60 ± 0.25 b	0.403 ± 0.022 a	0.119 ± 0.006 a
2021	1.63 ± 0.28 b	9.29 ± 0.94 a	0.338 ± 0.009 b	0.051 ± 0.003 c
Significance[1]				
F	**	***	***	*
Y	***	***	***	***
F*Y	NS	***	**	*

[1]NS, *, **, and ***, Not significant or significant at $p < 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests ($p \leq 0.05$).

3.3 Must chemical analysis

Must chemical analysis of the four vineyards in the three years (2019-2020-2021) is reported in table 3. The main effect of field (F) was significant for all parameters analyzed with the exception of Catechins. For reducing sugars SL and AC showed significant lower values than CA, with GR showing an intermediate value. For pH, the AC must showed a higher value than GR, which in turn had a higher value than SL, with CA showing an intermediate value. For YAN, the musts of SL, CA and AC showed a significant lower values than GR. Calcium content was significantly higher in the must of CA than GR musts, which in turn showed significantly higher values than AC; SL showed intermediate values. Anthocyanin content in the SL must was significantly higher than in CA, GR and AC. Total polyphenols content was significantly lower in the must of SL and GR comparedto CA, with AC must showing intermediate values. The main factor of year (Y) was signif-icant for all the studied parameters but Catechins and Anthocyanins. The Reducing sugarsshowed higher value in 2020 compared to 2019 and 2021. For pH, in 2021 there was a higher value than in 2019, which in turn showed higher value than 2020. For YAN, in 2021there was a significant lower value than 2019 and 2020. For Calcium, in 2020 there was a significant lower value than 2019 and 2021. For total polyphenols, the year 2019 showed asignificant higher value than the subsequent years. The interaction F ×Y was significant forYAN, Calcium, Antocyanins and total Polyphenols, with significant differences showed in the table of supplemental materials. Reducing sugars showed the significant highest val- ues for CA 2020, AC 2020, AC 2021 and the lowest for SL in the three years. The significant highest values of APA were found in GR 2019 and GR 2020. Calcium showed the significant highest value in CA 2019. Anthocyanin showed the significant highest values in SL 2020 and AC 2020. Total polyphenols were significantly higher in 2019 than 2020 and 2021 for all the field (Table S3).

Table 3. Effects of field (F), year (Y) and their interaction (F x Y) on reducing sugars, pH, YAN, calcium, catechins, antocianin, total poliphenols of V. vinifera subsp. vinifera 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Mean values and standard errors are shown.

	Reducing sugars	pH	YAN	Calcium	Catechins	Anthocyanin	Total polyphenols
	g/l		*mg/l*	*mg/l*	*mg/l*	*ppm*	*ppm*
Field (F)							
SL	201 ± 4.90 b	3.11 ± 0.01 c	88.4 ± 15.7 b	58.8 ± 7.49 bc	5.49 ± 1.24 a	69.8 ± 15.4 b	628.5 ± 254.2 b
CA	230 ± 12.07 a	3.14 ± 0.05 bc	81.0 ± 10.2 b	103 ± 16.4 a	8.78 ± 1.61 a	230 ± 41.6 a	1011 ± 233.5 a
GR	213 ± 6.88 ab	3.21 ± 0.04 b	144 ± 22.8 a	66.0 ± 7.02 b	6.98 ± 1.35 a	225 ± 20.8 a	708.2 ± 218.8 b
AC	208 ± 25.47 b	3.32 ± 0.05 a	82.0 ± 13.0 b	46.6 ± 9.01 c	7.47 ± 1.48 a	218 ± 69.1 a	854.4 ± 120.4 ab
Year (Y)							
2019	196 ± 16.08 b	3.14 ± 0.03 b	117 ± 17.5 a	81.5 ± 15.4 a	7.40 ± 1.23 a	160 ± 42.9 a	1467 ± 123.6 a
2020	236 ± 8.60 a	3.32 ± 0.04 c	125 ± 11.3 a	42.4 ± 6.13 b	6.49 ± 1.19 a	207 ± 39.5 a	543.4 ± 130.0 b
2021	207 ± 9.72 b	3.13 ± 0.03 a	53.7 ± 6.39 b	82.3 ± 2.01 a	7.65 ± 1.35 a	190 ± 40.7 a	391.3 ± 105.1 b
Significance[1]							
F	*	***	***	***	NS	**	*
Y	***	***	***	***	NS	NS	***
F*Y	***	NS	*	***	NS	**	***

[1]NS, *, **, and ***, Not significant or significant at $p < 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests ($p \leq 0.05$).

3.4 Must organic acids

The analysis of must organic acids analysis of the four vineyards in the three years (2019-2020-2021) is reported in table 4. The main effect of field (F) was significant for all the parameters with the exception of Gluconic acid. Titratable acidity was significantly higher in must of SL, CA, GR than in AC. Volatile acidity in must of CA showed a higher value than both GR and SL, with must of AC showing intermediate values. Malic acid in must if SL showed a significantly higher value than AC, with must of GR showing an intermediate value ; CA must showed the significant lowest value. For citric acid, the must in CA showed a higher value than SL, GR and AC. For tartaric acid, the must CA showed a higher value than SL and GR, which in turn showed a higher value than must of AC. The effect of the main factor year (Y) was significant for all parameters but Gluconic acid. Titratable acidity in must of 2020 showed a significantly lower value than 2019 and 2021. The volatile acidity in must of 2020 showed a significantly higher value than 2019, which in turn was higher than in must of 2021. Malic acid was significantly higher in must of 2019 than in 2020 and 2021. Citric acid showed a significant lower value in must of 2021 compared to 2019 and 2020. Tartaric acid showed a significant lower value in must of 2020 compared to the other two years. The interaction F × Y was significant for all analyzed parameters, but Gluconic acid. . The interaction effects are shown in the table of supplemental materials. Titratable acidity showed the significantly highest values for SL 2019, CA 2019 and GR 2019. Volatile acidity showed values significantly highest in CA 2020 and AC 2020. Malic acid content was significantly highest in SL 2019, GR 2019 and AC 2021, wheras Citric acid only in CA for all the three years of study. Tartaric acid showed significant highest values for CA 2019, CA 2021 and GR 2019 (Table S4).

Table 4. Effects of field (F), year (Y) and their interaction (F x Y) on titratable acidity, volatile acidity, malic acid, gluconic acid, citric acid, tartaric acid of *V. vinifera* subsp. *vinifera* 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Mean values and standard errors are shown.

	Titratable acidity	Volatile acidity	Malic acid	Gluconic acid	Citric acid	Tartaric acid
	g/l	*g/l*	*g/l*	*g/l*	*g/l*	*g/l*

Field (F)						
SL	6.80 ± 0.44 a	0.067 ± 0.012 b	2.81 ± 0.36 a	0.350 ± 0.033 a	0.290 ± 0.023 b	5.09 ± 0.24 b
CA	6.56 ± 0.49 a	0.091 ± 0.018 a	1.93 ± 0.13 c	0.462 ± 0.088 a	0.396 ± 0.017 a	6.06 ± 0.56 a
GR	6.34 ± 0.37 a	0.064 ± 0.111 b	2.53 ± 0.29 ab	0.281 ± 0.058 a	0.285 ± 0.042 b	5.38 ± 0.26 b
AC	4.64 ± 0.44 b	0.082 ± 0.022 ab	2.33 ± 0.23 b	0.408 ± 0.111 a	0.161 ± 0.054 b	3.22 ± 0.17 c
Year (Y)						
2019	6.88 ± 0.58 a	0.233 ± 0.004 b	2.98 ± 0.30 a	0.436 ± 0.079 a	0.340 ± 0.037 a	5.47 ± 0.52 a
2020	5.12 ± 0.27 b	0.127 ± 0.009 a	1.97 ± 0.11 b	0.348 ± 0.067 a	0.360 ± 0.026 a	4.07 ± 0.20 b
2021	6.26 ± 0.24 a	0.078 ± 0.007 c	2.23 ± 0.18 b	0.343 ± 0.059 a	0.276 ± 0.036 b	5.28 ± 0.38 a
Significance[1]						
F	***	*	***	NS	***	***
Y	***	***	***	NS	**	***
F*Y	***	*	***	NS	***	**

[1]NS, *, **, and ***, Not significant or significant at p < 0.05, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests (p≤0.05).

A correlation analysis was carried out to highlight the possible relationships among biometrical, yield berry and must quality traits for each season of trial (table5). Concerning the data collected in the season 2019, a significant negative correlation was observed between bunch weight and SSC, pH and Anthocyanin, whereas a significant positive correlation was found between SSC and pH. The pH and TA (as well as the content of organic acids) were positively correlated with Total polyphenols. In 2020, bunch weight was significantly positively correlated with total SLA. SSC was positively correlated with pH, Citric Acid, Total Polyphenols and negatively correlated with TA, Malic Acid and Tartaric Acid. The parameter pH was significantly correlated with total polyphenols. In 2021, bunch weight was negatively correlated respectively with SSC and Citric Acid. SSC was positively correlated respectively with total polyphenols and organic acids with the exclusion of tartaric acid.

Table 5. Correlations among the main analyzed parameaters during the three year study.

Year 2019				Year 2020				Year 2021			
		r	**s**			**r**	**s**			**r**	**s**
BW	*TLA*	-0.111	NS	*BW*	*TLA*	0.585	****	*BW*	*TLA*	0.054	NS
BW	*SSC*	-0.749	****	*BW*	*SSC*	-0.198	NS	*BW*	*SSC*	-0.615	****
BW	*pH*	-0.551	****	*BW*	*pH*	-0.475	**	*BW*	*pH*	0.226	NS
BW	*GLU*	-0.421	NS	*BW*	*GLU*	-0.112	NS	*BW*	*GLU*	-0.051	NS
BW	*CIT*	-0.359	NS	*BW*	*CIT*	0.001	NS	*BW*	*CIT*	-0.650	***
BW	*ANT*	-0.555	**	*BW*	*ANT*	-0.427	NS	*BW*	*ANT*	-0.216	NS
SSC	*pH*	0.546	****	*SSC*	*pH*	0.620	****	*SSC*	*pH*	-0.252	NS
SSC	*TA*	0.180	NS	*SSC*	*TA*	-0.368	*	*SSC*	*TA*	0.327	NS
SSC	*MAL*	-0.386	NS	*SSC*	*MAL*	-0.497	*	*SSC*	*MAL*	0.642	***
SSC	*GLU*	0.390	NS	*SSC*	*GLU*	0.206	NS	*SSC*	*GLU*	0.688	****
SSC	*CIT*	0.373	NS	*SSC*	*CIT*	0.718	****	*SSC*	*CIT*	0.860	****
SSC	*TAR*	0.394	NS	*SSC*	*TAR*	-0.683	****	*SSC*	*TAR*	-0.374	NS
SSC	*TPO*	0.320	NS	*SSC*	*TPO*	0.864	****	*SSC*	*TPO*	0.791	****

pH	*MAL*	-0.017	NS	*pH*	*MAL*	0.197	**	*pH*	*MAL*	-0.064	NS
pH	*GLU*	-0.010	NS	*pH*	*GLU*	-0.188	**	*pH*	*GLU*	-0.209	NS
pH	*CIT*	0.464	*	*pH*	*CIT*	0.600	**	*pH*	*CIT*	0.090	NS
pH	*TAR*	0.607	**	*pH*	*TAR*	-0.644	***	*pH*	*TAR*	-0.239	NS
pH	*TPO*	0.450	*	*pH*	*TPO*	0.628	***	*pH*	*TPO*	0.024	NS
TA	*CIT*	0.672	****	*TA*	*CIT*	-0.102	NS	*TA*	*CIT*	0.150	NS
TA	*TAR*	0.686	****	*TA*	*TAR*	0.255	NS	*TA*	*TAR*	0.718	****
TA	*TPO*	0.760	****	*TA*	*TPO*	-0.329	NS	*TA*	*TPO*	-0.253	NS
MAL	*GLU*	-0.021	NS	*MAL*	*GLU*	-0.267	NS	*MAL*	*GLU*	0.888	****
MAL	*CIT*	0.551	**	*MAL*	*CIT*	-0.001	NS	*MAL*	*CIT*	0.494	*
MAL	*TAR*	0.386	NS	*MAL*	*TAR*	-0.030	NS	*MAL*	*TAR*	-0.718	****
MAL	*TPO*	0.429	NS	*MAL*	*TPO*	-0.301	NS	*MAL*	*TPO*	0.572	**
GLU	*CIT*	0.257	NS	*GLU*	*CIT*	0.432	NS	*GLU*	*CIT*	0.596	**
GLU	*TAR*	0.374	NS	*GLU*	*TAR*	0.028	NS	*GLU*	*TAR*	-0.579	**
GLU	*TPO*	0.561	**	*GLU*	*TPO*	0.391	NS	*GLU*	*TPO*	0.534	**
CIT	*TAR*	0.943	****	*CIT*	*TAR*	-0.658	***	*CIT*	*TAR*	-0.118	NS
CIT	*TPO*	0.946	****	*CIT*	*TPO*	0.851	****	*CIT*	*TPO*	0.741	****
TAR	*TPO*	0.942	****	*TAR*	*TPO*	-0.582	**	*TAR*	*TPO*	-0.338	NS

[1]NS,*, **, ***,****: Non-significant or significant at $P \leq 0.1$, 0.05, 0.02, 0.01, respectively.

4. Discussion

Falanghina grapevine is one of the most economically important and cultivated varieties of the Campania region [23]. Since climate changes are expected to affect vines growth and quality of the production, the variability of growth and must quality traits was investigated in vineyards characterized by different microclimatic and pedological characteristics. Vine growth, yield and grape quality in the four analyzed experimental vineyards were characterized by significant differences suggesting different regulation of the source-sink balance according to the different microclimatic and pedological conditions which can differentially influence water availability for the vines [26]. In general, along the three years the SL vineyard showed to be the more performant in terms of vegetative growth compared to the other four vineyards, in agreement with the morphophysiological and carbon isotopic analyses performed in the 2019-2020 season of the same vineyards [26,27]. Indeed, in a previous study Damiano et al. [26] found that SL and AC showed the lower values of $\delta^{13}C$ in must indicating that the two vineyards experienced reduced stress compared to the other two vineyards CA and GR. Plants grown under water-limited conditions, are known to experience a strong stomatal regulation, which leads to partial or total stomatal closure determining a decrease in $^{13}CO_2$ discrimination and an increase of $\delta^{13}C$ values [33]. In SL the high vegetative vigor was also accompanied by the highest shoot leaf area. In CA the lowest values of both parameters showing how different level of biomass accumulation during the three years of growing are strictly related to the specific area of the vineyards. On the contrary, the grape of vineyard CA showed significant higher value of SSC, suggesting that the more limiting drought condition experienced by vines at this site may have significantly accelerated the ripening dynamics. Probably, as reported in [34] the period after veraison is the period for which the thermal conditions have a more significant effect on the technical ripening process. Usually in drought conditions there is a common reaction of the plants which consist in the closure of leaf stomata in order to avoid an excess of water loss and the increase of soluble solid concentration in the berries but probably when the drought is strong this mechanism it is not enough to avoid a concentration effect of soluble solids. Grape berry water loss in ripening stage, known as berry dehydration, is an irreversible regression process in berry fresh weight accelerated by hot and dry growing conditions, which occurs as a consequence of berry water depletion through xylem back-flow and transpiration that exceeds the import of water and solutes into the berry [35,36]. Another indicator of the advanced metabolic process in the ripening stage of the CA vineyard is the level of malic acid in grape, significantly lower than in grapes of the other three vineyards. Malic acid breakdown is not an intrinsic part of the veraison program because external parameters like temperature and internals like carbon balance could be determinants of malate breakdown. Normally, malic acid is the main organic acid that is actively metabolised throughout ripening of grapes and the degradation of grape berry malate occurs after an earlier period of accumulation [16] compared to tartaric acid that is less dependent by increasing temperature [37]. Under high-temperature regimes, malate content decreases as soon as sugar accumulates in the berry. On the contrary in cool conditions, the malic acid decreasing is only evident when sugar content has reached 500 mM [38]. Observing the interaction among the analyzed parameters, a negative correlation for Bunch Weight-Brix° and Bunch Weight-Anthocyanin was observed along the three years confirming the dilution effect of the grape chemical compounds with the increase in bunch weight. Concerning the yield of grape production observing the bunches weight, in CA there is the lower significant value in accordance with the significant lower values of leaf area. The leaf area is crucial for carbohydrates allocation in reproductive organs of grapevine for reaching and maintaining high productivity in terms of fruit yield [39,40]. For this reason CA may have a reduced yield compared to the other three vineyards, while in SL vineyards the high shoot leaf area have been able to hold higher yield.

About the mineral nutrition, the vines need an adequate supply of macro- and micronutrients in order to achieve their normal physiological and biochemical function. Basic mineral nutrients are considered to be essential for plant metabolic processes seeing that are cofactors and/or activators of many metabolic enzymes [41]. The nutrients are required for vine life cycle from budburst to leaf senescence, and generally they limit grape production [42]. Excessive nutrient supply and deficiencies can both lead to physiological disorders. Deficiencies occur when plants cannot reach sufficient availability of nutrients for their basic metabolism in the surrounding environment, while the abundance of minerals, in particular trace metals (e.g. zinc, copper, manganese) can induce sometimes toxicity phenomena [43]. In the case of CA a significant higher level of the cations K^+, Mg^{2+}, Ca^{2+} and anions SO_4^{2-}, PO_4^{3-} was found compared to the other three vineyards. A high level of salt in soils generate both ionic and osmotic disruption in plant function [44,45]. The osmotic effect with a similar symptoms of water deficit [45] characterized by low water potential in the soils due to the increased salt concentration, could be responsible for the low vigor of the vines in these vineyards. This reduction of soil water potential restrains water uptake into the plant, decreasing growth and nutrient uptake consequently [46]. Another indicator of the drought stress conditions in the vineyards CA is the high content of Total polyphenols in the berries (table 3). Usually, water stress cause relevant losses of berry weight affecting also the concentration of several polyphenols [47]. Because of the drought stress probably in CA plant may have reacted with polyphenols accumulation, being them part of the chemical resources mediating the adaptive response to abiotic stress, by acting against oxidative damages through the scavenging Reactive Oxygen Species produced during drought stress [48]. The YAN was significantly higher in GR compared to the other three vineyards indicating an high presence of nitrogen in the must of this vineyard.

Observing the table 5 with the correlations among biometrical, berry yield and must quality traits for each season, with bunch weight tending to increase there is a decreasing of SSC and pH respectively, showing a negative correlation between the parameters due both to a wll known crop load effect and a dilution effect. This negative correlation is significant for the year 2019 and 2021 that are also the years with the significant high bunch weight compared to the year 2020.

The optimal grape maturity is cultivar specific and defined by a specific combination of three main factors: i) technological maturity (i.e., sugar, acids or their ratio); ii) phenolic maturity (i.e., quantity and quality of all tannins and pigments); iii) aromatic ripeness (i.e., typical olfactory features reached without appearance of untypical aging or excessive veggie-green aromas). The decoupling between the above three factors is strongly aggravated under a global warming scenario [49] Higher temperatures increase the speed of sugar accumulation, hasten acid degradation, alter flavor compounds [7, 50, 51,] and affect the synthesis/degradation of certain compounds as polyphenols and anthocyanins [17, 52, 53,54,55].

In conclusion, the overall results showed differences in growth, yield, and must quality characteristics for the four vineyards, with the field CA and GR showing pedoclimatic conditions constraining vegetative growth and berry production, compared to SL and AC. These findings are in line with data from previous studies in which a different leaf anatomical development was evidenced in the four vineyards, being the reason for different eco-physiological behavior. Therefore, it is clear that microclimatic and spatial variability in soil water availability can prime a different vine development and eco-physiological behavior that is reflected in yield and berry quality and must be taken into account when designing strategies for Falanghina cultivation management in the sight of precision viticulture in a climate change scenario.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1, Data of interaction analysis F x Y for bunch weight and SSC; Table S2, Data of interaction analysis F x Y for Reducing sugar, APA, Calcium, Antocianin, Total poliphenols; Table S3, Data of interaction analysis F x Y for K^+, Mg^{2+}, Ca^{2+}, Tartrate, Citrate, Isocitrate; Table S4, Data of interaction analysis F x Y for Titratable acidity, Volatile acidity, Malic acid, Gluconic acid, Citric acid, Tartaric acid.

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Appendix

Table S1. Data of interaction analysis F x Y for bunch weight and SSC. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

	Total leaf area	Bunch number	Y/LA	Yield	SSC
	m² vine⁻¹	*n° bunches vine⁻¹*	*Kg m-2*	*kg bunch⁻¹*	*°Brix*
Field (F)					
SL 2019	10.31±1.15 a	21.5±1.47 c	0.83±0.10 d	7.32±0.55 a	17.6 ± 0.64 bcd
SL 2020	7.45±0.45 bc	15.6±0.99 d	0.82±0.06 d	5.91±0.45 b	16.1 ± 0.62 cde
SL 2021	3.81±0.52 ef	32.5±1.60 a	2.31±0.37 a	7.52±0.39 a	15.2±0.26 e
CA 2019	6.38±0.51 cd	16.1±0.93 d	0.47±0.04 d	2.84±0.23 efg	22.3±0.72 a
CA 2020	4.64±0.31 de	11.0±1.02 e	0.58±0.55 d	2.62±0.28 fg	18.5±0.88 b
CA 2021	3.21±0.27 ef	14.4±1.13 de	0.54±0.08 d	1.29±0.12 h	17.1±0.55 bcde
GR 2019	8.51±0.86 b	17.2±1.11 d	0.51±0.07 d	3.78±0.36 de	22.1±0.71 a
GR 2020	4.98±0.32 de	16.8±1.25 d	0.47±0.05 d	2.2±0.22 gh	15.4±0.96 de
GR 2021	4.51±0.53 de	27.3±1.61 b	1.38±0.33 c	4.99±0.33 bc	15.9±0.32 cde
AC 2019	3.35±0.22 ef	16.1±0.85 d	1.72±0.11 bc	5.5±0.33 b	18.1±0.93 bc
AC 2020	4.89±0.37 de	13.4±1.13 de	0.78±0.07 d	3.49±0.3 def	21.4±0.81 a
AC 2021	2.48±0.30 ef	21.7±1.46 c	2.01±0.20 ab	4.12±0.26 cd	15.6±1.10 de
Significance[1]					
F*Y	***	***	***	***	***

[1]NS.*. **. ***: Non-significant or significant at P ≤ 0.05. 0.01. 0.005. respectively.

Table S2. Data of interaction analysis F x Y for Reducing sugar, APA, Calcium, Antocianin, Total poliphenols. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

	Reducing sugar	YAN	Calcium	Anthocyanin	Total polyphenols
	g/l	*mg/l*	*mg/l*	*ppm*	*ppm*
Interaction FxY					
SL 2019	197 ± 5.24 cd	118 ± 11.0 bc	62.0 ± 0.38 bcd	7.99 ± 3.34 ab	1639 ± 51.57 a
SL 2020	212 ± 6.83 cd	119 ± 8.95 bc	30.5 ± 5.22 d	3.22 ± 0.53 b	69.00 ± 32.72 c
SL 2021	194 ± 10.6 d	28.0 ± 4.58 f	84.1 ± 1.61b	5.28 ± 1.13 ab	177.0 ± 45.72 c
CA 2019	247 ± 3.05 ab	105 ± 2.91 cd	162.5 ± 18.99 a	11.15 ± 2.01 ab	1849 ± 29.33 a
CA 2020	255 ± 13.1 a	95.0 ± 7.21 cde	60.9 ± 8.72 bcd	6.32 ± 0.85 ab	833.3 ± 204.6 b
CA 2021	187 ± 13.2 d	43.3 ± 9.68 ef	86.7 ± 1.76 b	8.86 ± 4.53 ab	351.3 ± 165.7 c
GR 2019	231 ± 6.99 abc	190 ± 44.5 a	66.5 ± 9.61 bc	6.74 ± 1.54 ab	1560 ± 94.19 a
GR 2020	218 ± 2.52 bcd	169 ± 15.9 ab	44.7 ± 7.33 cd	4.91 ± 1.34 ab	402.0 ± 74.59 bc
GR 2021	189 ± 7.69 d	71.7 ± 0.88 cdef	86.9 ± 1.11 b	9.31 ± 3.57 ab	162.7 ± 42.27 c
AC 2019	110 ± 8.82 e	56.7 ± 3.18 def	34.8 ± 11.12 d	3.74 ± 1.20 ab	819.7 ± 148.9 b
AC 2020	257 ± 23.6 a	118 ± 30.6 bc	33.7 ± 19.62 d	11.51 ± 3.05 a	869.3 ± 324.7 b
AC 2021	257 ± 7.94 a	71.7 ± 8.65 cdef	71.5 ± 1.54 bc	7.15 ± 0.56 ab	874.3 ± 212.8 b
Significance[1]					
F*Y	***	*	***	**	***

[1]NS.*. **. ***: Non-significant or significant at P ≤ 0.05. 0.01. 0.005. respectively.

Table S3. Data of interaction analysis F x Y for K^+, Mg^{2+}, Ca^{2+}, Tartrate, Citrate, Isocitrate. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

Must minerals	K^+	Mg^{2+}	Ca^{2+}	Tartrate	Citrate	Isocitrate
	(g kg^{-1} DW*)*	*(g kg*$^{-1}$ DW*)*	*(g kg*$^{-1}$ DW*)*	*(g kg*$^{-1}$ DW*)*	*(g kg*$^{-1}$ DW*)*	*(g kg*$^{-1}$ DW*)*
Interaction FxY						
SL 2019	0.94 ± 0.04 e	0.063 ± 0.004 ef	0.075 ± 0.003 def	6.36 ± 0.13 d	0.075 ± 0.003 cd	0.096 ± 0.008 cd
SL 2020	0.99 ± 0.05 de	0.081 ± 0.003 cd	0.089 ± 0.003 cd	6.24 ± 0.14 d	0.089 ± 0.003 cd	0.101 ± 0.016 bcd
SL 2021	0.98 ± 0.13 de	0.054 ± 0.001 fgh	0.079 ± 0.005 cdef	7.32 ± 0.69 cd	0.079 ± 0.005 d	0.041 ± 0.003 f
CA 2019	1.78 ± 0.08 a	0.072 ± 0.005 de	0.078 ± 0.013 cdef	9.20 ± 0.87 b	0.077 ± 0.013 ab	0.127 ± 0.013 ab
CA 2020	1.27 ± 0.12 c	0.105 ± 0.004 a	0.124 ± 0.006 b	6.62 ± 0.34 d	0.124 ± 0.006 d	0.107 ± 0.009 bc
CA 2021	1.76 ± 0.03 ab	0.088 ± 0.004 bc	0.183 ± 0.004 a	14.24 ± 0.85 a	0.183 ± 0.004 d	0.06 ± 0.004 ef
GR 2019	1.22 ± 0.05 cd	0.058 ± 0.001 fg	0.067 ± 0.001 def	6.70 ± 0.53 cd	0.067 ± 0.001 a	0.106 ± 0.016 bc
GR 2020	1.47 ± 0.17 c	0.089 ± 0.002 bc	0.104 ± 0.005 bc	7.63 ± 0.36 bcd	0.104 ± 0.005 ab	0.141 ± 0.002 a
GR 2021	1.33 ± 0.12 c	0.048 ± 0.005 gh	0.088 ± 0.017 cde	8.55 ± 0.99 bc	0.088 ± 0.017 cd	0.054 ± 0.004 ef
AC 2019	1.31 ± 0.01 c	0.063 ± 0.003 ef	0.058 ± 0.008 f	6.46 ± 0.10 d	0.058 ± 0.008 cd	0.076 ± 0.006 de
AC 2020	1.51± 0.09 bc	0.098 ± 0.008 ab	0.086 ± 0.006 cde	5.93 ± 0.58 d	0.086 ± 0.006 bc	0.126 ± 0.007 ab
AC 2021	1.34 ± 0.04 c	0.044 ± 0.001 h	0.061 ± 0.011 ef	7.05 ± 0.40 cd	0.061 ± 0.011 d	0.047 ± 0.002 f
Significance[1]						
F*Y	**	**	***	***	**	*

[1]NS.*. **. ***: Non-significant or significant at P ≤ 0.05. 0.01. 0.005. respectively.

Table S4. Data of interaction analysis F x Y for Titratable acidity, Volatile acidity, Malic acid, Gluconic acid, Citric acid, Tartaric acid. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

	Titratable acidity	Volatile acidity	Malic acid	Gluconic acid	Citric acid	Tartaric acid
	g/l	*g/l*	*g/l*	*g/l*	*g/l*	*g/l*
Interaction FxY						
SL 2019	8.39 ± 0.41 a	0.030± 0.006 de	4.18± 0.29 a	0.353 ± 0.046 ab	0.373 ± 0.003 abc	5.85 ± 0.14 bcd
SL 2020	5.85 ± 0.31 cd	0.106± 0.009 b	2.03± 0.29 c	0.327 ± 0.072 ab	0.310 ± 0.032 bc	4.40 ± 0.10 efg
SL 2021	6.15 ± 0.32 cd	0.063± 0.009 cd	2.21± 0.07 c	0.370 ± 0.07 ab	0.187 ± 0.026 d	5.01 ± 0.39 def
CA 2019	7.91 ± 0.11 ab	0.033± 0.003 de	2.34± 0.21 c	0.617 ± 0.035 a	0.433 ± 0.007 a	7.23 ± 0.34 a
CA 2020	4.97 ± 0.44 de	0.147± 0.02 a	1.76± 0.17 c	0.553 ± 0.202 ab	0.4 ± 0.037 ab	4.10 ± 0.39 fgh
CA 2021	6.80 ± 0.69 bc	0.093± 0.019 bc	1.69± 0.04 c	0.217 ± 0.064 ab	0.354 ± 0.018 abc	6.84 ± 0.75 ab
GR 2019	7.55 ± 0.37 ab	0.027± 0.003 e	3.6± 0.33a b	0.453 ± 0.062 ab	0.420 ± 0.006 a	6.09 ± 0.36 bc
GR 2020	5.62 ± 0.55 cd	0.103± 0.003 b	2.12± 0.21 c	0.22 ± 0.114 ab	0.297 ± 0.030 c	4.64 ± 0.16 efg
GR 2021	5.86 ± 0.36 cd	0.063± 0.003 cd	1.88± 0.08 c	0.170 ± 0.032 b	0.140 ± 0.015 d	5.41 ± 0.38 cde
AC 2019	3.67 ± 0.12 f	0.003± 0.003 e	1.83± 0.16 c	0.320 ± 0.32 ab	0.133 ± 0.030 d	2.70 ± 0.04 i
AC 2020	4.04 ± 0.21 ef	0.150± 0.015 a	2.010.25 c	0.290 ± 0.091 ab	0.433 ± 0.067 a	3.13 ± 0.06 hi
AC 2021	6.22 ± 0.58 cd	0.093± 0.012 bc	3.160.23 b	0.613 ± 0.08 a	0.423 ± 0.015 a	3.84 ± 0.11 gh
Significance[1]						
F*Y	***	*	***	**	***	**

[1]NS,*, **, ***: Non-significant or significant at P ≤ 0.05, 0.01, 0.005, respectively.

CHAPTER 4

Retrospective reconstruction of the ecophysiological grapevine behaviour through the analysis of tree-ring series to validate an approach to extract data from space-born and UAV techniques

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Retrospective reconstruction of the eco-physiological grapevine behaviour through the analysis of tree-ring series to validate an approach to extract data from space-born and UAV techniques

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***Abstract*—Climate change is intensifying the need to improve the use efficiency of farm resources and to increase crop yield and quality, especially in high profitability crops as grapevine. The achievement of these challenges requires the realization of a continuous crop monitoring in the field to identify and forecast possible anomalies in plant growth and health status due to short- and long-term environmental constrictions (e.g. climate change). Several indicators are currently used to evaluate plant growth, based on *in situ* data collection or remote sensing. In this study, we propose a multi-scale approach to assess and interpret plant growth indicators in vineyard systems. We not only monitor plant growth and eco-physiology *in-vivo* during cultivation, but also reconstruct past eco-physiological behavior by transferring the approach of dendro-sciences, typical of the forest science domain, to viticulture. More specifically, we extract anatomical and stable isotope traits (linked with hydraulic and resource efficiency parameters) from tree-ring series to evaluate plant plasticity to past fluctuations in environmental conditions and to changes in the vineyard management. We also check whether the reconstructed eco-physiological behavior corresponds with the indexes obtained from the retrospective analysis of space-borne and UAVs data.**

Keywords*—*ecophysiology, functional plant traits, precision agriculture, Sentinel-2A, stable isotopes, tree rings, UAV, wood anatomy

I. Introduction

Climate change is one of the major challenges for agriculture and forestry since it is expected to drastically modify plant growth, with possible negative effects especially in arid and semi-arid regions of Europe. The Mediterranean region is a biodiversity and a climatic change hotspot [1], where climate models consistently project significant increase in temperature and high irregularities in precipitation patterns [2]. Such conditions will be responsible for an increase in the frequency, duration and severity of drought events as well as a shift in time of their occurrence [3].

These variations in climatic conditions will likely induce plastic adaptive responses in plants, thus affecting growth and productivity of agricultural and forestry systems, and ultimately biogeochemical cycles [4, 5]. Increasing temperature will have also effects on soil nutrient availability with direct impact on soil organic matter and nitrogen cycle. These effects will depend on interactions and processes involving the whole soil-plant-atmosphere *continuum*, where changes in temperature and precipitation can have additive effects or may work in opposite directions for plant nutrition, with a direct effect on soil carbon stock. Therefore, a correct evaluation of possible plant responses to environmental changes cannot be realized without taking into account the soil characteristics and their effects on the processes involved in the *continuum* soil-plant-atmosphere (SPA system).

Several studies focused on the effects of climate change on forest and the related vegetation dynamics [6]. Within the forest science domain, there is consolidated awareness of the need for the application of multiple proxies to understand the mechanisms leading to worrying phenomena such as die-back [7, 8]. Concomitantly, there is increasing awareness of the impact that climate change can have on the distribution, productivity and quality of the most important crops as it is happening in the case of grapevine, which is one of the most widespread crops worldwide (with about 38% of vineyards areas located in Europe) [9, 10]. Indeed, it has been forecasted a dramatic change in the landscape (expansion or contraction of the wine regions as well as geographical shifting) for grapevine production in Europe, especially in seasonally dry zones [11].

Within this framework, we propose a multi-scale and multi-disciplinary approach to assess and interpret indicators of plant growth and health in vineyard systems. Apart from the application of *in-vivo* plant growth monitoring and eco-physiology assessment, we propose to transfer dendro-sciences (denro-ecology, -anatomy and -isotopes) tools from forestry to viticulture in order to reconstruct past vine behavior after fluctuations in environmental conditions and changes in the cultivation management.

Indeed, being photosynthesis considered the main driver for plant growth, studies aiming to assess the effect of stressors on plant growth performance and health are based on the evaluation of the photosynthetic performance. The growth efficiency of plants can be monitored *in-vivo* by measuring gas-exchange and chlorophyll-a fluorescence at the leaf level. Changes in photosynthesis and in heat dissipation induced by environmental stressors result in the fluctuation of the chlorophyll fluorescence, which is an indicator of photosynthetic efficiency. Under non-stressful conditions, the light energy absorbed by chlorophyll is mostly converted into photochemical energy, but still a fraction is dissipated as heat or reemitted as fluorescence. Moreover, biomass accumulation, fruit set and final yield are also evaluated in different phenological phases. As regards, retrospective analyses, information on past growth performance of plants, can be “read” in wood by analyzing tree rings. Anatomical and isotopic signals in grapevine as in every woody species, are a synthesis of the various environmental factors and inter-connected processes occurring during plant growth [12, 13]. The combined action of environmental and cultivation factors determines the unique structure of a tree ring formed at a specific time and position within plant architecture. Wood adaptive traits are connected to plant physiological status at the time of wood formation, and linking them with past climatic conditions provides information on species vulnerability to predicted climate changes, thus being useful for forest and crop management.

The *in-situ* health monitoring analyses are generally limited to sample points or small areas and are time-consuming. To overcome these limitations, remote sensing technologies can be applied since they offer means to assess indicators in an effective, repetitive and comparative way that support long-term monitoring and provide valuable information about environmental changes and trends. One of the main advantages of implementing remote sensing techniques for plant growth and health assessment, is the possibility of repeatedly acquiring standardized information over large areas, at low costs, with a high temporal coverage [14].

In this study, traits measured *in-vivo* are analyzed together with traits linked with plant hydraulic behavior, water status and resource use efficiency which can be derived from tree-ring series. The information on plant growth and health status, derived from the overall data, is compared with indexes obtained from space-borne and UAVs data.

II. Experimental Design

A. Study sites

The study area is located in a hilly environment in southern Italy (Guardia Sanframondi, Benevento, Campania region) (Fig. 1a). The experimental sites were selected within the vineyards of the La Guardiense farm, as follows: 1) SL-Santa Lucia (lat: 41.246357; lon: 14.570825,194 m a.s.l.); GR-Grottole (lat: 41.240120; lon: 14.584056, 158 m a.s.l.); CA-Calvese (lat: 41.237675; lon: 14.587291, 163 m a.s.l.); AC-Acquafredde (lat: 41.229231 lon: 14.592362, 84 m a.s.l.) (Fig. 1b).

Fig. 1 Location of the study area in southern Italy (a) and of the four study sites (b). Source: Google Earth Pro.

The general idea was to select vineyards as much as possible similar for plant material and cultivation techniques, apart from water availability. Therefore, in the four vineyards, the same cultivar *Vitis vinifera* L. subsp. v*inifera* ‘Falanghina’ (Controlled designation of origin – DOC/AOC), is grafted on the same rootstock (157-11 Couderc) and vines are characterized by similar age, training system and pruning management (double arched Guyot system for the GR, CA and SL sites, while single arched Guyot system for AC). In all the vineyards, vines are spaced by about 2.2 × 1 mspacing (≈ 4545 vines/ha), and E-W row orientation for sitesGR, CA, AC, while N-S for the SL site. However, the soil type and management are different at the four sites leading todifferent conditions of water availability.

B. *In-vivo plant analyses*

Vine growth is monitored in the main phenological phases in terms of morphological development and eco-physiological parameters.

Morphology and yield. At each site, 2 shoots per 20 plants are selected and monitored in terms of biometrical and production parameters including: shoot length, number of leaves and anticipated branches per shoot, leaf area, number of leaves per anticipated branch, number of bunches per plant, fruit set percentage, and other morphological parameters.

In order to minimize the number of leaves to be sampled for the measurement of the leaf area, an allometric estimation model is applied to estimate leaf area from the lamina width [15].

The fruit set percentage is calculated as the ratio between the number of berries per bunch and the number of flower buds per inflorescence. In the case of the estimation of the number of flowers / berries per cluster, in order to reduce production losses, we proceed by applying digital image analysis methods. In particular, regression curves are constructed between the number of manually counted flowers / berries on 10 bunches collected per each site (on plants not subject to growth monitoring) and the number of flowers / berries counted by digital images of the sameinflorescences / clusters sampled. The obtained equations allow calculating the number of real flowers / berries startingfrom the flowers / berries estimated by image analysis.

Eco-physiology. At each site, at least 20 healthy leaves, fully expanded and characterized by similar position and exposition within the canopy, are analyzed for gas-exchanges and chlorophyll "*a"* fluorescence emission. Gas-exchanges measurements include net photosynthesis (Pn), stomatal conductance (gs), transpiration (E); they are carried out between 10.00 and 14.00 through a portable IRGA system (LCA4, ADC Bioscientific Ltd, Hoddesdon, UK), equipped with a leaf chamber of 6.25 cm^2 (PLC 2). Chlorophyll "*a"* fluorescence emission measurements are aimed to calculate parameters such as the maximum quantum yield of PSII photochemistry (F_v/F_m), the quantum yield of PSII electron transport (Φ_{PSII}) and the photochemical (q_P) and non-photochemical quenching (q_N); they are carried out between 10.00 and 14.00 as well, through a portable fluorometer (Plant Stress Kit, Opti-Sciences, Hudson, NH, USA).

C. *Retrospective analyses through tree-ring series*

Past eco-physiological behavior of vines is reconstructed by analyzing tree-ring series. Common dendro-ecological techniques to build tree-ring chronologies are applied. Core sampling is carried out by means of a small increment borer (diameter 5 mm) at a 30 cm height. Wood cores are seasoned in a fresh-air dry store and sanded with different grain size paper. Semi-thin (15-20 μm) cross sections from each core are obtained through the sliding microtome and observed under an epi-fluorescence microscope (BX60 Olympus, Germany) with settings to detect the autofluorescence of lignified cell walls [16, 17]. Digital images of the sections are collected with a digital camera (XC50, Olympus) and the microphotographs are analyzed with software programs to quantify wood anatomical traits. Measured parameters include: ring width, vessel frequency, vessel lumen area, hydraulic diameter (Hd) and potential hydraulic conductivity (Kh). The C stable isotope composition is also measured by continuous-flow isotope ratio mass spectrometry to calculate the intrinsic water use efficiency (WUEi) per each ring or pools of rings.

D. *UAV imagery*

UAV images across VIS-NIR-SWIR bands have been collected to set up the convolutional neural network (CNN) for pan-sharpening of Sentinel-2A images [18]. Among numerus spectral vegetation indices (VIs), the following are calculated: NDVI (normalized difference vegetation index), GNDVI (green NDVI) [18], RENDVI (red-edge NDVI), and NDII (normalized difference infrared index).

III. Results and Discussion

This paper reports the first results of the monitoring of vine growth and health, with different approaches, at four experimental sites, useful to set and apply the convolutional neural network (CNN) as reported in Brook et al. (2019) [18].

In this specific study case, the four vineyards, although being characterized by the same cultivar and rootstock, similar age, training system, pruning and cultivation management, showed different sensitivity to the climatic constraints of the growth season. The analyzed vineyards showed different plant vigor, as evidenced by both morphological and physiological measurements *in-vivo* and UAV images (examples of data analyzed are in Fig. 2, 3). More specifically, vines at the SL and AC sites were characterized by higher vegetative vigor (average NDVI in September was: 0.9±0.04). Instead, vines at GR and CA (average NDVI in September was: 0.8±0.1) sites showed stress signs at the leaf level, culminating in earlier senescence compared with vines from the other two sites.

At harvest, collected data on vineyard production highlighted that the average bunch size, expressed in grams, is significantly higher at SL and AC (342 g per bunch) than in the two more stressed vineyards (CA and GR) (Fig. 4a). However, the highest total yield/ha was recorded at SL that, despite a similar vine density and average bunch size compared to AC, produced a 33% more than the AC vineyard (Fig. 4b). On the contrary, yield harvested at the CA and GR sites appeared strongly influenced by the generallower health status of the vines, resulting reduced on average by 50% compared to the most productive SL and AC (Fig. 4b). Further information will be available soon, including eco-physiological and vine fertility data, allowing to better explain the recorded differences in yield performances at the four sites. Moreover, the obtained results will allow to set up the CNN.

It is important to highlight that a multi-scale approach is an accurate and efficient way to assess and interpret growth and health indicators of crop systems. The analysis of plant systems performance at large spatial scale and continuously over a period of time is challenging and many parameters can be evaluated as indicators of growth efficiency at different scale levels (from single plants up to large populations), following different consolidated techniques. Most *in-vivo* techniques are time consuming and focused on very precise measurements on a small scale.

On the other hand, by selecting appropriate parameters of remote sensing tools, it is possible to obtain indicators of the physiological status of the plant system, provided that a set-up and calibration of the different systems is performed to avoid bias due to inter-site and inter-species variability [19]. However, integration of information as a whole is still missing, and ground data are needed for calibration and validation of indicators derived from remote sensing. The application of dendro-sciences methods has proven to be useful in previous studies for such a calibration, because they allow extracting series of eco-physiological data from tree-ring series [18, 20, 21].

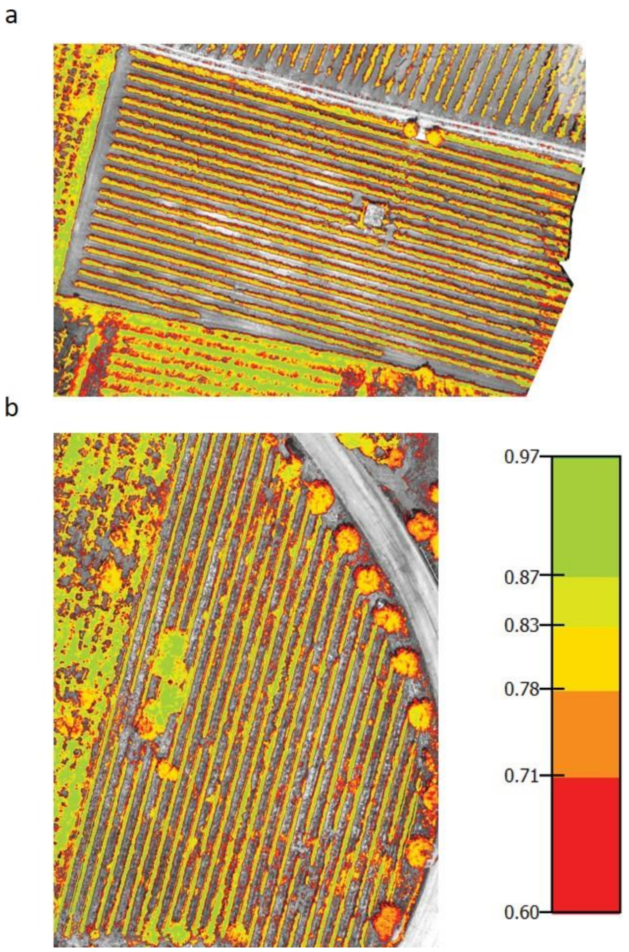

Fig. 3 Normalized Difference Vegetation Index (NDVI) of 26/06/2019 at SL (a) and GR (b) vineyards.

Specific anatomical traits (e.g. conduit size and cell wall thickness) as well as stable isotopes composition are linked with plant physiological status during xylogenesis and especially with water use and photosynthetic performance [20]. Therefore, they are indicators of adaptive capability of plants under conditions of limited water availability. The analysis of wood anatomical traits is efficiently combined with the analysis of carbon and oxygen isotopes in plant tissues, especially in tree-rings also to estimate WUEi and carbon use efficiency [22, 23].

The tree-ring series of the vines have been analyzed, identifying and dating the rings. The parameters derived by vessel lumen area and frequency suggested that the four vineyards have different hydraulic conductivity in the different years at different sites, likely due to different pedoclimatic conditions.

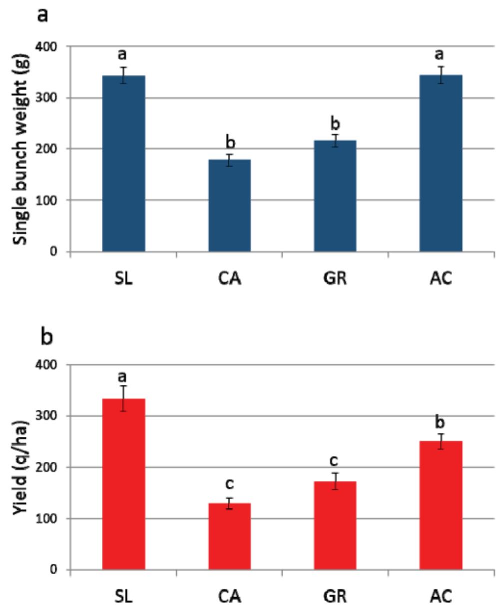

Fig. 3 Average weight of single bunch (a) and total yield per unit area (b)recorded in the four vineyards

In conclusion, the first results of the reported study casess confirmed the value of both *in-vivo* measurements and retrospective analyses in grapevine because they allow the assessment of current status and the reconstruction of past eco-physiological behavior, in agreement with indexes obtained from UAVs data. Moreover, they furnish fundamental information to validate and apply CNN approach to monitoring, with high spatial resolution, the plant health status with Sentinel 2 images.

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CHAPTER 5

Stable isotopes application to evaluate plant water status, in viticulture

1. Introduction

From an etymological point of view, the word isotope, has a Greek origin, were *iso* means "same" and *topos, means "*place", and refers to chemical elements which are located in the same position of the periodic table. Isotopes of an element have in common the same number of protons and therefore the atomic number, but their physical properties are not the same because of different atomic mass, as the number of neutrons in their nuclei is different. Isotopes are usually classified into two groups, namely stable and radioactive (i.e. unstable). Although radioactive isotopes are atoms that, at predictable and measurable rates, disintegrate to other isotopes forms by emitting a nuclear electron and radiation, they maintain a constant concentration on Earth over time. In environmental sciences, the stable isotopes most frequently considered are those included in the elements C, O, H and N (Marshall et al. 2007). The relevance of these isotopes depends on their abundance on the Earth's surface and their implications in natural biological processes (Adams and Grierson 2001). Instead, stable isotopes of heavier elements, such as B, S, Sr and Mg, are less used in plant research. Since the isotopic differences among materials are small, the isotopic composition needs to be reported with reference to international accepted standards, usually expressed as per mill or parts per thousand deviation from that standard according to the formula:

$$\delta(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

Rsample and *Rstandard* are the heavy-to-light isotope ratio of the sample and the standard, respectively. More details on the isotopic composition of the international standards can be found in Sulzman (2007). Isotopic composition is sometimes expressed by the Δ value as well, defined as:

$$\Delta a{-}b\ (\%) = \delta\ a - \delta\ b$$

where *a* and *b* are the reactant and the product of a process, respectively. Isotope ratio mass spectrometry (IRMS) is the technique typically used to separate charged atoms or molecules on the basis of their mass-to charge ratio. Two basic types of IRMS, are used: continuous flow (CF-IRMS) and dual inlet (DI-IRMS). Usually, DI system is more precise when samples are analysed, whereas CF systems allow to introduce multiple component samples and obtain isotopic information for individual elements or compounds.

In this chapter, the attention is concentrated on the applications of the analysis of stable isotopes in viticulture by presenting the elements from the most to the least studied. Then, a focus is done on a study case aimed to analyse the variability of $\delta^{13}C$ in different organs of grapevine.

2. Carbon isotopes

Carbon of plant tissues comes from CO_2 molecules of the atmosphere, fixed through the photosynthesis. In nature, there are two stable carbon isotopes, ^{12}C and ^{13}C, representing respectively 98.93% and 1.07% (Hoefs et al. 2009). The carbon isotope discrimination in plant tissues of these two isotopes depends on two processes: the capacity of the ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) to fix the CO_2 and the diffusion rate of $^{13}CO_2$ across the stomatal pore and boundary layer, that is lower than $^{12}CO_2$ (Farquhar et al. 1989). The different amount of carbon isotopes in the plants dry matter is the results of a multiple effect between photosynthetic rate and stomatal aperture during the growth period. The closure of stomata reduces the kinetic preference and ^{13}C /^{12}C ratio increases in the substomatal chamber: as a consequence, Rubisco reduces the discrimination between the two isotopes and the primary photosynthetic products are enriched in ^{13}C (Farquhar et al. 1984). Therefore, the carbon isotope ratio in plant tissues reflects the plant water status; a higher quantity of $\delta^{13}C$ in tissues of plants growing under conditions of water deficit is frequently observed, compared to non-water stressed plants. The analysis of $\delta^{13}C$ in grapevine is more and more applied since it reflects the effect of the plant water status on photosynthesis throughout the growing and reproductive season (Gaudillere et al. 2002, De Souza et al. 2003, Santesteban et al.2011, Brillante et al. 2018). Several organs have been used to analyse $\delta^{13}C$ in grapevine as indicators of plant water status, including main shoot leaves, lateral shoot leaves (De Souza et Al. 2005, Bchir et al. 2016), whole berries (Santesteban et al. 2013), parts of berries (seed, skins and pulp) (De Souza et al. 2005), must (Gomez Alonso et al. 2010), sugars extracted from berries (Gaudilierre et al. 2002, Brillante et al. 2018) and wine (ethanol) (Costantini et al. 2010, Guyon et al: 2006). The relations among water potentials, photosynthesis and $\delta^{13}C$ have been investigated to gain insight on water useefficiency in different grapevine cultivars, under several environmental conditions and cultivation frameworks. In

De Souza et al. 2005, the researchers evaluated the deficit irrigation effect on the intrinsic WUE and carbon isotope composition (^{13}C) of Castelao and Moscatel grapevine cultivars growing in a vineyard in Portugal. In this two cultivars, $\delta^{13}C$ of the pulp showed the best associationwith the integrated intrinsic water use efficiency throughout the growing season (measured as net CO_2 assimilation rate (A) divided by stomatal conductance) since the determination coefficient was higher (R^2=0.74) compared with that calculated with $\delta^{13}C$ of whole grapes (R^2=0.62) or leaves (R^2=0.17). Therefore, the latter matrix is reported as the least representative organ, probably due to their early formation during the season before the occurrence of any significant water deficit (De Souza et al. 2003, De Souza et al. 2005, Bchir et al. 2016). Concerning water potential used as a water stress indicator, in Gaudillerre et al. (2002) a Merlot grapevine cultivar of Bordeaux (France), sampled on three different soils (a trial of sandy, clay and stony soil) showed a high linear relationship ($P<0.001$) between minimum predawn leaf water potential (Ψpd) and must sugars $\delta^{13}C$. Santesteban et al. (2012), in grapevine cultivar Tempranillo of Navarra (Spain) foundsignificant relationships between stem water potential and $\delta^{13}C$ of whole berries. In addition, the berry $\delta^{13}C$ was shown to depend more on vine water status during the ripening period than on water status during berry herbaceous development (Santesteban et al. 2012). Brillante et al. 2018 showed that sugar berry $\delta^{13}C$ of Chardonnay grapevine cultivated in Burgundy (France) was related to the minimum predawn leaf water potential (Ψpd) and confirmed such a relation as a good integrator of grapevine water status . However, a comparison among published works showed that the relationship between $\delta^{13}C$ and the predawn leaf water potential (Ψpd) is not stable. The slope of the relationshipbetween $\delta^{13}C$ and minimum predawn leaf water potential (Ψpd) was stable, while the intercept and then the values of $\delta^{13}C$ at equivalent minimum predawn leaf water potential (Ψpd), vary between studies. The relation could be affected by varieties and grape-growing regions, therefore more studies in future should be performed to understand the variation (Brillante et al. 2018).

3. Oxygen Isotope

The oxygen in nature has three stable isotopes, ^{16}O , ^{17}O and ^{18}O , with the abundance level of 99.757%, 0.018% and 0.205% respectively (Hoefs et al. 2009). In nature, most of the plant oxygen comes from soil water, and during the adsorption of soil water and xylem vessels transport, no significant isotopic fractionation occurs. Therefore, the ratio of different oxygen isotopes in plants, depend on water source and heavy isotopic enrichment at the leaf level (Marshall et al.2007). The plant water source is meteoric water (rainfall, rivers, lakes and glaciers), which is isotopically depleted compared to ocean water. When water evaporates from the ocean surface, the ^{16}O of the H_2O has a higher vapour pressure than ^{18}O and a depletion occurs in the vapour. Such depletion becomes smaller with the increasing of temperature. In the same way, when rainfall occur, heavier water is more abundant in the raindrops and evaporation process occurs either from surface of falling drops implying ^{18}O enrichment. Instead, the remaining water vapour in clouds become depleted in ^{18}O (Hoefs et al.2009).

$\delta^{18}O$ of water present in deeper soil layers is related to the average $\delta^{18}O$ of the rainfall at the specific site across the year, instead a variation of the correlation occurs in upper layers, because of the effect of any rainfall event on water composition and the consequent evaporation enrichment. In any evaporation process heavier molecules tend to be left behind (Farquhar et al. 2007) and also in leaves the transpiration process implies an isotopic enrichment of the heavier ^{18}O molecules in the leaf water. The isotope composition of atmospheric water on the leaf to air vapour pressure difference (VPD) affects the degree of isotopic enrichment in the plant. The higher the VPD was on leaf, the higher was the heavy isotope enrichment of leaf water. At a constant VPD, the observed leaf water isotopic composition was relatively enriched in heavy isotopes when exposed to atmospheric water vapour that had a large heavy isotope content and relatively depleted in heavy isotopes when exposed to atmospheric water vapour that had a low heavy isotope content (Flanagan et al. 1991). In grapevine,most of the studies on oxygen isotope are made on must (Ingraham and Caldwell 1999) . In Gomez- Alonso et al. (2010), the $^{18}O/^{16}O$ isotopic ratios was quantified for grape juices of La Mancha (Spain) on four indigenous grapevines [Airén, Macabeo (white grapes), Tempranillo and Garnacha (red grapes)], and four foreign [Chardonnay, Sauvignon Blanc (white grapes), Cabernet Sauvignon and Merlot (red grape)]. $\delta^{18}O$ resulted different between indigenous and foreign grapes, with values usually higher in foreign than in indigenous Spanish varieties. This suggests some physiological differences that lead to a different isotopic discrimination in the indigenous and non-indigenous varieties. The uptake of water by the roots of the vine and the subsequent transport of water to the leaves are not associated with any significant isotope fractionation, which takes place mainly in the leaves and also in the grape skin because of evaporation and the exchange with atmospheric vapour (Martin et al.1988, Tardaguila et al. 1997).

Then, there are some studies on the isotopic composition of the leaf water (Ingraham and Caldwell 1999), petioles and shoots (Dunbar et al.1983). The values of $\delta^{18}O$ obtained from leaves show substantial changes throughout the day with an isotopic enrichment during the part of the day when transpiration takes place. For leaves, $\delta^{18}O$ values have been reported to change from 2 to 15.4‰ within a day in a single vineyard (Förstel and Hützen 1984). Instead, no significant change has been reported at a daily scale either for petioles or shoots or berries (Förstel and Hützen 1984). The isotopic ratio $^{18}O/^{16}O$ of water in wine is used also to gain information on irrigation management, even if little is known about how the $\delta^{18}O$ isotopic ratio is affected by irrigation and the potential of its use as an indicator of water supplement (International Organisation of Vine and Wine 2006). More recently, Raco et al. 2015 performed analysis on wine with the idea that the content of $\delta^{18}O$ in the water of vegetative organs, grapes and wine, may reflect the geographical origin from where the wine is originated. The analysis were performed on Merlot, Sangiovese and Cabernet cultivated in Gavoranno and Grosseto (Italy). The impact of the same climatic conditions during the ripening and harvesting of grapes of different varieties influenced differently the isotopic values of water in wines: $\delta^{18}O$ Cabernet > $\delta^{18}O$ Merlot > $\delta^{18}O$ Sangiovese likely because of their different ripening period. Indeed, grape varieties which mature early (i.e. Cabernet, Merlot) usually lead to higher $\delta^{18}O$ values of the wine water, as compared to late ripening grape varieties (i.e. Sangiovese).

The analysis of $\delta^{18}O$ is often conducted together with $\delta^{13}C$ to get more precise information of plant water use in the so-called dual isotope approach. Indeed, $\delta^{13}C$ of plants is a good indicator of WUEi, which is given by the ratio between leaf net photosynthetic rate (A) and stomatal conductance (gs), while $\delta^{18}O$, being inversely related to the ratio of atmospheric to leaf intercellular water vapour pressure, is indicator of gs. Therefore, measuring plant $\delta^{18}O$ can help separate the independent effectsof A and gs on $\delta^{13}C$ (Scheidegger et al. 2000). In grapevine, it has been recently applied in several red and white grape varieties (Aligote, Rkatsiteli, Sauvignon Zeleny, Chardonnay, Cabernet Sauvignon, Sauvignon Blanc, Merlot, Risling, Pinot Noir, Cabernet Franc, Sira, Krasnostop), indicating their inter-cultivar, spatial and inter-annual variability in Crimea peninsula and South West Coast of the Greater Caucasus (Kolesnov et al. 2019). However, limitations dual-isotope approach model have been identified in several species due to the model assumption (Roden and Farquhar, 2012) and further studies are needed to verify its efficacy in grapevine.

4. Hydrogen isotope

Hydrogen has two stable isotopes in nature, ^{1}H and ^{2}H (or D, for deuterium) and their relative abundance is 99.9885% and 0.0115% respectively (Hoefs et al. 2009). There is also a third naturally occurring but radioactive isotope, ^{3}H (tritium), with a half-life of approximately 12.5 years. Considering that hydrogen is always present in terrestrial environments, the H/D ratios is relevant for plant physiology and it is the element with the largest mass difference in relative terms between its two stable isotopes (Hoefs et al. 2009).

The applications regarding the analysis of hydrogen isotopic values in plants are similar to those of the oxygen. Therefore, the differences observed for δD in the water of plant tissues are caused by different source water (depleted in D with regards to ocean water)and to the discrimination process occurring during transpiration but no significant hydrogen discrimination occur during water absorption through the roots. Rainwater δD values are more negative in winter in comparison to summer; moreover, they decrease with increasing of latitude, altitude and distance from the ocean (Marshall et al. 2007). During the transpiration process, lighter water is favoured and the enrichment in δD is dependent on the isotopic composition of atmospheric water vapour and the leaf-air VPD (Flanagan et al. 1991). The discrimination of D during the leaf transpiration process is higher than O discrimination, because there is a higher difference in the vapour pressures between H_2O and HDO than $H_2{}^{16}O$ and $H_2{}^{18}O$, as the case of the evaporation from the surface of the ocean, where the water vapor is enriched in H and ^{16}O because $H_2{}^{16}O$ has a higher vapor pressure than HDO and $H_2{}^{18}O$ (Hoefs et al. 2009). The Craig equation or 'meteoric water line' reflects the relationship between the discrimination occurring for each element ($\delta D = 8\delta^{18}O + 10$) that applies to most locations except to those where extensive evaporation occurs (Craig 1961). Hence, δD values reported in grapevine tissues are more negative than those reported for $\delta^{18}O$ (Ingraham and Caldwell 1999). Measurements have been performed in leaf (Martin et al.1989, Ingraham and Caldwell 1999) and must water (Martin et al. 1989, Ingraham and Caldwell 1999, Martin et al.2003). As the case of $\delta^{18}O$, research that measures wine water δD may also be significant from a viticultural point of view; even though conversely to what happen with $\delta^{18}O$, an increase in δD occurs during the fermentation process of wine water compared with that of must water ($\Delta \sim 40‰$) (Martin 2003), with a higher increase when must sugar content is higher (Martin et al. 1988, 1989). In conclusion, a moderate relationship has been detected between δD in berry water and in ethanol (Martin et al. 1989), and a relationship between annual weather conditions and δD has also been reported. More precisely, the water D content is incorporated during the biosynthesis of sugars concentrated through the evapotranspiration process and the degree of enrichment is a function of the climatic conditions in the growing area. The degree of enrichment will change from year to year on the basis of the annual climatic differences as a consequence of the fractionation that occurs during the hydrologic cycle. The deuterium content of the rainfall progressively decreases with inverse proportionality between rainfall and deuterium content (Yurtsever et al. 1981). The isotopic distribution is maintained both in the groundwater taken up by grapevines and in surface water. Furthermore, isotopic ratios increase proportionally with temperature, so that a higher concentration of the heavier isotope is connected toa warmer climate as a result of more extensive evapo-transpiration. (Bigwood et al., 1998, Aghemo et al. 2011). The interpretation of δD wine values in viticultural terms is complex, because δD in ethanol depends partly on δD of must water, δD in must glucose, fructose, and on the characteristics of the fermentation medium (Martin et al. 1986). A fermentation in diluted media (e 100 g L^{-1}), shows thatthe deuterium content of the final water has differences on the direct analysis on glucose but is significantly dependent (coefficient 0.04) and variations in the deuterium content of the initial water are transmitted with a factor 0.96 to the final water. These results offer direct mechanistic information on fractionation effects occurring in the course of the fermentation. Thus, it is verified that no directconnection with the hydrogen atoms of glucose occurs in the mechanism of formation of the methylene group of ethanol. In Monetti et al. (1994) it is also confirmed that sugars samples of different site-specific D content are highly correlated with the site-specific properties of ethanol D content after fermentation. The methyl group content is strongly dependent on the isotopic concentration of the non-exchangeable C-H group in peculiar positions of glucose and reflects the geographical and botanical origin of the sugar. The methylene group, on the other hand, depends more on the D content of the fermentation water. These relationships are important for determining the botanical origin of some sugars and to discover certain types of adulteration (Martin et al. 1986, 1988, 1989, Monetti et al. 1994, Pionnier et al. 2003, Zhang et al. 2003).

5. Nitrogen

Nitrogen has two stable isotopes in nature, ^{14}N and ^{15}N, and their relative abundance are 99.634% and 0.366% respectively (Hoefs et al. 2009). Not significant fractionation is induced by plant uptake during the absorption process, particularly under conditions of nitrogen shortage in the soil or substrate of cultivation: under limiting conditions, no isotopic fractionation occurs). Only under higher nutrient concentration, some fractionation (between −3 and +1‰) can occur (Billy et al. 2010). On the opposite, there are substantial differences in the $\delta^{15}N$ between the different sources of N, with organic matter usually showing much higher $\delta^{15}N$ values than inorganic fertilisers (Bateman and Kelly 2007). For instance, ammonium nitrate fertilisers show a range of $\delta^{15}N$ between −1.4 and 2.6‰, instead of manure and compost whose $\delta^{15}N$ ranges between 3.5 and 16.2‰. Therefore, the source ofN is the main factor determining the $\delta^{15}N$ values observed in plant tissues (Kendall et al. 2007). The interpretation of $\delta^{15}N$ values in plant tissues is not straightforward because of the complexity of the N dynamics in the soil (Evans 2007, Billy et al. 2010). Indeed, the following processes occur: (i) denitrification which induces ^{15}N enrichment of the residual nitrate, the enrichment factor ranging between −15 and 30‰; (ii) volatilisation and (iii) nitrification which can also cause large isotopic depletion, their mean enrichment factors being −20‰ and −25‰, respectively; and (iv) ammonification, (i.e. the production of NH_4^+ from soil organic matter) which is usually responsible for a small fractionation (−1‰). There is little research on $\delta^{15}N$ in grapevine compared to carbon or oxygen isotopes (Stamatiadis et al. 2007, Santesteban et al. 2014). Stamatiadis et al. (2007) studied the within-field variability observed for leaf $\delta^{15}N$ at two Merlot vineyards (one flat and the other a hillside) during two consecutive seasons, establishing 25 to 32 sampling sites per vineyard depending on the season. The vineyard and the season considerably influenced leaf $\delta^{15}N$, relevant within-field variability (from 0.42 to 9.12‰) also being observed. The highest values observed at the upper parts of the hillside vineyard were attributed to a greater dependence of the plants at those positions to organic sources of N because of a greater N fertiliser leakage occurring in spring at the upland parts of the vineyard. Santesteban et al. (2014), when comparing $\delta^{15}N$ values in berries sampled at three vineyards of Traibuenas (Spain) at a single location during five consecutive seasons, reported consistent differences between vineyards. For instance in gravelly soil, the highest $\delta^{15}N$ values were always recorded, probably as a consequence of greater N leakage in spring. Inter-annual variability was lower than inter-vineyard one, and such a variability was attributed to differences in the soil mineralisation dynamics in spring. Theoretically, $\delta^{15}N$ values could be used to discriminate grapevines grown according to organic farming practices, as no inorganic N sources are permitted, and $\delta^{15}N$ would be expected to be higher. This approach has been used in other crops (Camin et al. 2011, Flores et al. 2011, Laursen et al. 2013), where it has been shown to have some limitations, because some organic fertilisers (particularly those coming from green leguminous covers) have an isotopic signature similar to that of the main inorganic fertilisers not authorised in organic growing (Bateman and Kelly 2007). However, certain threshold values could probably be established at regional level in order to detect the unauthorised use of synthetic fertilisers as N source (Camin et al. 2011, Laursen et al. 2013). In the case of the comparison of organic and conventional wine, the protocols for analysing the N signature should be precisely tuned as yeast metabolism can cause some isotopic effect and in particular, because of the N nutrients addition during fermentation – not prohibited in organic winemaking – which would greatly modify the isotopic signature of wines.

6. Other elements

Isotopic ratios of other elements as strontium, boron, copper and sulfur have also been considered in viticultural research with different purposes. In the case of strontium, $\delta^{87}Sr$ can be used to indirectly study the cation sources and fluxes in plant systems because of the chemical similarity between Ca and Sr (they have similar ionic radius and the same valence) and to the lack of discrimination by the plants for Sr uptake (none of the isotopes is favoured in plant absorption). Green et al. (2004) compared $\delta^{87}Sr$ of grapes, soil solution, soil solids and water in two grape growing sites of Australia, evaluating the impact of irrigation on the strontium isotopic ratio under both growing conditions. In grape samples, $\delta^{87}Sr$ was confirmed to be much more related to that in the soil solution at the rooting zone rather than to that in soil solids. Likewise, when $\delta^{87}Sr$ of soil, shoot and juice samples from four vineyards in Modena, Italy, was compared, an improved correlation was found between them when the biologically active fraction of the soil (extracted with NH_4NO_3) was considered (Durante et al. 2013). However, $\delta^{87}Sr$ of shoot and juice samples was much more closely related to each other, which makes Sr isotopic ratio a more powerful tool for juice or wine traceability if plant instead of soil samples are considered (Durante et al. 2013). Green et al. (2004) showed in a similar way the influence that irrigation had on grape $^{87}Sr/^{86}Sr$, showing the limitations of the use of Sr isotopic ratio has to trace grapes to their soil of origin, in particular under irrigated conditions.

Concerning boron, Coetzee et al. (2011) reported that grapevine leaves clearly reflect the changes in $\delta^{11}B$ that occur in the growing substrate where the grapevine is placed. . The soil properties such as clay composition and pH may affect $\delta^{11}B$ value in plant tissues, because clay minerals show the preference for light isotope (^{10}B) adsorption, and the pH-affect the isotopic exchange between the plant-assimilable form [boric acid, $B(OH)_3$] and non-assimilable form [borate ion $B(OH)_4$] (Vengosh et al. 1994). . Coetzee et al. (2011) suggest that the cultivar and rootstock can affect the discrimination ratio for this element even though, these findings and their physiological relevance have to be further investigated to provide definitive conclusions.

The sulfur isotope ratio ($\delta^{34}S$) is not commonly used in plants because the analysis for its determination is complex, but also because the sources of variability are not completely clear (Finlayet al.2007). In the areas with low atmospheric inputs of sulfur, plant tissue $\delta^{34}S$ values usually reflectthe values of the soil sulfate, but when higher atmospheric inputs occur, they can also include direct incorporation of sulfur dioxide through the stomata. Hinckley and co-workers have approached the study of $\delta^{34}S$ in vineyards, but their research was focused on how this ratio changed in soil water and leachates after S fungicide application, irrigation and storm events (Hinckley and Matson 2011). According to these authors, it is possible to use variations in S and $\delta^{34}S$ dynamics to trace the applied S and water, showing the different dynamics of their flow depending on amount of irrigation applied or after a storm (Hinckley and Matson 2011, Hinckley et al. 2011).

7. Study case. Application of carbon isotopes in grapevine: variability throughout the plant up to must

7.1 Context

Current climate change in the Mediterranean region is endangering plants due to to more and more extreme environmental conditions, such as increasing temperatures, prolonged and severe periods of water scarcity and strong winds (IPCC 2021). As requested by the production specifications for quality labels in Italian viticulture, the grapevine (*Vitis vinifera* L. subsp. *vinifera*) is a crop cultivatedin rainfed regime in many cultivation areas. Therefore, due to drought-stress during summer, this crop is facing increasing problems, when high evapotranspiration is accompanied by very low precipitation. In the next future, this problem might endanger the traditional wine producing areas, with the risk of a reconfiguration of the regions suitable for viticulture especially for the Mediterranean cultivation areas (Naulleau et al. 2021). Thus, in order to adopt suitable cultivation strategies to mitigate drought-stress in viticulture, there is need to develop and fine-tune methods formonitoring the vine water status in response to the environmental variability. An important method for evaluation of the plant water status and specifically water-use efficiency (WUE) is the analysis of carbon isotopes composition (Bchir et al. 2016, Brillante et al. 2020). In the case of grapevine as described in the previous paragraphs, different plant organs/tissues/matrixes have been used, with significant relations evidenced between d13C and growth and yield parameters (Gibberd et al. 2001, Gaudillere etal.2002, De Souza etal.2003, Virgona et al. 2003; De Souza et al.2005, Brillante et al.2018, Koundouras et al.2008, Van Leeuwen et al.2009, Costantini et al.2010).

Despite the analysis of carbon isotope ratio of dry matter ($\delta^{13}C$) is considered an important tool for the measurement of the intrinsic water-use efficiency, which provides important information on the water status of the vines, there is still a debate on which plant organ is more representative of the plant water status (Farquhar etal.1982). The carbon isotope composition of plant materials depends on the discrimination against $^{13}CO_2$ during photosynthetic process, due to fractionation events happening first during CO_2 stomata assimilation and then by Rubisco (Ribulose 1,5-bisphosphate carboxylase/oxygenase) activity (Farquhar etal.1982). The more the stomatal closure (usually occurring in limited-water-availability conditions), the higher the $\delta^{13}C$ values in plant tissues (Altieri etal.2015). Moreover, the carbon isotopic composition in the various plant compartments is variable due to possible remobilization of plant reserves and to the non-homogeneus isotopic distribution within metabolites (Tcherkez et al., 2011). In the present study case, the attention was focused to the analysis of $\delta^{13}C$ on three different matrixes, namely wood, leaf and must in order to evaluate which is the most representative of the vine water status. In order to do this, materials from vines growing in vineyards characterised by the occurrence of different water availability in the soil (due to different pedoclimatic conditions) were analysed.

7.2Materials and methods

7.2.1 Study site

The sampling of different plant matrixes was performed in the four vineyards in the study area of Guardia Sanframondi, as detailed in the previous chapters. In brief, the experimental vineyards were, as follows: (1) SL-Santa Lucia (41° 14′ 45″ N; 14° 34′ 16″ E, 194 m a.s.l.); (2) CA-Calvese (41° 14′ 19″ N; 14° 35′ 11″ E, 163 m a.s.l.); (3) GR-Grottole (41° 14′ 21″ N; 14° 34′ 56″ E, 158 m a.s.l.); and (4) AC-Acquafredde (41°13′44″ N; 14°35′33″ E, 84 m a.s.l.). For details on plant material, cultivation management and pedo-climatic conditions, please refer to previous chapters and to Damiano et al., 2022a, 2022b).

In brief, the pedoclimatic conditions and agronomic management of the four selected vineyards determine the differentiation of the four vineyards into two groups: SL and AC where vines showed more vigorous growth and yield, and CA and GR where vines showed morpho-physiological traits and yield reduced as signs of stronger water stress (Damiano et al., 2022a, 2022b).

The samples of wood, leaf and must in the four selected vineyards SL, CA, GR and AC were collected over three years: 2019, 2020 and 2021. Concerning the wood samples, ten vine cores per vineyard were taken at the end of December 2021 from the main stem at a height of 20 cm above the graft union point, by using a 0.5 cm Pressler increment borer. Leaf samples were collected from six vines per vineyards in veraison, the stage of maximum vegetative activity, corresponding to mid-end of July in the study area. For must samples, grape production from six vines per vineyards was sampled at the ripening stage and the must from each plant was stored at -20 °C.

7.2.2 Meteorological Data

Daily weather information (temperature, rainfall, wind, solar radiation, etc.) were collected during the experiment, in 2019, 2020 and 2021 from the Guardia Sanframondi (BN) weather station (41°14017.200 N; 14°35049.800 E) of the Campania region weather network (www.agricoltura.regione.campania.it/meteo/agrometeo.htm). In the year 2019, the climate of Guardia Sanframondi area (BN) was characterized by the annual mean temperature of 17.26 °C, with the hottest period occurring between June and August (monthly average mean temperature 27.55 °C) and the coldest month was January (monthly average mean temperature 5.95 °C). The cumulative annual precipitation was 821 mm, the wettest month was November with the cumulative monthly precipitation of 244 mm while the lowest value was reached in May with (cumulative monthly precipitation of 10.2 mm). The aridity period lasted from May to July (Figure 1A). In the year 2020, the annual mean temperature was 16.74°C, with the hottest period occurring between July and August (monthly average mean temperature 26.31 °C) and the coldest month was January (monthly average mean temperature 8.10°C). The cumulative annual precipitation was 950.5 mm, the wettest month was December with the cumulative monthly precipitation of 226.2 mm while the lowest value was reached in August with (cumulative monthly precipitation of 9 mm). The aridity period corresponded to July and August (Figure 1B). In the year 2021, the annual mean temperature was 16.55 °C, with the hottest period occurring between July and August (monthly average mean temperature 26.60 °C)and the coldest month was January (monthly average mean temperature 7.69°C). The cumulative annual precipitation was 1206.8 mm, the wettest month was January with the cumulative monthly precipitation of 330.8 mm while the lowest value was reached in August (cumulative monthly precipitation of 2.8 mm). The aridity period corresponded to July and August (Figure 1C).

A focus on the period 1 June - 30 September is reported n Table 1, where the maximum (T Max) , minimum (T Min), medium (T Med) average temperature and the cumulative precipitations (CP) are reported.

Table 1. Meteorological data of the years 2019, 2020 and 2021 referred to the period starting from the 1 June until 30 September with mean values of temperatures, maximum (T Max) minimum (T Min) and medium (T Med) and cumulative precipitation (CP). Standard deviation for temperatures are reported.

Year	T Med *°C*	T Max *°C*	T Min *°C*	CP *mm*
2019	26.8±2.46	33.3±2.72	20.3±1.97	214.6
2020	24.4±2.35	32.3±3.08	17.9±1.82	285.6
2021	25.0±1.89	33.3±1.85	18.2±1.59	167.6

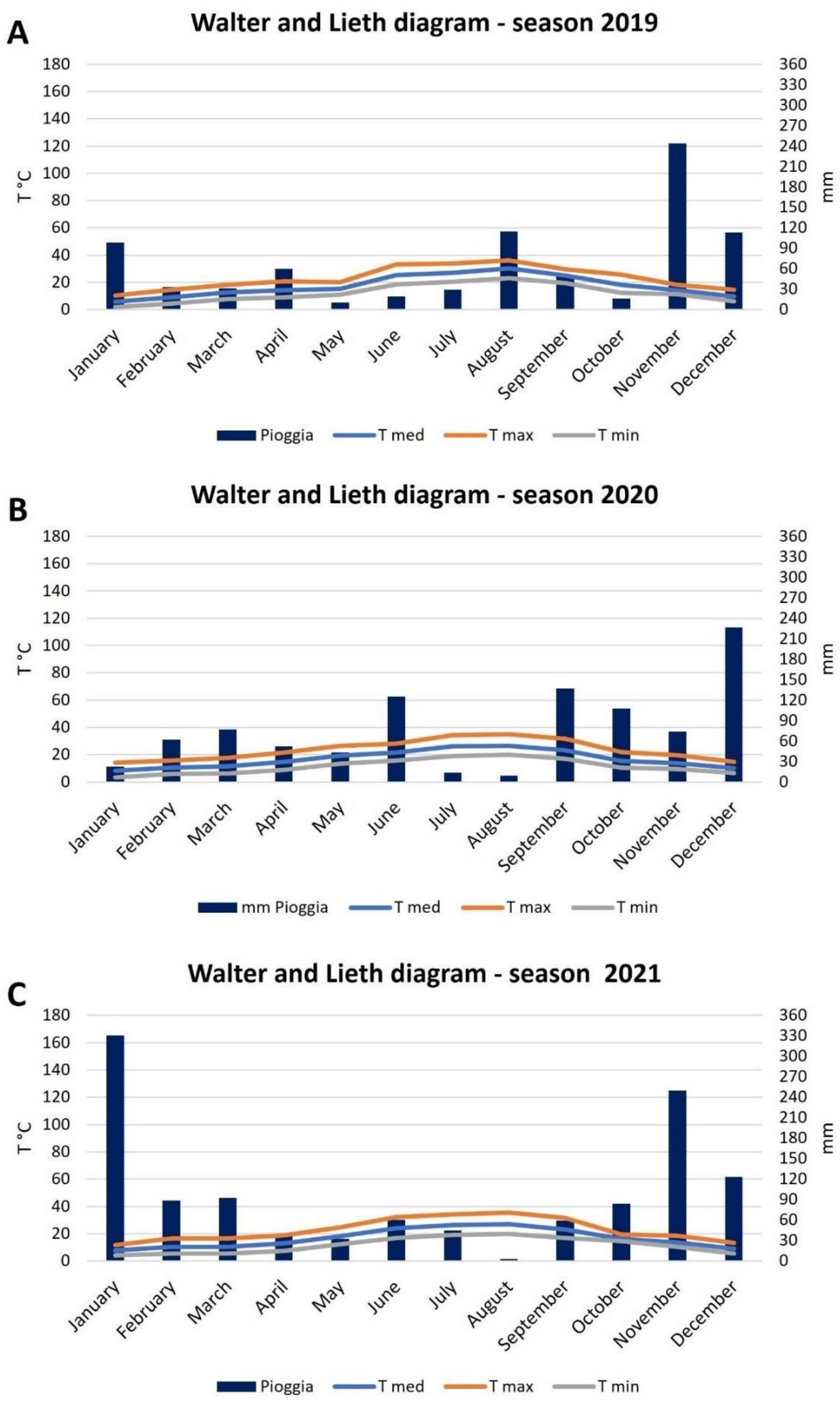

Figure 1. Walter and Lieth diagram of meteorological data referred to the period starting from the 1 January until 31 December for the years 2019 (A), 2020 (B) and 2021 (C). Monthly mean values of temperatures, and monthly cumulative precipitation (CP) are reported.

7.2.3 Samples preparation

For wood samples, the cores were analysed under a dissection microscope Wild M32 (Leica, Germany), in order to dissect single rings corresponding to the years 2019, 2020 and 2021 with a blade cutter. Each ring was ground in a centrifugal mill (ZM 1000, Retsch, Germany) with a 0.5 -mm mesh size to ensure homogeneity as in leaves.

Concerning the leaf samples, they were dried in oven at 70 °C for 72 h making sure they reached constant weight. Dry leaves were then ground in a tiny powder with a centrifugal mill (ZM 1000, Retsch, Germany) with a 0.5-mm mesh size to ensure homogeneity.

As regards must samples, 10 mL of defrosted must was collected and freeze-dried in a VirTis wizard 2.0 SP Scientific (New York, USA) for 2 days and stored away from light, in cool and dry place.

7.2.4 Carbon Isotope Analysis

Per each sample 0.95-1.05 mg of material were weighed and preserved in a tin capsule (3.3x5mm). The $\delta^{13}C$ isotopic analyses were performed using an isotope-ratio mass spectrometer with Delta V Advantage (Thermo Scientific, Waltham, MA, USA) (Ricci et al. 2016) connected, in continuous flow mode (CONFLO III), with an elemental analyzer Flash EA 1112 series (Thermo Scientific, Waltham, MA, USA), following international standards used to calibrate the samples data: IAEA C3($\delta^{13}C$ VPDB = - 24.91 ± 0.49‰), IAEA CH6 ($\delta^{13}C$ VPDB = -10.45 ± 0.03‰), and IAEA CH3 of 10 ($\delta^{13}C$ VPDB = - 24.72 ± 0.04‰). All results are expressed in delta notation, as computed in Equation (1):

$$\delta^{13}C = [(Rsample/Rstd) - 1] \times 1000 \quad (1)$$

where Rsample and Rstd are the absolute $\delta^{13}C/\delta^{12}C$ ratios for sample and standard, respectively; values of $\delta^{13}C$ are reported in parts per thousand (‰), relative to the Vienna Pee Dee Belemnite (VPDB) international reference**.**

8. Results

The results of $\delta^{13}C$ analysed in the year 2019 on the three matrixes, wood, leaf and must are reported in the figure 2. For the vineyard SL, there were no significant differences among the three matrixes analysed, namely wood (-27.791 ‰ vs. PDB ± 0.410, mean value and standard error), leaf (-27.063 ‰ ± 0.493) and must (-26.865 ‰ ± 0.205). Similarly in AC, there were no significant differences among the three matrixes analysed, namely wood (-28.379 ‰±0.361), leaf (-28.045 ‰±0.214) and must (-28.038±0.247). In CA, the $\delta^{13}C$ values analysed in wood (-26.986 ‰ ± 0.424) and leaf (-27.862 ‰ ± 0.155) were similar and significantly lower than in must (-25.037 ‰ ± 0.081). Finally, in GR the value of $\delta^{13}C$ analysed in leaf (-27.859 ‰ ± 0.558) showed a significant lower value than must (-24.932 ‰± 0.123), with wood (-26.260 ‰± 0.504) showing an intermediate value.

In the year 2020 (Figure 3), for the three vineyards SL, CA and GR showed similar trends of d13C variation among the three matrixes. More specifically, in SL there were no significant differences between $\delta^{13}C$ values analysed in wood (-27.229 ‰± 0.232) and leaf (-26.654 ‰± 0.178), which were significant lower than in must (-25.666 ‰± 0.580). In filed CA, the $\delta^{13}C$ values analysed in wood (-26.916 ‰± 0.147) were similar to those in leaf (-27.280 ‰± 0.191), and were both significantly lower than in must (-24.434 ‰± 0.756). In GR, the value of $\delta^{13}C$ in the three matrixes showed the similar trend registered in the previous year although the significance was not the same. More specifically, d13 C analysed in wood (-26.300 ‰± 0.400) tended to be higher, although not significant, than leaf (-27.166 ‰± 0.221), while significantly higher values were found in must (-24.008 ‰± 0.373) compared to the other two matrixes. In AC, there were no significant differences among the three matrixes analysed wood (-27.619 ‰± 0.202), leaf (-27.621 ‰± 0.344) and must (-27.940 ‰± 0.122) as occurred in 2019.

In the year 2021, as in 2020, the three vineyards SL, CA and GR, showed similar trends of d13C variation among the three matrixes (Figure 4). For the vineyard SL, there were no significant differences between $\delta^{13}C$ values analysed in wood (-26.979 ‰± 0.158) and in leaf (-26.819 ‰± 0.204), which in turn were significantly lower than in must (-24.557 ‰± 0.360). In CA, the results showed no significant differences between the $\delta^{13}C$ values analysed in wood (-26.610 ‰± 0.210) and leaf (-26.948 ‰± 0.136), which were significantly lower than in must (-21.888 ‰± 0.061). In GR the value of δ13C analysed in wood (-25.710 ‰± 0.278) was higher, although not significantly, than in leaf (-26.522 ‰± 0.266), and both values were significantly lower than in must (-22.524 ‰± 0.204). In AC, d13C in leaf (-28.370 ‰± 0.489) was significantly lower than in must (-26.245 ‰± 0.122), with wood showing intermediate values (-27.880 ‰± 0.394).

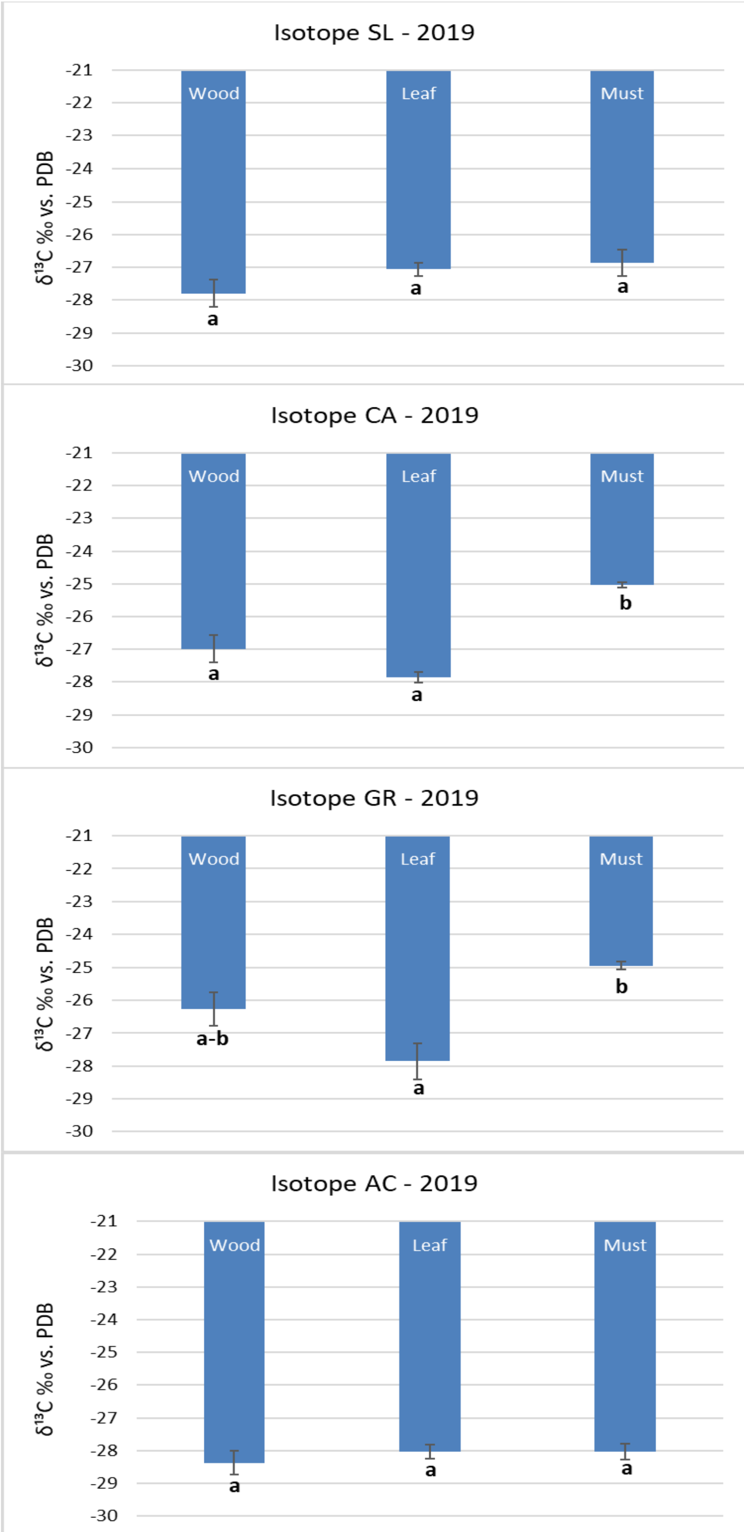

Figure 2. Comparison between the three matrixes (wood, leaf and must) in terms of δ13C values in the four vineyards (SL, Santa Lucia; CA, Calvese; GR, Grottole; AC, acquefredde) in the year 2019. Mean values and standard errors are shown. Different letters indicate significant different values according to Duncan's multiple-range test ($P \leq 0.05$).

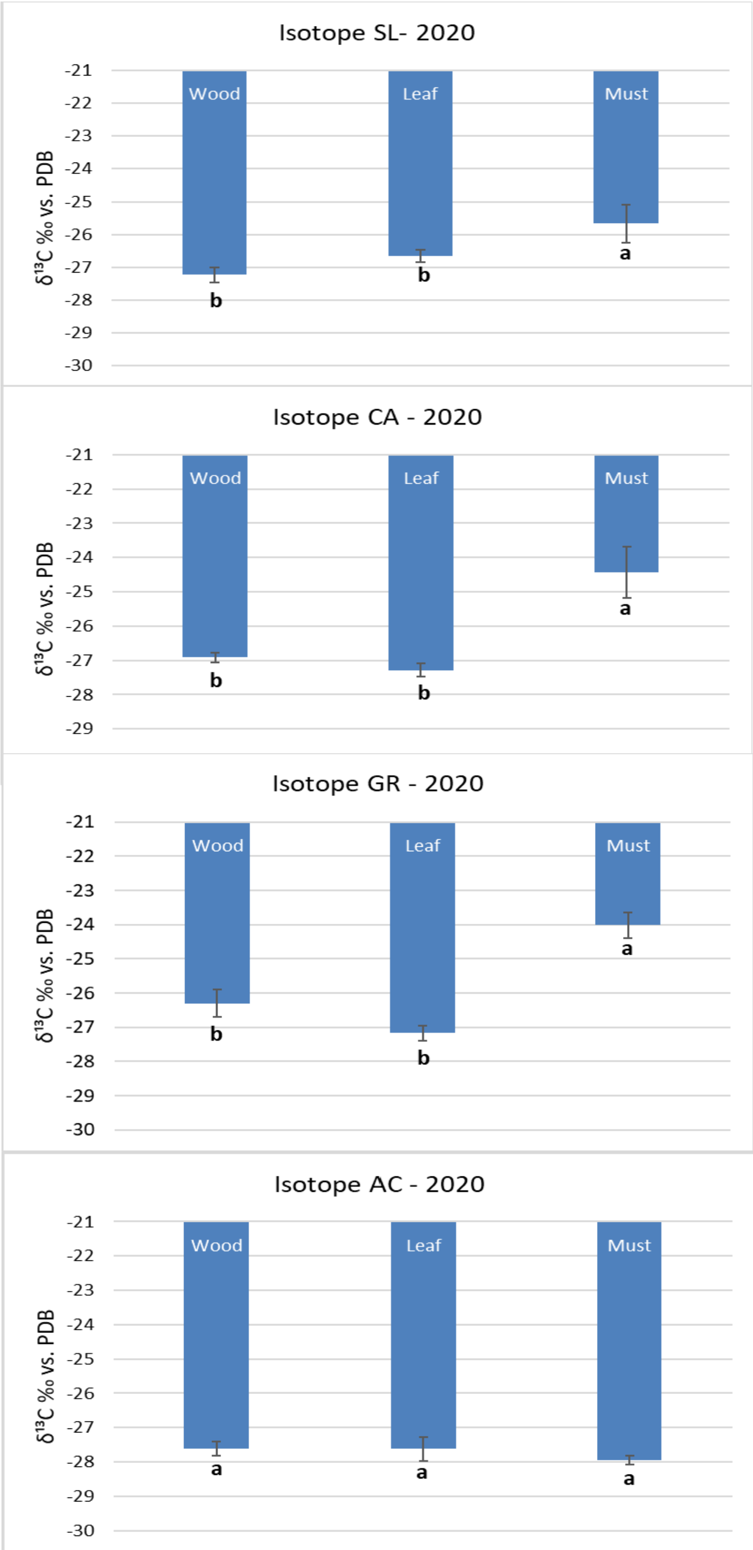

Figure 3. Comparison between the three matrixes (wood, leaf and must) in terms of δ13C values in the four vineyards (SL, Santa Lucia; CA, Calvese; GR, Grottole; AC, acquefredde) in the year 2020. Mean values and standard errors are shown. Different letters indicate significant different values according to Duncan's multiple-range test ($P \leq 0.05$).

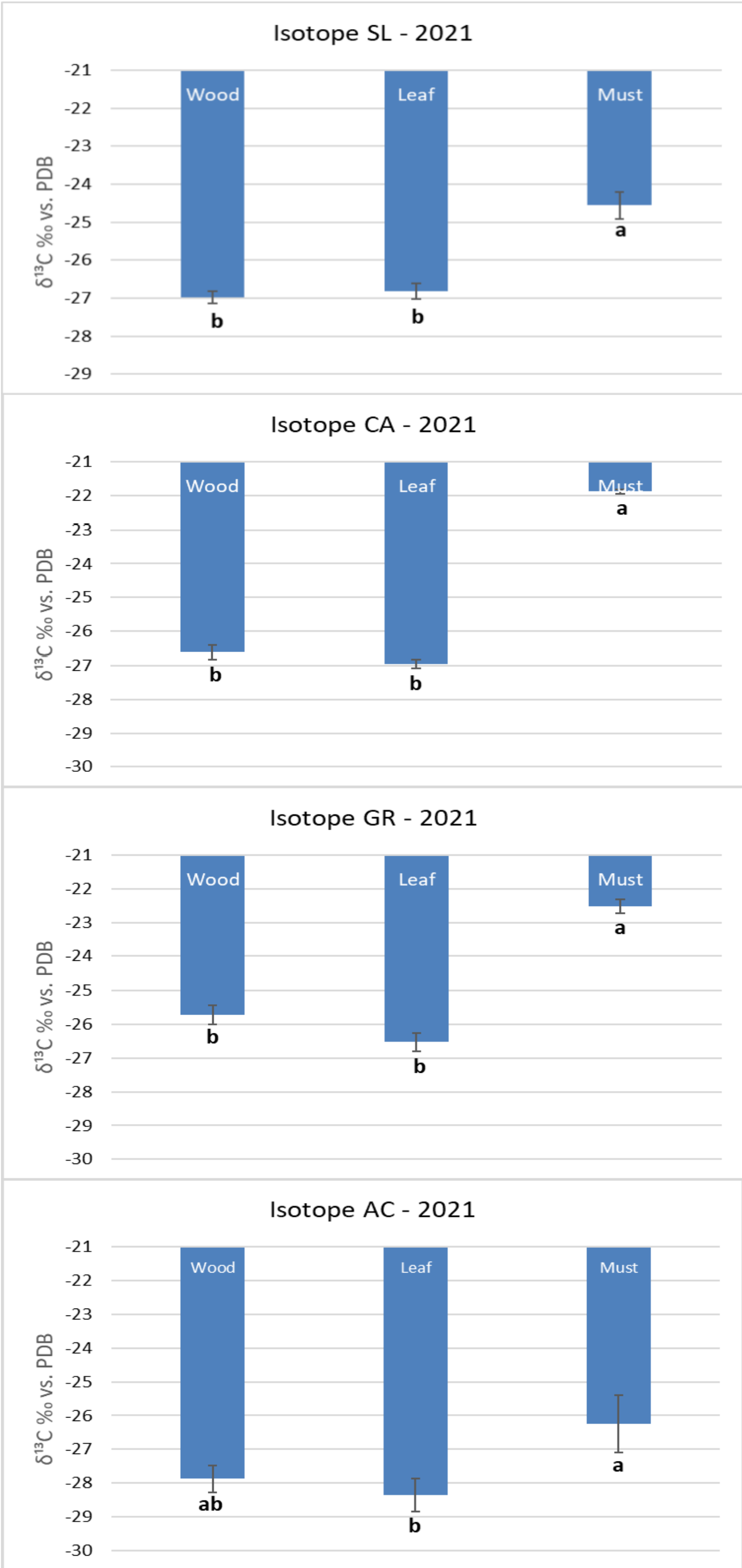

Figure 4. Comparison between the three matrixes (wood, leaf and must) in terms of δ13C values in the four vineyards (SL, Santa Lucia; CA, Calvese; GR, Grottole; AC, acquefredde) in the year 2021. Mean values and standard errors are shown. Different letters indicate significant different values according to Duncan's multiple-range test ($P \leq 0.05$).

9. Discussion

This study highlighted the different sensitivity of the three matrixes in variations of $\delta^{13}C$ as indicator of water availability in Falanghina vineyards characterised by different water availability in the soil over three years (2019-2020-2021)in vineyards. More specifically, in 2019, the vineyards characterized by wetter conditions, SL and AC, showed a tendency to have lower values of $\delta^{13}C$ in all matrixes compared to the respective matrixes of the other vineyards confirming that CA and GR were experiencing stressful conditions leading to stomata closure (Gerzon et al. 2015, Brodribb et al. 2016) (Rif. Also to chapters 3 and chapter 7 of this thesis). Indeed, the higher values of $\delta^{13}C$ in must, compared to wood and leaf, suggests that the must holds a stronger signal than the other two matrixes. Moreover, the three matrixes did not show different signals as they values were comparable. In 2020, the tendency of variation are similar, with a stress signal starting to be evidenced in the must of SL vineyard, while AC still appeared not stressed likely because a supplemental irrigation allowed for the period characterized by the extreme drought stress, would have mitigated the water shortage stress (Damiano et al. 2022b). On the contrary the field CA and GR that are the more stressed ones showed always significant differences between must values and the two matrix wood and leaf with differences even more marked in the year 2021, which was characterized by the lowest level of rainfall among the three years, in the referred period June-September (table 1). Indeed, the higher sensitivity of carbon isotope composition of the must was more evident in the more arid year, 2021, also in the wetter vineyards: in SL too, the δ13C values of must were quite higher than in wood and leaves; even in AC a stress signal was hold in must, and started to be read in wood, as if supplemental irrigation was not enough to avoid water shortage. It seems that the must $\delta^{13}C$ signal is much more sensitive to the very low precipitation in August (as occurring in 2021), while a drought stress experienced in late spring/beginning of summer (as occurring in 2019) does not leave a strong isotopic trace in must especially at the wetter sites.

These results confirm that in Falanghina grapevine, the must, thus grapes, is a more sensitive matrix for the evaluation of seasonal drought stress faced by the vines in agreement with findings on other authors on Castelao and Moscatel grapevine cultivars in a vineyard in Portugal, and Tempranillo cultivar in Spain (De Souza et al. 2003, 2005, Bchir et al. 2016)) and wood. These findings can be explained by the fact that wood and leaf are built also using carbon stock synthetized in the previous year, while must sugars mainly rely on carbon fixed in the seasonal photosynthesis which in case of drought stress preserves the trace of the enrichment in ^{13}C (Santesteban et al. 2011, Bchir et al. 2016). In conclusion is possible to confirm how the analyses of the $\delta^{13}C$ on must is a valuable method to estimate the level of drought stress occurred in a vineyard, more precise than the analyses performed on wood or leaf matrixes.

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<u>CHAPTER 6</u>

Comparing Methods for the Analysis of $\delta^{13}C$ in Falanghina Grape Must from Different Pedoclimatic Conditions

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Technical Note

Comparing Methods for the Analysis of δ13C in *Falanghina* Grape Must from Different Pedoclimatic Conditions

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Abstract: Agroforestry applications in viticulture are considered a promising strategy to improve vine water status by mitigating the threats of increasing drought due to climate change. The analysis of δ13C is often used in viticulture to understand vine water use. In this study, the analysis of δ13C was performed on the must of Falanghina grapevines growing in different pedoclimatic conditions. The aim was to compare the results obtained by the application of two different methodologies, using the whole must or extracted sugars as the matrix. The results showed that the δ13C values obtained by applying the two methodologies were comparable in all analyzed vineyards independently from the pedoclimatic conditions. Indeed, the proposed method of extraction of the δ13C on the must as a whole can be both cost- and time-saving for the analysis. This is valuable, considering that the δ13C of must is becoming more and more used as indicator of vines' water use. Therefore, the possibility to utilize a simplified method of extraction would enhance the application of the δ13C at a larger scale to evaluate vine adaptation in the context of climate-change-driven increases in drought.

Keywords: agroforestry; carbon isotopic discrimination (δ13C); must analysis; sustainable viticulture; *Vitis vinifera*; drought-stress; water-use efficiency (WUE)

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1. Introduction

Ongoing climate change in the Mediterranean region is exposing plants to more and more extreme environmental conditions, such as frequent, prolonged, and severe periods of water scarcity, high temperatures, and strong winds [1]. Grapevine (*Vitis vinifera* L. subsp. *vinifera*) is a crop cultivated in the rainfed regime in many Mediterranean cultivation areas, as requested by the production disciplinary of quality and origin labels. Therefore, this crop is facing increasing problems due to drought-stress during summer, when high evapotranspiration is accompanied by very low precipitation. In the near future, this phenomenon might endanger viticultural suitability of the traditional wine-producing areas, with the risk of a reconfiguration of vineyard locations, especially in Mediterranean cultivation areas [2]. It has been recently underlined by Favor and Udawatta et al. [3] that agroforestry applications in viticulture are instead promising, although severely overlooked. Indeed, the incorporation of trees in vineyards may ameliorate grapevine water status and mitigate stress conditions due to many mechanisms that are mainly linked to the alteration of microclimate and belowground interactions [3]. The main objective of agronomic techniques is to maintain environmental conditions to guarantee a balanced vegetative growth directed towards quality production [4]. Indeed, it has been recently shown that the control of vine water status can help achieve a rebalance of the gap between technological and aromatic maturity [4]. Therefore, in order to adopt suitable cultivation strategies to mitigate vines drought-stress, the monitoring of vine water status is needed over the course of years to understand the vines' responses to year-to-year environmental variability, including possible agroforestry applications [3,5]. An important current challenge in the biological research applied to viticulture systems is to identify an analytical method that is quick to perform and effective for evaluating the plant water status and specifically water-use efficiency (WUE). In the water-stressed vines, the decrease in leaf area and photosynthesis leads to physiological and biochemical disorders, which can have a negative impact on plant growth, structure and chemistry of the leaves, (soil-)nutrient uptake, and berry ripening [6,7]. All of these aspects affect the yield and berry composition (e.g., content of organic acids, sugars, and polyphenols also responsible for aroma) and are ultimately associated with lowering wine quality. Therefore, the improvement of WUE is among the main aims of viticulture to achieve an environmentally friendly and sustainable viticulture.

The concept of WUE always reflects a balance between gains (carbon acquisition or crop yield, AN) and costs (water consumed by transpiration and water applied, E). This balance can be measured at different levels from instantaneous fluxes of CO_2 and water vapor at the leaf, plant, and crop levels; however, in a wider context, this concept is also applied to whole agricultural systems [8]. At the leaf level, WUE can be assessed with an infra-red gas-analyzer, allowing us to determine the "instantaneous water use efficiency (AN/E, WUE_{inst})" and the "intrinsic water use efficiency (AN/gs, WUE_{i}-gs stomatal conductance)". These are in-vivo measurements which, although repeated during the growth cycle at specific intervals (often in correspondence of specific phenological stages), are not representative of the annual water status of the vines [9]. The carbon isotope ratio of dry matter ($\delta^{13}C$) is instead used as an integrated measurement of intrinsic water-use efficiency and can provide important information on the water status of the different plant- organs or of the whole plants [10]. The carbon isotope composition of plant material depends on the discrimination against $^{13}CO_2$ during photosynthetic process, due to fractionation events happening first during CO_2 stomata assimilation and then by Rubisco (Ribulose 1,5-bisphosphate carboxylase/oxygenase) activity [10]. More stomatal closure, i.e., in limited-water-availability conditions, determines increasing $\delta^{13}C$ values in plant tissues [11]. Therefore, the analysis of carbon isotopes composition in different plant tissues allows us to determine the integrated value of WUE, and, thus, the increase of $\delta^{13}C$ corresponds to an increase of WUE. In the case of grapevine, significant relations between plant water status and the carbon isotope ratio of grapevine organs have been observed under both glasshouse [12,13] and field conditions [14–20].

Several approaches to analyze the $\delta^{13}C$ isotope in viticulture have been performed by using different matrices with different procedures. Various plant tissues can be taken as samples for $\delta^{13}C$ detection, with leaves [16] and berries [17] being the most common ones. Concerning representativeness, under field conditions, leaves have been reported to be the least representative organ, as their carbon isotope ratio is less related to water-use efficiency [16], or to water potential [15,16] (R^2 = 0.17), compared to the data from pulp (R^2 = 0.74) and from whole grape (R^2 = 0.62). This is probably due to the fact that leaves are formed early in the season, before any significant water deficit is experienced [15,16]. In Santesteban et al. [21], it was showed that the vine water status of the Tempranillo cultivar was related to $\delta^{13}C$ more strictly during the ripening period (from veraison to harvest) than during the berry herbaceous development (from fruit-set to veraison). Seeds were included in the $\delta^{13}C$ whole-berry analysis that could indeed have caused a slight decrease in the $\delta^{13}C$ values as an average, considering that values are 1–2‰ lower in seeds than in berry flesh [16,22] and seeds represent just a 10–15% of berry dry weight [23,24]. The range of $\delta^{13}C$ observed in the study was also very broad, ranging from − 23‰ to − 29‰. In Coulouma et al. [25], the samples of whole berries were ground, centrifuged, and oven-dried, and the resulting powder was analyzed by a continuous-flow isotope-ratio mass spectrometer. As expected, the driest year (2016) presented a significantly higher $\delta^{13}C$ mean and the highest maximum value. In conclusion, berry pulp appears to be the most sensitive tissue [16], although whole berries follow a similar trend [16]. Indeed, it is not clear yet if the whole berry's pulp can be considered the most representative matrix of plant water status during the growing season. A translocation of sucrose from leaves to fruits occurs during berries' maturation, with a subsequent conversion in glucose and fructose. This might indicate that sugar berries' carbon isotope signature better reflects the leaf carbon photosynthetic discrimination [14]. If so, the $\delta^{13}C$ of must sugars should be considered the most representative physiological indicator of grape water status [26]. However, sugar extraction is a time-consuming procedure, and, to our knowledge, only a few reports, limited to a single study case, have compared the different methodologies to find the more convenient method to assess grapevine water status over the growing season and in a large number of plots [14].

In this study, the attention was focused on methods for $\delta^{13}C$ determination in must in order to evaluate the possibility to perform the analysis of isotopes on must as a whole and not on the extracted sugars. Our hypothesis is that no fractionation occurs during the formation of sugars and that, since the must contains more that 20% sugar, the $\delta^{13}C$ signal, if present at the photosynthesis level, it will be present at the level of must, too.

The method was evaluated in vineyards growing in different pedoclimatic conditions in order to extend the methodology in a larger number of conditions.

The possibility to perform the analysis directly on must would allow us to save time and resources to perform the analysis and to expand the isotope analysis on vineyards at a larger scale.

2. Materials and Methods

2.1. Sampling Vineyards

The study area is located in a hilly environment in Southern Italy (Guardia Sanframondi, Benevento, Campania region) (Figure 1). The experimental sites were selected within the vineyards of the La Guardiense farm, as follows: (1) SL-Santa Lucia (41° 14′ 45′′ N; 14° 34′ 16′′ E, 194 m a.s.l.); (2) CA-Calvese (41° 14′ 19′′ N; 14° 35′ 11′′ E, 163 m a.s.l.); (3) GR-Grottole (41° 14′ 21′′ N; 14° 34′ 56′′ E, 158 m a.s.l.); and (4) AC-Acquafredde (41°13′44” N; 14°35′33” E, 84 m a.s.l.).

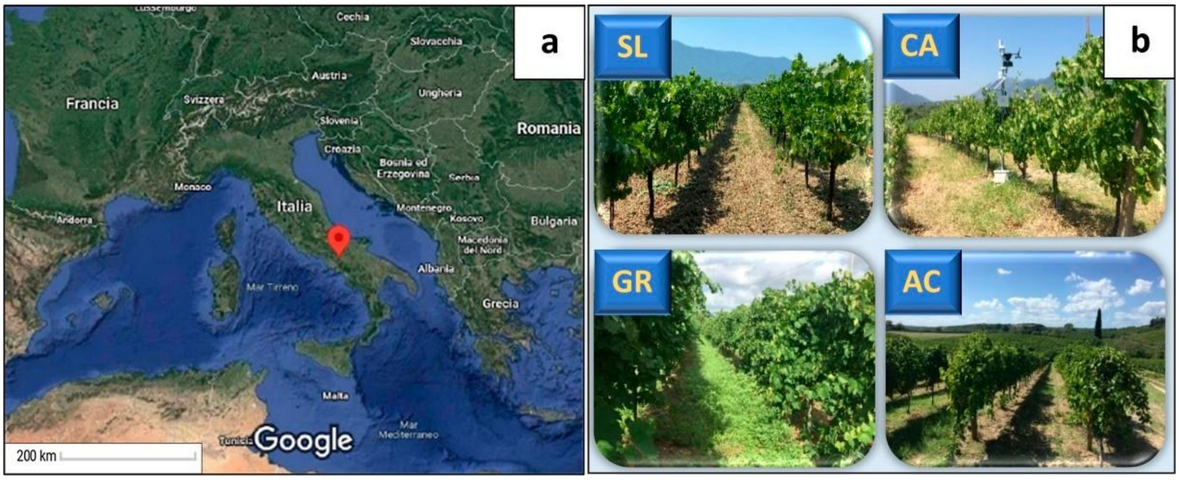

Figure 1. Location of the study area in Southern Italy (**a**) and images of the four study sites: SL, Santa Lucia; CA, Calvese; GR, Grottole; and AC, Acquefredde (**b**). Source: Google Maps ©2021.

The vineyards were selected, as much as possible, based on the use of similar plant material and cultivation techniques, apart from the water availability [27]. Therefore, in the four vineyards, the same cultivar, *Vitis vinifera* L. subsp. *vinifera* 'Falanghina' (Controlled designation of origin—DOC/AOC), is grafted on the same rootstock (157-11 Couderc), and vines are characterized by similar age, training system (double Guyot), pruning management, and spacing (∼4545 vines/ha). Some information on environmental characteristics, soil type and management, vineyard agronomic management, and vine productivity of the four sites are reported in Table 1. The pedoclimatic conditions (data reported from Bonfanteet al. [28,29] and Terribile et al. [30]) and agronomic management determine different plant vigor and productivity measured as yield (bunch weight per plant, kg) divided by TCSA (trunk cross-sectional area, cm^2) in 10 vines per site (Table 1).

Table 1. Information on environmental and productivity at the four sites (SL—Santa Lucia, CA—Calvese, GR—Grottole, and AC—Acquefredde). For yield efficiency, mean values and standard errors are shown; different letters correspond to significant differences.

Site and Vineyards Information	SL	CA	GR	AC
Row orientation	N-S	E-W	E-W	E-W
Landscape Systems *	Hills	Hills	Hills	Ancient alluvial terraces
Soil type **	Typic calciustolls	Typic calciustolls	Typic calciustolls	Typic calciustolls
Soil series	Consociazione dei suoli Pennine	Consociazione dei suoli Pennine	Consociazione dei suoli Pennine	Consociazione dei suoli Taverna Starze
Soil management	Tillage	Natural coverage	Natural coverage	Tillage
Average Amerine & Winkler index (DDA) ***	1697	1697	1697	1827
Average Potential CWSIcum (%)—Total stress ***	6	6	6	20
Irrigation management	Rainfed	Rainfed	Rainfed	Supplemental irrigation
Yield efficiency (Kg/cm^2)	0.951 ± 0.115 a	0.288 ± 0.024 c	0.591 ± 0.038 b	0.924 ± 0.126 a

* Data from Bonfante et al., 2018 [29]. ** Data from Terribile et al., 1996 [30]. *** Data from Bonfante et al. 2011 [28]. DDA, degree-day average; CWSIcum, Crop Water Stress Index.

For sampling, in the year 2019, all the bunches from 3 vines per each vineyard were collected, and the berries were pressed to achieve the must (3 samples × 4 vineyards). Selected vines did not show any sign of disease and/or mechanical stress.

2.2. Freeze-Drying of Samples

Starting from frozen samples, 10 mL of defrosted must was taken and placed in a 50 mL plastic tube and stored in freezer at −20 °C for one day. The samples were freeze-dried in a VirTis wizard 2.0 SP Scientific for 2 days and stored away from light, in cool and dry place.

2.3. Soluble Sugars Extraction and Carbon Isotope Analysis

A modified version of the method proposed by Devaux et al. [31] and Perini et al. [32] was used. Starting from samples of freeze-dried must, 45–55 mg of sample was taken with the help of a spatula, and 15 mL of deionized water was added. The samples were stirred for 60 min at temperature, i.e., 4 °C, and then placed in a thermostatic bath at a temperature of 95 °C for 10 min (protein denaturation and precipitation phase). After the thermostatic bath, the samples were centrifuged at 1200 rpm for 10 min, twice. With the help of a pipettor, the final supernatant (the extracted sugars) was removed and placed in the freezer at a temperature of −20 °C and freeze-dried again for subsequent isotopic analysis.

Carbon isotope composition was measured on must's extracted sugars (ES) in mature grapes and on must (M) before the sugar extraction, weighing about 0.1 mg of both sample types in tin capsules. The $\delta^{13}C$ isotopic analyses were performed at the iCONa lab of the University of Campania Luigi Vanvitelli, using an isotope-ratio mass spectrometer Delta V Advantage (Thermo Scientific, Waltham, MA, USA) (for details see Ricci et al. [33]) connected, in continuous flow mode (CONFLO III), with an elemental analyzer Flash EA 1112 series (Thermo Scientific, Waltham, MA, USA). IAEA C3 ($\delta^{13}C$ VPDB =

$-24.91 \pm 0.49‰$), IAEA CH6 ($\delta^{13}C$ VPDB = $-10.45 \pm 0.03‰$), and IAEA CH3

(δ^{13}C VPDB = −24.72 ± 0.04‰) international standards were used to calibrate samples data. All results are expressed in delta notation, as computed in Equation (1):

$$\delta^{13}C = [(R\text{sample}/R\text{std}) - 1] \times 1000 \quad (1)$$

where *R*sample and *R*std are the absolute $\delta^{13}C/\delta^{12}C$ ratios for sample and standard, respectively; values of δ^{13}C are reported in parts per thousand (‰), relative to the Vienna Pee Dee Belemnite (VPDB) international reference.

2.4. Data Analysis

Data were analyzed with the SPSS 13 statistical software (SPSS Inc., Chicago, IL, USA). A two-way ANOVA was performed by using the site and matrix as main factors and using the Duncan's coefficient for multiple comparison tests. Pearson's rank correlation coefficient was calculated between data series from must (M) and extracted sugars (ES).

3. Results

The data analysis showed that the site had a significant effect as main factor ($p < 0.001$), while interaction with the matrix (Site × Matrix) did not have significant effect (Table 2). There were no significant differences between the δ^{13}C values obtained with the two methods (must as a whole or extracted sugars) for all the analyzed vineyards (Figure 2). The most negative values were found in the AC vineyard, i.e., −28.04‰ ± 0.25 on must (M) and −27.82‰ ± 0.24 on extracted sugars (ES), which were significantly lower ($p < 0.001$) than those for SL vineyard, i.e., −26.87‰ ± 0.40 on M and −26.87‰ ± 0.36 on ES. The SL values were, in turn, significantly lower ($p < 0.001$) than CA −25.03‰ ± 0.08 on M and −25.02‰ ± 0.11 on ES and GR −24.93‰ ± 0.12 on M and −24.94‰ ± 0.12 on ES, respectively, with no differences among them. The graph in Figure 3 shows a linear correlation between the δ^{13}C ‰ values calculated on must and sugars extract. The correlation between data from the two matrixes was significant ($R^2 = 0.9935$, $p < 0.001$).

Table 2. Results of the two-way-ANOVA of δ^{13}C values (‰ vs. PDB), using the matrix (must and sugars) and vineyards (SL, Santa Lucia; CA, Calvese; GR, Grottole; AC, Acquefredde) as factors. Mean values and standard errors are shown. Different letters correspond to significant differences.

	d^{13} C
	‰ vs. PDB
Site	
SL	−26.87 ± 0.24 ^{b}
CA	−25.03 ± 0.06 ^{a}
GR	−24.94 ± 0.08 ^{a}
AC	−27.93 ± 0.16 ^{c}
Matrix	
Must (M)	−26.22 ± 0.41 ^{a}
Extracted sugars (ES)	−26.16 ± 0.38 ^{a}
Significance	
Site	***
Matrix	NS
Site × Matrix	NS

NS, *, **, and ***, Not significant or significant at $p < 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan multiple comparison tests ($p \leq 0.05$).

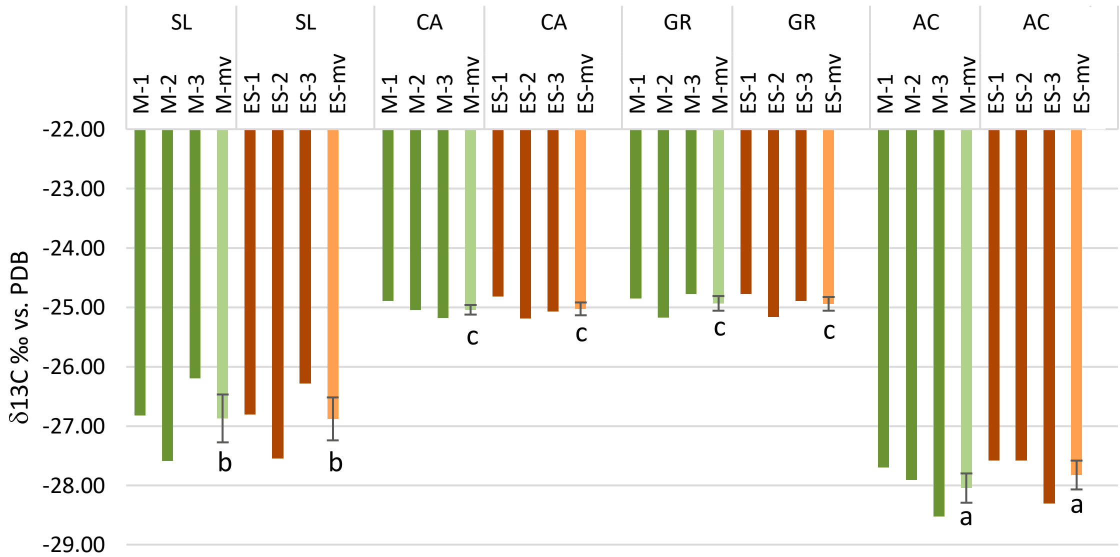

Figure 2. Comparison of $\delta^{13}C$ values (‰ vs. PDB) in must (M, 1-2-3 replicates) and extracted sugars (ES, 1-2-3 replicates) in the four vineyards (SL, Santa Lucia; CA, Calvese; GR, Grottole; AC, Acquefredde). Raw data, mean values (mv), and standard errors are shown. Different letters correspond to significant differences among vineyards according to Duncan's multiple comparison tests of one-way ANOVA.

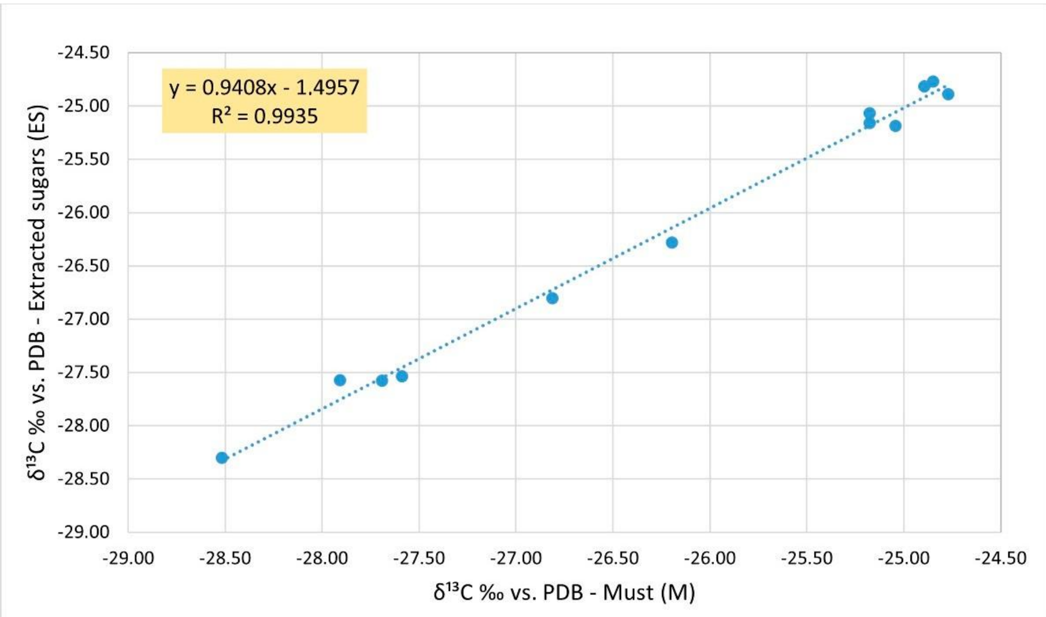

Figure 3. Relation between $\delta^{13}C$ values obtained from the analysis of must (M) samples with the corresponding extracted sugars (ES) samples, considering all the samples collected in the four study sites.

4. Discussion

In plants, such as *V. vinifera*, sugar fraction (mainly glucose and fructose) is the largest carbon pool in berries, since it is the primary photosynthetic product that is produced during the current growing season. Thus, it is important to understand how much it can influence the $\delta^{13}C$ signal of the whole must, where other components (such as organic acids) are present and where a carbon remobilization from reserves could occur [34].

In this study, the δ13C values on the two matrices, M and ES, collected in Falanghina vineyards did not show significant differences. A linear correlation between δ13C calculated on M and ES was found (R^2 = 0.9935). The comparison between data obtained by the two matrixes showed that not only the same trends of δ13C values among the samples from the different pedoclimatic conditions were found, but also no statistical differences in the absolute values, thus reinforcing the idea to utilize directly must as a whole for δ13C analyses. The result is in agreement with a previous study performed by Gaudillere et al., [14] who proposed to use must without sugar extraction in Merlot, Carbernet Souvignon, and Cabernet Franc but recommend to test the procedure in a broader range of grapevine plots. Perini et al. [32] arrived at the same results when comparing different procedures, but they underlined the necessity to extract sugar when aiming to improve the detection of authenticity of grape must.

In the challenge to develop a powerful analytical tool for quantifying how climate changes and water scarcity affect or may affect the plant water status, the application of the carbon isotopes is a tool with great potential demonstrated by numerous researches on this subject. Santesteban et al. [35] proposed a correspondence between δ13C and the water deficit via the vine water status measured in a set of studies. The water deficit is considered as weak or null with a δ13C lower than – 26‰; conversely, the water deficit is considered severe with a δ13C higher than –24‰ [35]. Concerning water-use efficiency, it is a parameter that is often used to summarize the water state of the plant, and its correlations with δ13C were studied by several scientists. In Amani Bchir et al. [36], in Spanish cultivars Tempranillo and Grenache, a correlation was found between berries δ13C and the WUE achieving values of 0.98. In this study, dried berry powder was used and analyzed in an isotope ratio mass spectrometer, as in Farquar et al. [10]. In De Souza et al. [16], the researchers evaluated the effect of deficit irrigation on intrinsic WUE and carbon isotope composition (δ13C) of Moscatel and Castelao grapevine cultivars growing in a commercial Portugal vineyard. The results show for δ13C of the dried pulp and dried whole berry the best determination coefficient (respectively R^2 = 0.74 and R^2 = 0.62) with WUE as compared to the δ13C of leaves (R^2 = 0.17) in both cultivars and years considered, showing the less representativeness of this last tissue. In Gómez-Alonso et al. [37], the determination of δ13C was performed on the must of four Spanish autochthonous grapevines Airén, Macabeo (white grapes), Tempranillo, and Garnacha (red grapes), and four foreign ones, Chardonnay, Sauvignon Blanc (white grapes), Cabernet Sauvignon, and Merlot (red grapes), comparing irrigated and non-irrigated vines. The results showed significantly ($p < 0.001$) lower $^{13}C/^{12}C$ ratios of irrigated grapes, confirming the δ13C enrichment berries of drought- stressed vines. All of these studies show the importance of the δ13C use as an indicator of the crop water status when researchers tried to analyze different matrices with different results during the years.

In accordance with Santesteban et al. [35], who proposed thresholds of δ13C to evaluate weak/null and severe water deficit, in the present study case, the vineyards SL and AC seem to be not in drought-stressed conditions, while CA and GR would be classified as drought-stressed. Indeed, in water-limited conditions, there is a strong stomatal regulation, which leads to partial or total stomatal closure determining a decrease in $^{13}CO_2$ discrimination and an increase of δ13C values [10]. In our study, the lower values of δ13 C found in AC indicate that the supplemental irrigation at this site has likely reduced the stress experienced by the vineyard (also in line with higher yield efficiency), notwithstanding the higher potential stressful conditions, as suggested by the higher Average Amerine and Winkler index and CWSI, compared to the other sites. Indeed, supplemental irrigation is a practice applied to essentially rainfed crops to improve and stabilize yields under periods that are particularly dry [38]. In CA and GR, the lower yield efficiency, accompanied by higher δ13C compared to SL, with the same Amerine and Winkler index and CWSI, may be ascribed to the effect of different soil-management methods. Indeed, the natural coverage in CA and GR, in contrast to the tillage in SL, could have induced resources competition between the vines and cover crops during the period of water-stress conditions.

In fact, the use of cover crops is considered a strategy that could possibly have a positive influence on water-use efficiency in vineyards, but it can induce a negative effect on vineyard productivity in the case of limiting environmental conditions [39]. Our data confirmed our working hypothesis of there being a linear relationship between sugar and whole berries, and demonstrated that the two types of matrixes gave the same $\delta^{13}C$ values in all analyzed vineyards, characterized by different pedoclimatic conditions, reinforcing the idea to utilize directly must as whole for $\delta^{13}C$ analyses. This study aimed to evaluate the presence of correlation between must- and extracted-sugars-derived data in Falanghina vineyards, irrespective of different pedoclimatic conditions likely responsible for different levels of $\delta^{13}C$. Gained data suggested a different water use in the four vineyards that should be supported by further analyses considering also other growth and productivity traits. However, the methodological findings were valuable, given that the $\delta^{13}C$ of must is becoming more and more used as an indicator of vines' water use: the introduction of a simplified method of extraction would enhance the application of the $\delta^{13}C$ at a larger scale, allowing us to save both time and costs for the analysis.

5. Conclusions

Nowadays the negative effects of climate changes are increasing the need to implement strategies to monitor the water status of main crops, such as grapevine, in order to be able to implement corrective actions to improve the health status of the crops also in agroforestry systems. Therefore, seeing the representativeness of the carbon isotope ratio of grapevine organs on plant water status increasingly used to obtain such information, it is important to compare the protocols faster and more cost-effective to calculate the $\delta^{13}C$. There is a great diversity in the organs that have been chosen for carbon isotope analysis in the last decade. The proposed method of measurement of the $\delta^{13}C$ on the must as a whole—that is, without sugar extraction—allows us to save on both costs and time for the analysis; this result is promising, given that the application of the $\delta^{13}C$ analysis on grapevine must is being used more and more as an indicator of vines' water status, especially to evaluate vine adaptation ability in the context of climate-change-driven increases in drought.

As a result of this, in the future, we can evaluate the possibility to perform the analysis directly on must to spread the study of the $\delta^{13}C$ rate, a tool that is easy and quick in relation to determining the vine's water status.

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CHAPTER 7

Anatomical and isotopic traits in grapevine wood rings record pedoclimatic variability

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TITLE

Anatomical and isotopic traits in grapevine wood rings record pedoclimatic variability

SHORT TITLE

Wood anatomy and $\delta^{13}C$ in grapevine wood

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Summary

In the Mediterranean region, climate change is leading to an increase in temperature and in the frequency and severity of prolonged droughts, that are affecting grapevine vegetation, growth and reproductive cycles. Alterations in vine physiological processes, induced bychanges in environmental factors or in the cultivation management are recorded in wood anatomical and isotopic traits in grapevine stems. In this study, we measured anatomical traits and carbon stable isotope content in wood rings of *Vitis vinifera* L. subsp. *vinifera* 'Falanghina' plants cultivated in four vineyards located in southern Italy, which were characterised by different water availability. The aim was to evaluate the influence of local site conditions on wood plasticity in response to inter-annual climate variability.

Wood cores were extracted from the vine stem and subjected to both microscopy and carbon stable isotope analyses in order to quantify functional wood anatomical traits (e.g., vessel size and frequency) and water use efficiency of the plant. Wood traits were correlated to time series data of precipitation and temperature. Results showed that plants at the four vineyards are characterized by different wood structure which affect the vines behavior under different condition of water availability, due to pedoclimatic variability among the four vineyards. Overall, the analyses highlighted that the vineyards at the wetter sites show wood traits which favor the efficiency of water flow, while at the dryer sites safety against embolism is favoured. Depending on the interannual climate variability, either the site or the climatic conditions can prevail in influencing the anatomical traits, characterised by different sensitivity.

KEYWORDS
Carbon isotopes, drought, quantitative wood anatomy, Vitis vinifera, water use efficiency

1. Introduction

Environmental changes in the Mediterranean basin let predict a scenario characterized by an increase in drought frequency and severity, which is raising concerns for resource management in agriculture (Masson-Delmotte *et al.* 2021). Plant adaptation to drought and crop productivity rely on plant hydraulic traits and on their plasticity that are affected by complex interactions among multiple environmental factors, and cultivation techniques (Cirillo *et al.* 2014; Amitrano *et al.* 2019). The grapevine (*Vitis vinifera* L.) being mostly rainfed-cultivated in Italy, is particularly interesting from a crop management perspective since there is an increasing demand by stakeholders for cultivation techniques that improve water stress tolerance to counteract the negative effect of climate changes. Plant vascular architecture and hydraulics are severely affected by environmental conditions, and plant habit is greatly influenced by training techniques (i.e. canopy management), whose importance in the control of water use efficiency has been recently recognized under regimes of deficit irrigation (Cirillo et al. 2017). Water-use is a key factor of vine adaptation strategies to the environment and it is essential to understand the design of the vascular system involved in the vine water flow (Hacke & Sperry 2001a; Lovisolo *et al.* 2002, Gallo *et al.* 2022, Buesa *et al.*2021). Grapevine vascular architecture and hydraulic characteristics play a fundamental role in the adaptive strategies of plants that must be considered in water management. Concerning the influence of the soil type on the xylem hydraulic design, little is known in grapevines cultivars. Variations of diameter and density in xylem vessel, are relevant traits to evaluate plant responses to environmental conditions (Hacke *et al.* 2017a). It is known that slight changes in vessel lumen diameter, following Hagen-Poiseuille's law, cause considerable modifications in the rate of xylem-sap flow (Chavarria & dos Santos 2012; Hacke *et al.* 2017a, Pospíšilová & Zimmermann 1984). In plants, water transport along vessels is guaranteed in the plant-atmosphere continuum by the negative pressure through a continuous column, as result of leaf transpiration, as explained by the cohesive-transpiratory theory (Brodersen *et al.* 2010; Chavarria & dos Santos 2012). Usually, *V. vinifera* shows a ring porous, sometimes semi-ring porous wood anatomical structure characterised by the occurrence of very large solitary vessels in earlywood and very narrow latewood vessels grouped in radial files or small groups (Schweingruber 1990). Earlywood vessels promote efficient water transport but have low safety against embolism under conditions of water deficit (Hacke *et al.* 2006). Failures in the hydraulic system may occur under different circumstances, e.g., freezing and drought events (Baas *et al.* 2004; Hacke *et al.*2017b; Tyree & Sperry 1989)), producing obstruction of the hydraulic conduction system, andeventually death of the plant due to embolism (Brodersen *et al*. 2010), Therefore, keeping the reproductive and photosynthetic organs hydrated is crucial for survival. The possibility to maintain a functioning hydraulic system in periods of drought relies ultimately on the plasticityof the vascular cambium to create an apoplastic hydraulic system adapted to the environmentalgrowth conditions in which the wood is formed (Anderegg & Meinzer 2015; Hacke *et al.* 2001b;Hacke *et al.* 2017b; Islam *et al.* 2019). This is reflected by changes in vessel size at inter- and intra-species level, when plants are influenced by growth limiting factors, such as water shortage, salinity, early and late frosts or soil structure and fertility limitations (Hacke *et al.* 2017b; de Melo *et al.* 2018; Schmitz *et al*. 2006). Several drivers have been proposed to influence vessel size, including environmental factors and plant habitus and architecture (De Micco *et al.* 2008; Olson *et al.* 2014). Cultivation techniques (i.e. canopy training system and pruning) directly affect the crown structure, with consequences in the allometric relationship with the wood anatomical traits, but also with leaf area light interception and water flow resistances throughout the plant, thus ultimately influencing carbon assimilation and partitioning of resources (Cirillo *et al*. 2017; Souza *et al.* 2011; Willaume *et al.* 2004; Tyree & Evers, 1991). Variations in the latter are also consequent to the efficiency of water transport when water is available and therefore are largely influenced by vessel size, so far as larger vessels are less resistant than narrower ones (Davis *et al.* 1999). Therefore, vessel characteristics, including size, distribution and relations with the other cell elements contain important information to understand the effects of the environmental conditions to which plants are exposed (Robert *et al*. 2009). In this context carbon stable isotope can help to evaluate the balance between photosynthesis and stomatal conductance (Francey & Farquhar 1982), defined as intrinsic water use efficiency (Ehleringer *et al.* 1993). Change in water use efficiency is very important since it reflects the rate of carbon assimilation and thus tree productivity and its ability to face drought stress (Battipaglia *et al.* 2014; Altieri *et al.* 2015).

The aim of this study was to explore the inter-annual and inter-site variability of wood anatomical and isotopic traits in wood-ring series of grapevine cultivated in four vineyards located in different pedoclimatic conditions in Southern Italy. A previous study in the same vineyards indicated that vines adjusted their leaf anatomical traits, and specifically vein and stomata features, in response to the different pedo-climatic conditions and such traits corresponded to different eco-physiological behavior (Damiano *et al.* 2022a). Therefore, we aimed to check whether the different eco-physiological behavior might be recorded also in wood-ring traits. We also explored the relations with climate factors in order to hypothesize possible triggers of traits favoring either efficiency or safety of water transport likely indicating different strategies to cope with limiting environmental factors in the four pedoclimatic conditions.

2. Materials and methods

2.1 Study site and plant material

Wood stem of *V. vinifera* (Controlled designation of origin – DOC/AOC) was collected in 2019 and its anatomical and isotopic traits from vines cultivated in 4 vineyards within the farm "La Guardiense" in southern Italy (Guardia Sanframondi, Benevento) analyzed. The four vineyards are located at Santa Lucia (SL, 41° 14′ 45″ N; 14°34′ 16″ ,194 m a.s.l.); 2) Calvese (CA, 41° 14’ 19 N; 14° 35′ 11″E , 163 m a.s.l.); 3) Grottole (GR, 41°14′ 21″ N; 14°34′ 56″ E, 158 m a.s.l.); 4) Acquafredde (AC, 41° 13 ′ 44″N; 14° 35′ 33″E, 84 m a.s.l.). Vines (≈ 4545 vines/ha) were grafted on 157-11 Couderc (*Vitis berlandieri* × *Vitis riparia*) rootstock and were uniform for age, training system (double Guyot) and pruning management. The climate is Mediterranean with hot dry summer and wet mild winters. Metereological data of the period 2015–2019 are from the Guardia Sanframondi weather station (41°14’17.2”N;14°35’49.8”E) of the Campania region weather network (www.agricoltura.regione.campania.it/meteo/agrometeo.htm). The average temperatures of the periods 21 June - 23 September and 21 December - 20 March among the five years are 25.17 °C and 9.27 °C respectively. The average of the cumulative precipitation of the periods 21 June - 23 September and 21 December - 20 March among the five year are 191 mm and 238 mm respectively.

At the experimental sites the soil type is Mollisols, classified as Typic Calciustolls by the two principal soil series of the soil map of Valle Telesina area (1:50.000): i) Consociazione dei suoli Pennine (SL, CA and GR sites) and ii) Consociazione dei suoli Taverna Starze (CA site) (Terribile 1996). The horizons of the soil profile are Ap and Bw but the differences among the experimental sites are mostly due to the percentage of stones variability along the soil profile, and additionally the effect of vineyard planting which has modified the soil horizons depth and thickness among the sites. Previous studies have indicated that the vineyards can be classified based on water availability for vines into: two wetter sites, namely Santa Lucia (SL) and Acquefredde (AC), and two dryer namely Calvese (CA) and Grottole (GR) (Damiano *et al.* 2022a, 2022b).

2.2 Wood-cores sampling

Wood cores of ten vines per vineyard were taken at the end of December 2019, from their main stem at a height of 20 cm above the graft union point, using a 0.5 cm Pressler increment borer. The cores, seasoned in a fresh-air dry store and sanded with different grain-size paper, were cross-sectioned for wood anatomical analysis, with a sliding microtome (WSL Core Microtome, Switzerland). Cross sections (13-15 µm thick) were washed with distilled water and subsequently dehydrated using an ethanol series from 40 to 100 % (Gartner & Schweingruber 2013). Afterword, the microsections were stained with a blend 1:1 of safranin (0.8 g in 100 ml distilled water) and Astra Blue (0.5 g in 100 ml distilled water and 2 ml acetic acid). Thus, after washing with water and ethanol at increasing concentration, the stained microsections, they were mounted with EUKITT® and dried in the oven at 50 °C for 24 hours.

2.3 Wood-anatomical traits

Microphotographs of the cross sections were obtained with a digital slide scanner (Zeiss Axio Scan.Z1, Germany) to obtain digital images at 10x magnification and resolution of 1300

× 1030 pixels for whole tree-ring series. Images were analyzed to visually identify and date the rings in order to capture digital images of whole rings of the last five years (2015-2019) in five cores per site. Images of the last five years were captured with an EP50 camera (Olympus, Hamburg, Germany) on a BX51 transmission light microscope (Olympus, Hamburg, Germany). Images were analyzed with the CellSens 3.2 (Olympus) software program. The following anatomical features were quantified in each ring: vessel area % (VA), ray area % (RA), fiber area % (FA) over the total section analyzed, lumen area of each vessel, potential hydraulic conductivity (Kh), hydraulic diameter (Dh) (Colangelo et al. 2017). More specifically, as described in Colangelo et al. (2017) the Kh was estimated as $Kh = (\rho \times \pi \times \Sigma d^4)/(128 \times \mu \times Ar)$, where "ρ" is the density of water at 20 °C (998.2 kg m^{-3}), "d" is the vessel lumen diameter, "μ" is the viscosity of water (1.002×10^{-9} MPa s at 20 °C) and "Ar" is the area imaged; the Dh was calculated as the average of $\Sigma d^5/\Sigma d^4$, where "d" is the lumen diameter of each vessel. Considering that grapevine xylem is characterized by a large range of variation of vessel lumen area (VLA), in order to better analyze how different distributions of VLA are related to hydraulic properties, vessels were divided into three groups: lumen area <500 μm^2 (A), 500 μm^2 <lumen area<5000 μm^2 (B), lumen area >5000 μm^2 (C). Frequency distribution of the vessels in classes of lumen area within each group was calculated. In the three groups, the following parameters were quantified: average of minimum vessel area of group A (VAMin A), average of mean vessel area of group A (VAMean A), average of mean vessel area of group B (VAMean B), average of mean vessel area of group C (VAMean C), average of maximum vessel area of group C (VAMax C).

2.4 Stable Carbon isotope analysis

After sectioning, the cores were observed under a dissection microscope Wild M32 (Leica, Germany), in order to dissect single rings of the last five years, with a blade cutter. Each ring was ground in a centrifugal mill (ZM 1000, Retsch, Germany) with a 0.5-mm mesh size to ensure homogeneity. Stable C isotope composition was measured at the iCONa laboratory of the University of Campania (Caserta, Italy) by combustion in an elemental analyzer (Flash EA 1112 series, Thermo Scientific, Waltham, MA, USA) (connected via a CONFLO IV interface (Thermo Scientific, Waltham, MA, USA) to an isotope ratio mass-spectrometer (Delta V Advantage,Thermo Scientific, Waltham, MA, USA), operating in the continuous flow mode. Isotopic compositions are expressed in delta notation (‰) relative to an accepted reference standard: Vienna Pee Dee Belemnite for carbon isotope values. The standard deviation for the repeated analysis of an IAEA international standard (CH3, cellulose) was < 0.1‰. Finally, WUEi was estimated starting from the ^{13}C isotope of the respective vineyards (Gentilesca *et al*. 2021).

2.5 Meteorological data

Air temperature and rainfall data were collected from data recorded at the Guardia Sanframondi (BN) meteorological station of the Campania region weather network (41°14'17.2"N; 14°35'49.8"E) for the timeframe 2015-2019. The following parameters were considered: annual mean temperature (AMT), annual maximum temperature (AMaxT) and annual minimum temperature (AMinT), cumulative annual precipitation (CAP).

2.6 Statistical analysis

Anatomical data were subjected to a two-way analysis of variance (ANOVA) using the four vineyards (field, F) and the year (Y) as main factors, and analyzing their interaction (F × Y). Duncan's multiple range test (at $p \leq 0.05$) was applied to identify any significant differences among the four vineyards. Carbon isotopes data were analyzed by one-way ANOVA, grouping the 5 years per core and using F as factor. Shapiro–Wilk and Kolmogorov–Smirnov tests were performed to check for normality. The SPSS 27 software package (SPSS Inc., Chicago, IL, USA) was used for the analyses. Moreover, multivariate analysis was applied on anatomical, isotopic and climatic data. Line plots, correlation plots (*coreplot* package with the Spearman's method) were performed using the R software environment for statistical computing and graphics (version 4.4.1). For multivariate analysis, principal component analysis (PCA) and hierarchical cluster analysis (HCA), were performed using Past3 statistical software (University of Oslo, Norway). For HCA, the paired group (UPGMA) and Euclidean distances were used for clustering. Results of HCA were displayed as a tree-shaped dendrogram, where the horizontal distance between clusters represented data dissimilarities.

3. Results

Microscopy observations showed vines from the four vineyards were characterized by similar wood-ring anatomy (Fig. 1). Tree-ring boundaries were evident and typically undulating, and wood was ring- or semi-ring porous. In wide rings, the differentiation into earlywood, with very large vessels, and latewood, with very narrow vessels organized in radial files or small clusters, was evident. Such differentiation was less clear in narrow rings. Wide parenchyma rays occupy the cross section.

Fig. 1. Light microscopy views of cross sections of the trunk wood of vines from the four vineyards: A, Santa Lucia, SL; B, Calvese, CA; C, Grottole, GR; D, Acquefredde, AC. Images are at the same magnification. Bars = 500 μm.

The effect of the main factor Y and of the interaction F × Y were never significant in any anatomical parameters analyzed.

Concerning the area occupied by vessels, fibers and parenchyma cells in each tree ring, the main effect F was significant for Vessel area % (VA) and Fiber area % (FA). Concerning VA, the vineyard AC showed significantly higher values than SL and GR, with CA having intermediate values between AC and GR. The parameter FA was significantly higher in SL and GR compared to AC, with CA having intermediate values (Table 1).

Table 1. Main effects of field and year on average of vessel area % (VA), ray area % (RA), fiber area % (FA) on ring area. Mean values and significance of main factors interactions are shown. Different letters within column indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$).

	VA %	FA %	RA %
Field (F)			
SL	22.82±1.244 c	43.03±0.655 a	36.61±0.957 a
CA	27.63±0.945 ab	40.60±0.729 ab	33.76±0.512 a
GR	24.55±0.828 bc	41.81±0.903 a	36.14±1.002 a
AC	29.84±1.613 a	39.40±1.348 b	34.25±1.107 a
Year (Y)			
2015	25.51±1.141 a	42.79±0.813 a	34.31±0.919 a
2016	25.49±1.254 a	42.12±0.988 a	35.19±0.851 a
2017	27.33±1.967 a	41.31±1.484 a	35.13±0.943 a
2018	26.83±1.241 a	39.86±1.120 a	36.33±0.968 a
2019	27.09±1.571 a	40.77±0.967 a	35.67±1.485 a
Significance[1]			
F	***	**	NS
Y	NS	NS	NS
FxY	NS	NS	NS

[1]NS,*, **, ***: Non-significant or significant at $P \leq 0.05$, 0.01, 0.005, respectively.

As regards the mean vessel area calculated for the three VLA classes A, B and C (VAMean A, VAMean B, VAMean C), as well as the minimum and maximum vessel area calculated for the narrower (A) and larger (C) VLA classes respectively (VAMin A, VAMaxC), the main effect F was significant for all the analyzed parameters but VAMean B. VAMin A and VAMean A showed the same trend of variation, with CA and AC reaching higher values then SL, which in turn had a significantly higher value then GR. On the contrary, VAMean C and VAMax C were significantly lower in GR compared to the other three vineyards (Table 2).

Table 2. Main effects of field and year on average of minimum vessel area of group A (VAMin A), average of mean vessel area of group A (VAMean A), average of mean vessel area of group B (VAMean B), average of mean vessel area of group C (VAMean C), average of maximum vessel area of group C (VAMax C). Mean values and significance of main factors interactions are shown. Different letters within column indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$).

	VAMin A µm²	VAMean A µm²	VAMeanB µm²	VAMeanC µm²	VAMaxC µm²
Field (F)					
SL	83.84±2.981 b	245.2±7.459 b	1377±46.84 a	17569±1066.5 a	31944±2373.3 a
CA	135.6±7.463 a	268.9±8.021 ab	1374±41.83 a	20530±1373.8 a	38101±2731.1 a
GR	49.91±3.637 c	195.5±7.792 c	1584±104.9 a	9903.2±694.9 b	16051±1518.1 b
AC	152.1±11.71 a	287.2±11.15 a	1376±43.77 a	18371±1054.7 a	34646±2208.1 a
Year (Y)					
2015	94.92±9.746 a	250.5±11.64 a	1392±65.69 a	16409±1398.5 a	29862±3181.3 a
2016	105.0±11.71 a	251.5±11.52 a	1443±58.00 a	16433±1700.2 a	30387±3437.4 a
2017	101.8±11.48 a	249.0±12.40 a	1432±53.51 a	15080±1056.2 a	28627±2401.1 a
2018	111.4±14.29 a	245.9±13.33 a	1375±63.35 a	19346±1787.5 a	34705±3479.6 a
2019	113.7±13.98 a	249.2±13.89 a	1500±117.1 a	15698±1344.7 a	27346±3056.5 a
Significance[1]					
F	***	***	NS	***	***
Y	NS	NS	NS	NS	NS
FxY	NS	NS	NS	NS	NS

[1]NS,*, **, ***: Non-significant or significant at $P \leq 0.05$, 0.01, 0.005, respectively.

Finally, for the calculated potential hydraulic conductivity (Kh) and hydraulic diameter (Dh), the main effect of field (F) was significant for narrower lumen area (KhA and DhA) and for the larger lumen area (KhC and DhC) groups, but not for the group B (KhB and DhB). In particular, the vineyard GR showed significantly highest and lowest values of KhA and KhC respectively compared to the other vineyards. As regards the hydraulic diameter (Dh), GR showed DhA with the lowest value, whereas AC had the highest DhA. DhC was significantly lower in GR than all the other vineyards (Table 3).

Table 3. Main effects of field and year on potential hydraulic conductivity (Kh), hydraulic diameter (Dh), for the three group: lumen area <500 µm^2 (A), 500 µm^2 <lumen area<5000 µm^2 (B), lumen area >5000 µm^2 (C) of Vitis vinifera L. subsp. vinifera 'Falanghina'. Mean values and significance of main factors interactions are shown. Different letters within column indicate significant differences according to Duncan's multiple-range test (P ≤ 0.05).

	KhA (kg m^{-1}MPa^{-1} s^{-1}) ×10^{-2}	KhB (kg m^{-1}MPa^{-1} s^{-1}) ×10^{-2}	KhC (kg m^{-1}MPa^{-1} s^{-1}) ×10^{-2}	DhA µm	DhB µm	DhC Mm
Field (F)						
SL	0.488±0.074 b	10.68±1.595 a	483.7±159.1 a	34.05±0.619 bc	76.51±1.888 a	220.3±7.642 a
CA	0.388±0.046 b	7.168±0.467 a	425.5±41.54 a	36.54±1.046 b	76.19±1.188 a	239.9±8.636 a
GR	0.782±0.065 a	11.10±1.628 a	158.1±20.22 b	31.18±0.863 c	76.57±2.062 a	171.2±7.720 b
AC	0.541±0.052 b	9.729±1.001 a	443.5±66.84 a	40.41±2.073 a	76.91±1.707 a	221.8±8.017 a
Year (Y)						
2015	0.588±0.069 a	8.590±1.072 a	348.4±63.35 a	36.93±2.154 a	76.84±1.980 a	213.9±9.948 a
2016	0.609±0.097 a	10.27±1.422 a	368.5±79.67 a	36.92±1.551 a	77.44±1.597 a	212.4±11.20 a
2017	0.487±0.073 a	10.38±1.329 a	286.9±43.44 a	33.62±0.999 a	78.36±1.562 a	205.2±8.886 a
2018	0.517±0.062 a	9.711±1.791 a	534.4±186.6 a	36.74±1.953 a	73.88±1.890 a	231.3±10.94 a
2019	0.549±0.066 a	9.393±1.574 a	350.3±76.44 a	33.51±0.877 a	76.22±2.472 a	203.6±11.32 a
Significance[1]						
F	***	NS	*	***	NS	***
Y	NS	NS	NS	NS	NS	NS
FxY	NS	NS	NS	NS	NS	NS

[1]NS,*, **, ***: Non-significant or significant at P ≤ 0.05, 0.01, 0.005, respectively.

The distribution of vessels in classes of lumen size within each of the groups A, B, C was not similar in the four vineyards (Fig.2). In particular, for the group A of lumen area, SL showed a tendency towards a higher incidence of lumen area in the range 350-500 µm^2 and no vessels below 200 µm^2 (Fig. 2A). The field CA showed a tendency towards a higher incidence of lumen area in the range 200-250 µm^2 (Fig. 2D). GR showed the highest incidence of vessels in the class 100-150 µm^2 and was the sole vineyard to show vessels in the class 50-100 µm^2 (Fig. 2G). AC showed a vessel size distribution in the group A more similar to SL compared to the other two vineyards (Fig. 2L). For the distribution of vessels in the group B, the trends of lumen area distribution were similar in the four vineyards, with most of the water flow relying on vessels with lumen size in the range 750-1750 µm^2 (Fig. 2B, E, H, M). For the group C, all the vineyards with the exception of GR showed similar trends of VLA distribution, also with occurrence of vessels with VLA>50000 µm^2 (Fig. 2C, F, I, N). In GR, most of the water flow relied on vessels with lumen size in the range 7500-17500 µm^2 with no vessel frequency above 42500 µm^2. SL and AC showed a similar trend of the VLA distribution.

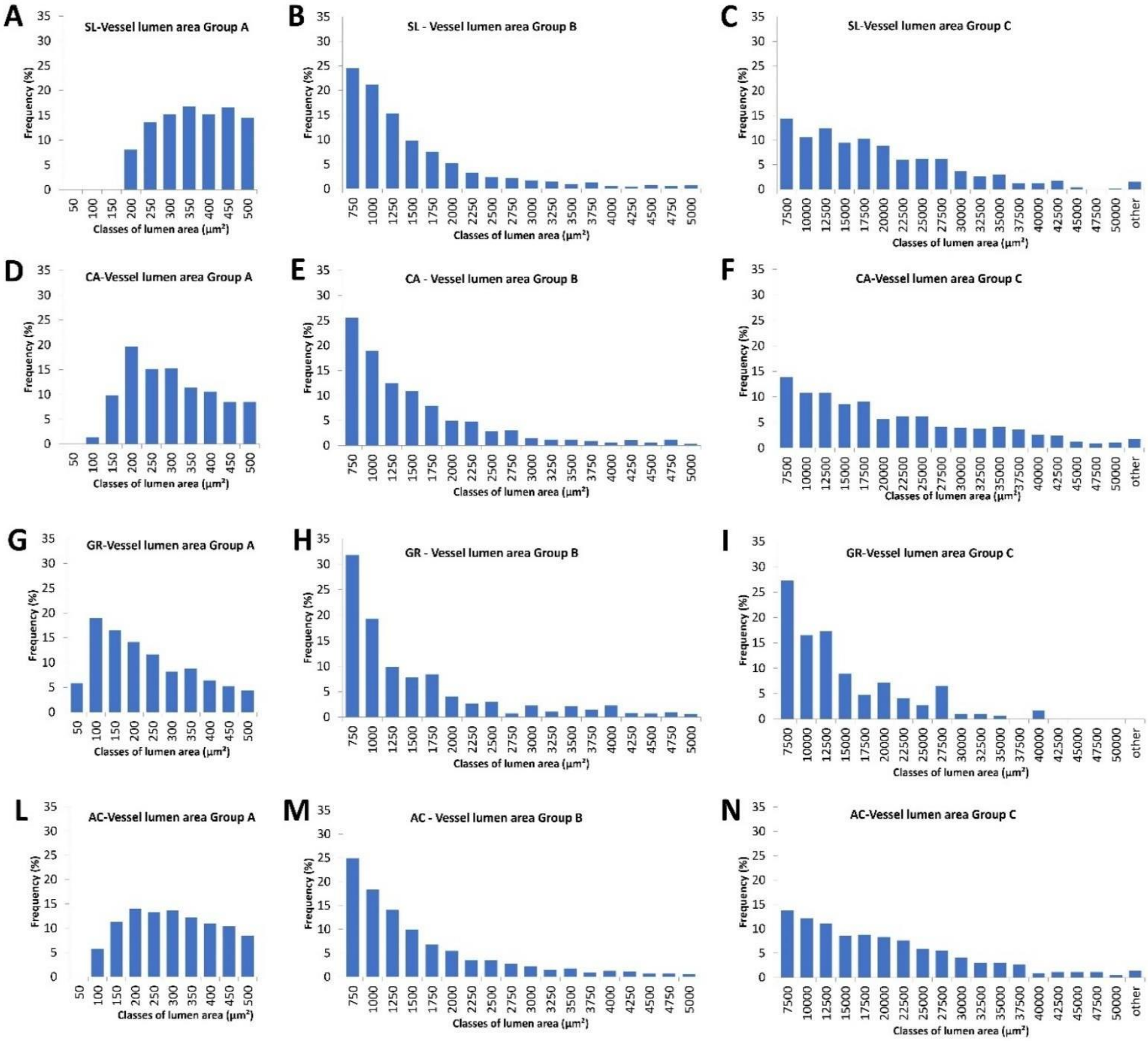

Fig. 2. Distribution of vessels in classes of lumen area in the three groups A (<500 µm^2), B (500 µm^2 <lumen area<5000 µm^2), C (>5000 µm^2) in the trunk wood of vines from the four vineyards: A, Santa Lucia, SL; B, Calvese, CA; C, Grottole, GR; D, Acquefredde, AC.

The carbon isotopes analyses indicated that in SL and AC values of WUEi were significantly lower than in CA and GR (Fig. 3).

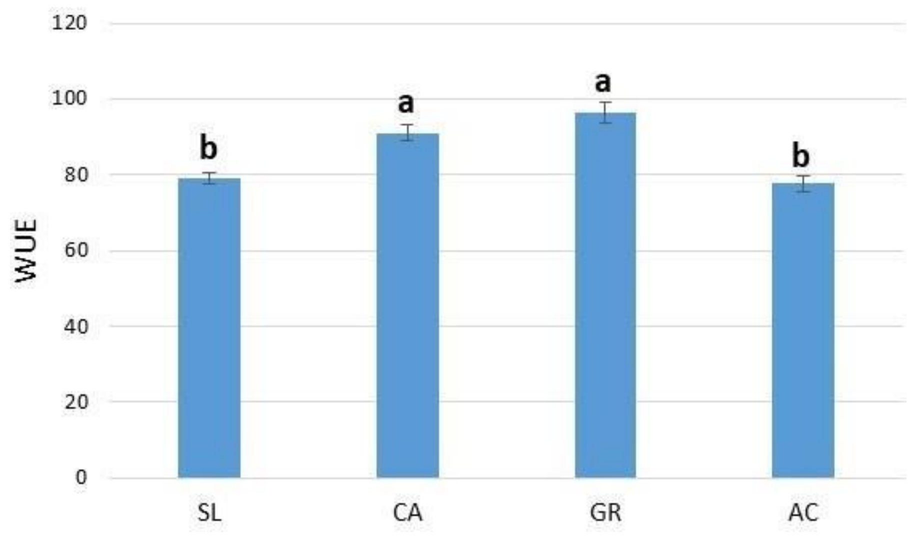

Fig. 3. WUEi values in trunk wood (rings corresponding to the years from 2015 to 2019) of vines from the four vineyards: A, Santa Lucia, SL; B, Calvese, CA; C, Grottole, GR; D, Acquefredde, AC. Mean values and standard errors are shown. Different letters corresponded to significantly different values according to Duncan HSD test ($p < 0.05$).

In the multiple scatter plots (Fig. 4) the correlations among meteorological variables and wood traits are reported. Many positive and negative correlations at different significant levels ($p < 0.001$ ***, $p < 0.005$ **, $p < 0.05$ *) were found. In SL and CA, the WUEi calculated from the ^{13}C values analyzed on wood samples was positively correlated with AMT, AMaxT and AMinT and negatively correlated with CAP. In GR, the WUEi was positively correlated with all the meteorological parameters. In GR, the KhA was negatively correlated with AMT, AMaxT and AMinT. The KhB in SL, CA and in GR was negatively correlated with AMT, AMaxT, AMinTand only in SL positively correlated with CAP. The KhC was negatively correlated with AMT, AMaxT and AMinT in SL, CA, AC and positively correlated with CAP. In GR, the KhC was positively correlated in AMT, AMaxT, AMinT and negatively with CAP. In the vineyard CA, the DhA was negatively correlated with AMT, AMaxT, AMinT and positively with CAP. DhB and DhC followed the same trends of correlations of KhB and KhC in the four vineyards. VA in the vineyards SL, CA, AC was negatively correlated with AMT, AMaxT, AMinT and positively correlated with CAP. RA was positively correlated with AMT, AMaxT, AMinT in the vineyards SL, CA, AC and negatively correlated in GR. RA wasnegatively correlated with CAP in SL and AC and was positively correlated with CAP in CA and GR. FA was negatively correlated with CAP in CA and GR positively with AMT, AMaxT, AMinT. VAMinA was negatively correlated with CAP in SL and AC and negatively correlated with AMT, AMaxT and AMinT in CA. VAMeanA was positively correlated with AMT, AMaxT, AMinT in SL and negatively with CAP in AC. VAMeanC and VAMaxC were positively correlated with AMT, AMaxT, AMinTin and positively with CAP in SL, CA and AC.

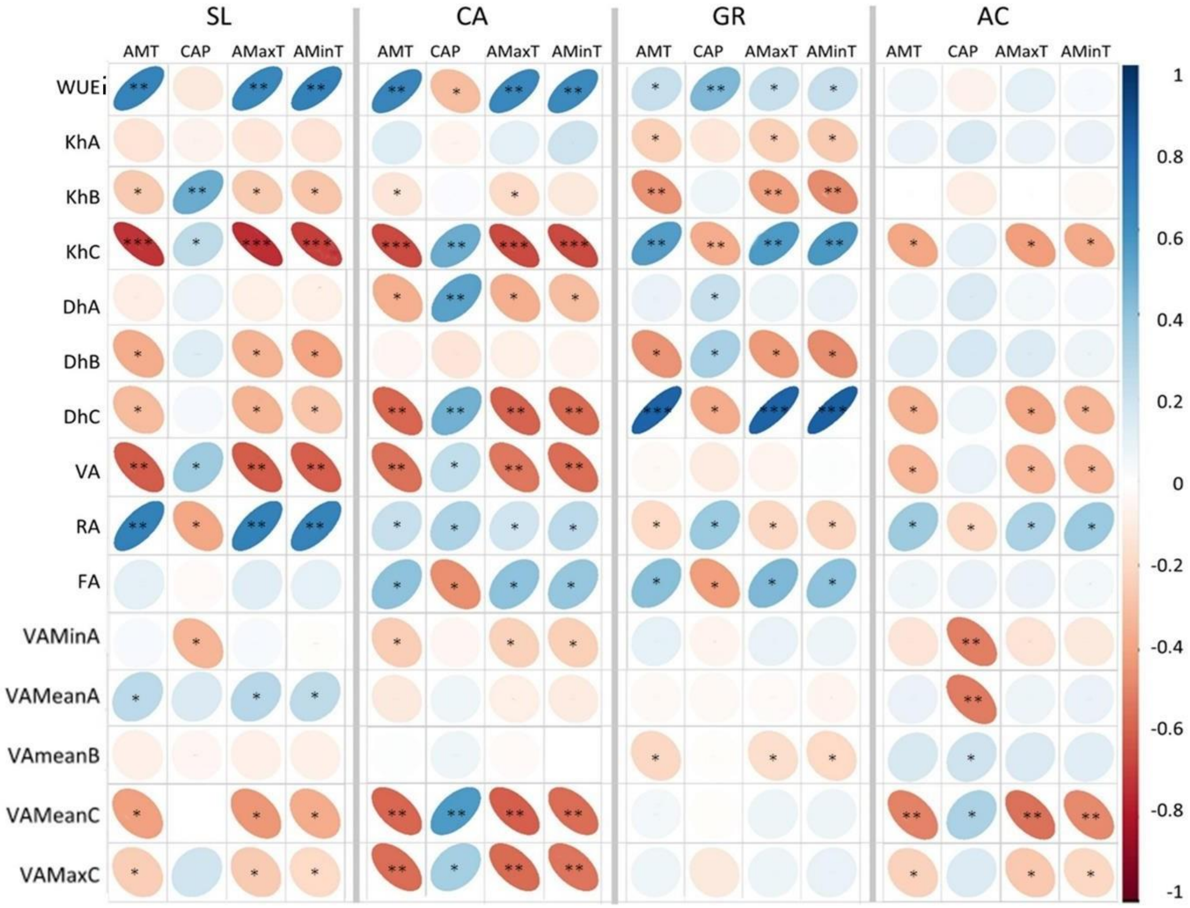

Fig. 4. Spearman's rank correlation coefficients among climatic variables and wood anatomical traits in the four vineyards: A, Santa Lucia, SL; B, Calvese, CA; C, Grottole, GR; D, Acquefredde, AC. Positive (blue) and negative (red) correlations are shown. *, **, ***, significant at p <0.05, 0.01 and 0.001 respectively.

In the dendrogram, the association between sites in the 5 years is reported considering only anatomical and isotopic traits (Fig. 5). It is possible to observe that the four sites were divided in two main groups, the group "a" with only GR samples in all the years analyzed and the other main "b" group with SL, CA and AC. In the group b there are three sub-groups respectively: "c" with 4_2019, SL_2017, SL_2019, SL_1016; "d" with CA_2017, AC_2015, AC_2017, SL_2015; "e" with CA_2015, CA_2019, CA_2016, CA_2016, CA_2018, SL_2018.

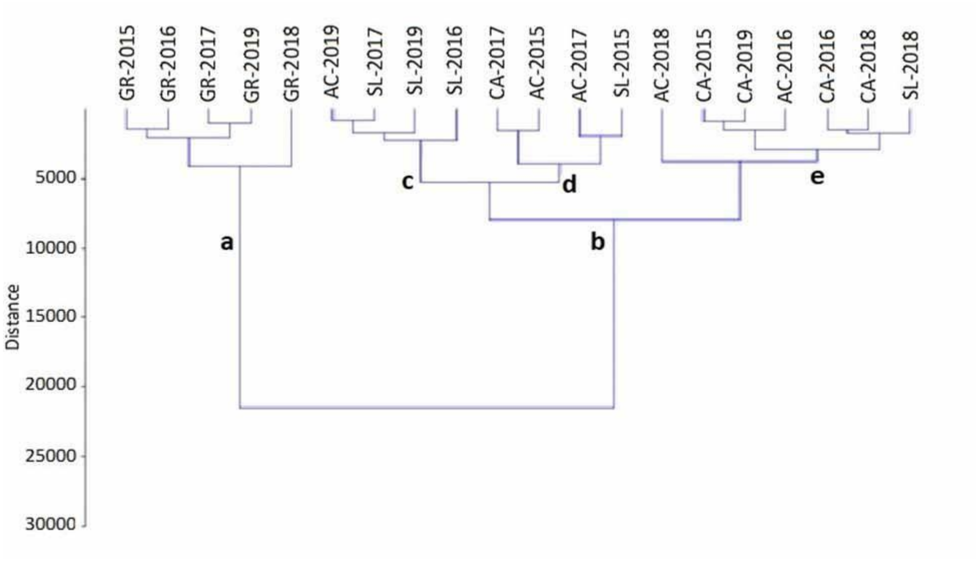

Fig. 5. Hierarchical cluster analysis (HCA) showing relationships between wood anatomical parameters and WUE among the four vineyards (A, Santa Lucia, SL; B, Calvese, CA; C, Grottole, GR; D, Acquefredde, AC) considering the effect of the year 2019, 2018, 2017, 2016, 2015.

The PCA scatterplot (Fig. 6) separates the wood anatomical parameters and WUEi for the four vineyards SL, CA, GR, AC, during the five year 2019, 2018, 2017, 2016, 2015. The first two components explained a total variance of 64.29 % (PC1) and 23.26% (PC2). From the PCA is evident that the vineyards GR (3) is the more different compared to the other three; whereas, CA and AC (2 and 4) are the more similar for wood characteristics.

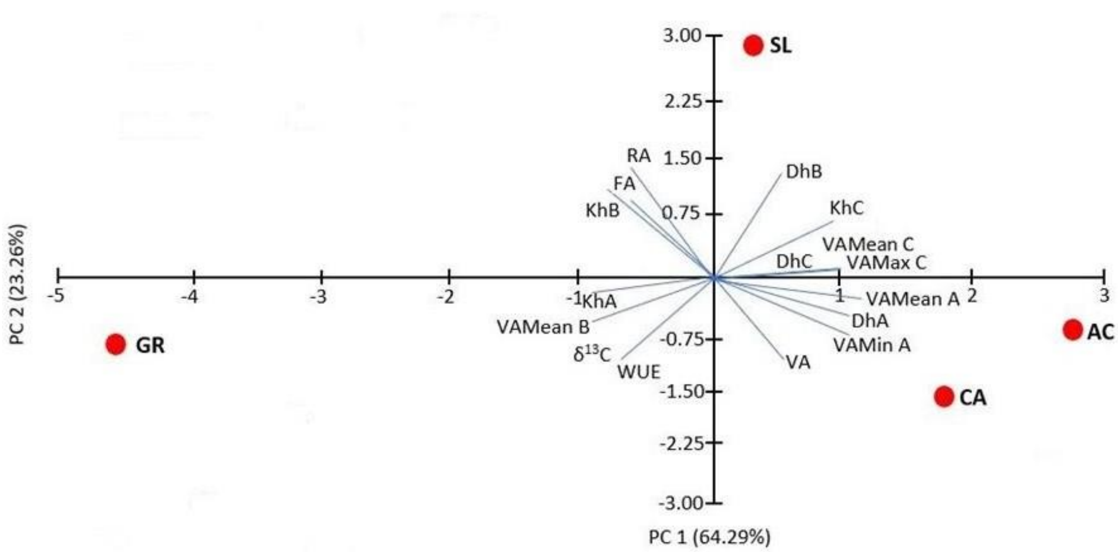

Fig. 6. Principal component analysis (PCA) biplot showing relationships between wood anatomical parameters and WUE among the four vineyards (A, Santa Lucia, SL; B, Calvese, CA; C, Grottole, GR; D, Acquefredde, AC) during the five years 2015-2019.

4. Discussion

This study highlighted how Falanghina grapevine growing under different pedoclimatic conditions develops wood anatomy in line with different photosynthetic behavior and productivity. Indeed, wood anatomical traits and hydraulic characteristics seemed to be differently coordinated according to the different pedoclimatic environment, suggesting a different water use efficiency in the four vineyards in the five analyzed years, likely triggered by spatial variability of temperature and precipitation amount. In general, the four vineyards showed two main patterns of development of the wood anatomical parameters and values of WUEi. In particular, SL and AC were characterised by overall anatomical traits more oriented towards the efficiency of water flow and associated to lower values of WUEi. On the opposite, GR showed safer quantitative anatomical traits against embolism, associated to higher values of WUEi, suggesting a decrease in $^{13}CO_2$ discrimination possibly due to partial or total stomatal closure. Finally, CA seemed to have an intermediate behavior, with anatomical traits more similar to SL and CA, while isotopic values closer to AC. The overall wood data are in line with the previous findings of SL and AC vines associated to a condition of higher water availability than CA and GR (Damiano *et al.*, 2022a, 2022b). The efficiency in water transportaffects the photosynthetic and reproductive organs hydration that is directly related to the vascular cambium plasticity to create an apoplastic hydrosystem adapted to the environmentalgrowth conditions (Hacke *et al.* 2017b; Islam *et al.* 2019; de Melo *et al.* 2018). This in turn causes changes in vessel size at intra- and inter-specific level (Hacke *et al.* 2017b) when plantsare affected by growth limiting factors, such as water shortage (de Melo *et al.* 2018; Schmitz *etal.* 2006). Analyzing the vessel size distribution among classes of diameter is important because changes in vessel lumen diameter may cause considerable modifications in the volume of xylem-sap flow according to Hagen-Poiseuille's law (Hacke *et al.* 2017b; Islam *et al.* 2019), and the larger are the vessels, the more sensitive is the impact on water flow of even a small increase in vessel size. While the analysis of larger vessels is important to gain information on water flow efficiency, there is increasing awareness that very narrow vessels in grapevine need to be considered in any study aiming to understand the vine water flow and ability to acclimate under limiting water availability. Indeed, a new paradigm has been recently enunciated in grapevine that xylem heterogeneity reduces flow rates in wider vessels by redirecting 15% of total flow towards narrow vessels, due to occurrence of transverse pressure gradients (Bouda *et al.*, 2019). Observing the data of VAMinA and vessel size distribution in the group of the narrowest vessel lumen (group A), GR wood tended to favor the occurrence of very narrow vessels with higher incidence of narrower vessels compared to the other vineyards, especially SL and AC. On the other hand, the VAMeanC, VAMaxC and vessel size distribution in the group of the largest vessel lumen (group C) suggest that water flow in GR wood mainly relies on classes of vessel lumen size up to a certain threshold, while the incidence of very large vessels is limited compared to the other vineyards. The xylem heterogeneity in vines at the four sites was reflected also in Kh which takes into account also vessel frequency. The potential hydraulic conductivity Kh of the three classes of dimension A, B and C among the four vineyards was different, indicating different water flow strategies. Indeed, wood anatomy is strictly related to the occurrence of hydraulic failure since wide xylem vessels are more prone to embolize than narrow vessels (Nardini *et al*. 2013). Embolism resistance is associated to a reduction in lumen area or to mechanical reinforcement of fibers, preventing xylem embolism and drought damage (Colangelo *et al*. 2017; Nardini *et al*. 2013). In our case, the distribution between cell types (vessels, fibers and parenchyma cells) was confirmed to be a quite stable character among vineyards suggesting no variability in mechanical resistance of the wood in the different pedo-climatic conditions. Therefore, GR vines present a trunk vessel structure that would guarantee continuous, although slow, water flow under conditions of severe water shortage (Colangelo *et al.* 2017). On the contrary, in presence of optimal conditions of temperature and precipitation vines at GR would still have reduced performances and growth compared to the other vineyards. The different wood hydraulic behavior suggested by anatomical and isotopic traits is in agreement with leaf anatomical traits (chapter 2). Indeed, vines at SL were characterised by leaf vein and stomatal traits (stomatal frequency, stomatal size, Minor VLA, Major VLA, Minor VAA, Major VAA, Total VLA, Total VAA), allowing the plants to maintain stomata open to sustain high photosynthetic rates, notwithstanding the increasing water losses through transpiration, even in conditions when vines at CA and GR promptly close stomata to limit transpiration although encountering lower net CO_2 assimilation rate (Damiano *et al*. 2022a). However, embolism risk in SL vines would be limited as well due to other adjustments at the leaf anatomical level as the occurrence of narrower leaf veins which are recognized tocontribute to vine drought tolerance and specific leaf venation controlling leaf hydraulic conductance (Broddrib *et al*., 2016; Creek *et al*., 2020; Dayer *et al*. 2020; Damiano *et al*., 2022a). In vineyards SL and AC the vine evapotranspiration demand seems to be continuouslysatisfied, differently from CA and GR where the higher levels of WUEi also in wood comparedto SL and AC, together with the overall quantitative anatomical data, suggest that they have been likely subjected to water shortage also in the 5 years analyzed (Damiano *et al*. 2022a). The stomatal closure occurring during drought stress normally determines increasing in WUEivalues in plant tissues and therefore an increase in WUEi (Altieri *et al*. 2015). This agrees withdata from the analysis of the isotopic trace in musts of the same vines and with low photosynthetic rate recorded in CA and GR (Damiano *et al*., 2022a, b). It is widely accepted that scarce water availability triggers the formation of narrow vessels because of a reduced turgor-driven enlargement during cell differentiation and as a strategy to reduce the cavitation risk (Hacke *et al.* 2006). Grapevine wood couples the occurrence of frequent wide vessels, typical of climbers, to high vessel size heterogeneity in a strategy favoring water transport efficiency, under favorable environmental conditions, still maintaining safety against embolism, during drought events as occurs in wood of many Mediterranean species (Baas & Schweingruber, 1987; De Micco *et al.* 2008). The occurrence in a wood of different vessel size classes, also known as vessel dimorphism, is considered an adaptive trait to drought, being also a well-established ecological trend in woods from boreal, via temperate up to Mediterranean ecosystems (Baas & Schweingruber, 1987). The distribution of vessels in different classes of vessel lumen size can be altered in response to environmental changes but also to local pedo-climatic conditions and cultivation practices (Cirillo *et al.* 2017). Comparing the two vineyards characterised by the lower water availability in the soil, namely CA and GR, wood traits in CA vineyard, especially the larger range of vessel size distribution compared to GR, would explain the better growth performances of the first especially in dryer years compared to the second (Damiano *et al.* 2022a). The analysis of correlations between wood parameters and climatic data supports the idea of a xylem plasticity triggered by temperature values and precipitation amount during the vegetative and reproductive seasons, but not with the same strength in all vineyards as if local pedo-climatic conditions would act as a buffer. Indeed, WUEi was positively correlated with temperature parameters (AMT, AMaxT, AMinT) in SL, CA and GR, and negatively correlated with precipitation (CAP) in SL and CA. In the two latter sites, data suggest that warm and dry conditions induce adjustments at wood level increasing the amount of CO_2 fixed per transpired water also granted by a slow, still continuous, water flow thanks to a tendency to reduce vessel size. This is supported also by the negative and positive correlations between hydraulic conductivity of classes of larger vessel lumen size (KhB and KhC) and temperature and precipitation respectively. Such trends are quite consolidated as a typical response to dry conditions also in other species (Abrantes *et al.*, 2013; Balzano *et al.*, 2020). It is interesting to observe that in GR, the correlation between WUEi and precipitation was positive, while increasing temperature and decreasing precipitation would increase the incidence of water flow relying on the classes of larger vessels (i.e. positive correlation between AMT, AMaxT and AMinT and KhC, while negative correlation between CAP and KhC. Such a strategy might be possible in GR vines because in case of severe drought such plants can respond with prompt stomata closure due to their leaf anatomical traits such as small and fast-responding guard cells (Damiano *et al.* 2022a). In the case of AC, significant relations between climatic data and WUEi were lacking, as well as the relations between wood anatomical parameters and climatic data followed the same trends as in SL and CA but they were less strong. This is likely due to the cultivation management allowing supplemental irrigation in case of severe drought which would have hidden the relations with temperature and precipitation data. The application of irrigation strategies combined with sustainable soil management practices (such as composting, mulching, cover crops, and reduced tillage) – can improve WUE in vineyards in semi-arid region having a great potential role in the adaptation to and mitigation of climate changes (Romero *et al.* 2022). Indeed, AC vineyard was classified as not drought stressed according to Santesteban *et al*. (2021), who proposed thresholds of $\delta^{13}C$ to distinguish between weak/null and severe water deficit and this is supported also by high photosynthetic levels and yield of vines at this site (Damiano *et al.* 2022a, b).

In conclusion, data shown supports the idea that of wood anatomical traits are likely modulated by pedoclimatic conditions during vine growth and may severely influence the physiological responses of a grapevine cultivar to short-term changes in water availability as supported by isotopic trace in wood-ring series. The different anatomical traits did not show a unique trend of variation according to temperature and precipitation parameters in the four vineyards, thus confirming that suites of anatomical features determine the acclimation capability of a species/cultivar to limiting environmental conditions (De Micco & Aronne, 2012). The suites of anatomical features found in the four vineyards, according to the spatial variation of pedoclimatic conditions, suggested the occurrence of different water use strategies between the two wetter and the two drier sites, which were also in agreement with leaf functional traits related to mesophyll and stomatal conductance highlighted in a previous study (Damiano *et al.* 2022a). In a gradient of water availability, SL is in the wettest end, thus acting as water-spender, and GR is at the driest end, thus being more water-saving. The importance of coordination between stomata and xylem traits for plant acclimation is recognized also at the evolutionary scale, and known as a "400 million year history of collaboration" (Brodribb *et al.*, 2016). Indeed, our overall analysis support the idea that to achieve a comprehensive understanding of vine water use and to develop appropriate cultivation management to promote vine acclimation to face drought conditions, wood anatomical traits cannot be disregarded and must be taken into account together with all leaf hydraulic traits, especially in the sight of a precision viticulture application.

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Chapter 8

Microvinifications of Falanghina grapes produced under different pedoclimatic conditions

1. Introduction

Climate is a determinant driver for grapevine geographical distribution, berry characteristics, must and wine quality around the world (Rosas et al., 2022). The Mediterranean region is threatened by climate change, where climate models project significant increase in temperature and high irregularities in precipitation patterns (Pörtner et al., 2022). Increase in the frequency, duration and severity of drought events as well as a shift in time of their occurrence will likely induce plastic adaptive responses in plants, expecting a negative impact on plant growth, as the case of grapevine, which is one of the most widespread crops worldwide, with about 38% of vineyards areas located in Europe (Bonfante et al., 2018; Tomás et al., 2014). Since it has been forecasted a dramatic change in the landscape with geographical shifting of the grapevine production regions, climate change is one of the major challenges for future viticulture, especially in arid and semi-arid regions of Europe (Chmielewski & Rötzer, 2001). Often, the combination of heat and severe water-deficit stress may compromise the berry maturation, with reduction in yield and atypical composition of grapes with problems during vinification. As a matter of fact the consequences for the organic acid composition of grapevines can be enormous with variation of The sour-sweet balance with risk for the wine stability against microbes as well as oxidation processes and for wine gustative equilibrium and then for typicity (Ribéreau-Gayon et al., 2006). Such a phenomenon is becoming more and more worrying in many areas of southern Italy where, alterations in titratable acidity content and in sugar accumulation dynamics have been detected in grapes notwithstanding the limited variations of pH values (Picariello et al., 2019).

Within this general framework, the aim of this study was to monitor if and to what extent pedoclimatic variability is reflected into wines produced in four vineyards of Falanghina grapevine growing in southern Italy over three years. The Falanghina grapevine is an autochthonous non- aromatic cultivar of Campania region in southern Italy (Boselli et al., 2000), characterized by middle trunk conical bunch, medium sized berries, (De Filippis et al., 2019; Di Vaio et al., 2020), used to obtain typical white wines characterized by the aroma of floral and fruity notes (Lamorte et al., 2008; Nasi et al., 2006). It is present in many Italian DOC (Designation of Controlled Origin) and IGT (Typical Geographical Indication) wine denominations ., as the vineyards of the study case placed in the area of DOC Falanghina del Sannio (DM 30.11.2011 G.U. 295 - 20.12.2011).

Therefore, within this context, the analytical and sensory evaluation of the wines were performed and interpreted together with growth, production and eco-physiological data to assess the relations between vegetative growth and berry and wine quality in order to have valuable information for the vineyard management targeted to specific oenological objectives.

2. Materials and methods

2.1 Harvesting and microvinifications

The micro vinifications were carried out in parallel for each of the four experimental vineyards (SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquefredde) over three years. Representative samples of grapes (180 kg per field) were harvested in the same day and processed by cellar winemaker applying the same protocol at La Guardiense farm.

The grapes were crushed and destemmed by a crusher-destemmer (NDC8, crusher-destemmer NDC series, Della Toffola) and immediately pressed. The obtained must was sulphited (potassium metabisulphite 8 g/hl + ascorbic acid 5g/hl) , and pectolytic enzymes 1 g/hl were added. Field temperature was lowered by CO_2 pellets. After 24 hours cleaned musts were transferred into 25 liter glass container to undergo alcoholic fermentation by the inoculation of Saccharomyces cerevisiae (Flavor 2000, Enologica Vason, Italy) at 20 g/hl. The fermentation was carried out at controlled temperature (16-18°C); yeast nutrients were supplied in order to have a regular yeast activity and sugar content were measured daily. All microvinifications were conducted in triplicate.

Finished wines were racked off, when all the trials reached a volumic mass of 0.995 gcm-3, and reducing sugars were lower than 2 gL^{-1}. Then, 20 mgL^{-1} of sulfur dioxide were added, and a clarification was carried out with 50g/hl of bentonite (TopGranPiù, Dal Cin Gildo Spa, Italy). Wines were stored at 15 °C in the tanks and then bottled at the end of the malolactic fermentation. The wines were not filtered and then bottled using 0.75 l bottles (European Bordeaux bottle 750 mL uvag) and subsequently capped with agglomerated corks.

2.2 Wines analyses

The analyses on the wines were performed with the WineScan ™ - FOSS analyzer (Padova, Italy). This instrument adopt the Fourier Transform Infrared spectroscopy (FTIR) based on the principle that functional groups within a sample will vibrate upon exposure to IR radiation allowing the measurement of the wine concentration constituents. Wine samples were also analysed with the instrument Dyonisos 150 SinaTech (Grottazzolina FM - Italy) consisting in enzymatic analysis using an automatic sequential sampler equipped with a spectrophotometer with absorption detector of UV radiations. The parameters measured with the WineScan ™-FOSS were: Alcohol, titratable acidity (TA), volatile acidity (VA), pH, Malic acid, Tartaric acid Citric acid, total polyphenols (PFT), and yeast assimilable nitrogen (YAN). The parameters analysed with Dyonisos 150 SinaTech were Malic acid, Amino nitrogen, NH_4^+, Calcium, Catechins and YAN.

Figure 1. Samples of the four wine in order from left to right site: SL-Santa Lucia, CA-Calvese, GR-Grottole and AC-Acquefredde filtered with a paper filter before to perform the analyses at the WineScan ™ - FOSS wine analyzer.

3. Results

Wine chemical analysis of the four vineyards in the three years (2019-2020-2021) are reported in table 1. The main effect of field (F) was significant for all parameters analysed. The Alcohol values in CA were significantly higher than the others with decreasing trend in GR, AC and SL. The titratable acidity (TA) showed significantly higher value in SL, followed by AC, GR and CA. The volatile acidity (VA) was in SL significantly lower that CA, GR and AC. The pH in AC and GR was significantly higher than CA, which in turn was significantly higher than SL.. The main factor of year (Y) was significant for all the studied parameters. Alcohol showed in year 2020 a value significantly higher than 2019 and 2021. TA and Tartaric acid in 2019 was higher than 2020, which in turn wassignificantly higher than 2021. The parameters VA and pH showed the highest values in 2021 followed by 2020 and 2019. The interaction FxY was significant for all parameters with significant differences showed in the table S1 of the appendix.

Table 1. Effects of field (F), year (Y), and their interaction (F x Y) on Alcohol, TA, AV , pH, of *V. vinifera* subsp. *vinifera* 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

	Alcohol	TA	VA	pH
	Vol %	*g/l*	*g/l*	
Field (F)				
SL	12.21±0.31 d	6.67±0.61 a	0.303±0.073 b	3.37±0.06 c
CA	13.50±0.19 a	5.61±0.39 d	0.356±0.025 a	3.41±0.04 b
GR	13.31±0.24 b	5.71±0.35 c	0.378±0.027 a	3.43±0.03 a
AC	13.09±0.18 c	6.45±0.49 b	0.355±0.057 a	3.45±0.03 a
Year (Y)				
2019	12.83±0.30 b	7.43±0.19 a	0.232±0.039 c	3.29±0.03 c
2020	13.49±0.15 a	6.47±0.27 b	0.304±0.011 b	3.40±0.02 b
2021	12.77±0.19 b	4.42±0.02 c	0.509±0.017 a	3.55±0.01 a
Significance[1]				
F	***	***	***	***
Y	***	***	***	***
F x Y	***	***	***	***

[1]NS, *, **, and ***, Not significant or significant at p < 0.05, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests (p ≤0.05).

Wine chemical analysis of the four vineyards in the three years (2019-2020-2021) are reported in table 2 in terms of PFT, YAN.. The main effect of field (F) was significant for all parameters analysed except YAN. Total polyphenols (PFT) in GR were significantly higher than AC, which in turn were significantly higher than both SL and CA. Malic acid showed in SL and AC significant higher values than GR, which in turn showed a value higher than CA. Tartaric acid in SL was significantly higher than AC, which in turn showed values significantly higher than both CA and GR. Citric acid in AC was significantly higher than SL, CA and GR. The main effect (Y) was significant for all the analysed parameters. PFT was in 2020 significantly higher than values of 2019, in turn significantly higher than 2021. YAN and Citric acid showed values in 2021 significantly lower than both 2019 and 2020. Malic acid was significantly higher in year 2019, than 2020 and 2021. Citric acid was significantly higher in 2021 than both 2019 and 2020. The interaction FxY was significant for all parameters but YAN, with significant differences showed in the table S2 of appendix.

Table 2. Effects of field (F), year (Y), and their interaction (F x Y) on PFT, YAN, Malic acid, Tartaric acid and Citric acid, , , of *V. vinifera* subsp. *vinifera* 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

	PFT	YAN	Malic acid	Tartaric acid	Citric acid
	ppm	*mg/l*	*g/l*	*g/l*	*g/l*
Field (F)					
SL	372.5±48.5 c	92.6±31.4 a	3.41±0.08 a	3.85±0.68 a	0.270±0.035 b
CA	368.5±18.2 c	84.8±8.02 a	2.3±0.14 c	2.16±0.11 c	0.278±0.032 b
GR	425.5±40.1 a	84.2±10.1 a	2.48±0.09 b	2.13±0.05 c	0.285±0.034 b
AC	410.0±82.6 b	60.7±12.9 a	3.33±0.12 a	3.15±0.46 b	0.304±0.042 a
Year (Y)					
2019	348.4±17.1 b	102±2.03 a	3.09±0.11 a	4.10±0.52 a	0.352±0.006 a
2020	573.2±31.8 a	95.1±23.9 a	2.81±0.20 b	2.28±0.13 b	0.357±0.010 a
2021	260.8±14.2 c	44.6±1.94 b	2.74±0.19 b	2.07±0.03 c	0.144±0.005 b
Significance[1]					
F	***	NS	***	***	***
Y	***	*	***	***	**
F x Y	***	NS	***	***	***

[1]NS, *, **, and ***, Not significant or significant at p < 0.05, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests (p ≤0.05).

In the table 3 are reported the results obtained from the analyses performed with the enzymatic analyses of Dyonisos 150 SinaTech . The results during the three year of analyses showed in SL a tendency for Malic Acid to reach higher value than CA, GR and AC. Amino nitrogen , $NH_4^{+,}$ YAN and Catechins showed variable values among the four vineyards depending to the year effect. Calcium in AC showed a tendency to reach higher values than those of SL, CA and GR during the three years.

Table 3. Results of the enzymatic analysis of Malic Acid, Amino Nitrogen, Nh4, Calcium, Catechins and YAN of *V. vinifera* subsp. *vinifera* 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde.

Field	Year	Malic acid	Amino nitrogen	NH_4^+	Calcium	Catechins	YAN
		g/l	*g/l*	*mg/l*	*mg/l*	*mg/l*	*mg/l*
	2019						
SL		3.81	77	75	121.36	18.12	138
CA		2.61	81	78	120.28	46.86	145
GR		2.79	97	76	118.44	52.84	160
AC		2.92	69	95	184.36	38.55	147
	2020						
SL		4.12	87	72	118.24	25.43	144
CA		2.77	77	74	119.28	44.34	140
GR		3.12	91	72	100.44	56.84	162
AC		3.77	80	93	156.36	48.12	135
	2021						
SL		3.84	83	77	116.73	31.24	147
CA		2.46	68	71	120.44	36.66	128
GR		2.76	94	83	117.44	40.44	158
AC		3.55	76	78	144.38	59.33	141

4. Discussion

The alcohol content of the wines obtained from the grapes harvested in the CA and GR sites, respectively 13.50 vol. % and 13.31 vol. %, was higher than the wines obtained from the grapes of the SL and AC sites respectively 11.19 vol.% and 12.24 vol.%. Anyway all the wines obtained an alcoholic volume higher than the minimum limit imposed from the DOC production disciplinary Falanghina del Sannio, which is 11.50 vol.% (DM 30.11.2011 G.U. 295 - 20.12.2011).

Alcohol content is one of the main parameters for the quality evaluation of wines, primarily for its antiseptic properties and then biological stability of wine. It contributes to sensory balance of wine strengthening the sensation of body and softness of the wine attenuating the effect of fixed acidity and mineral salts. On the other hand, low alcoholed wines are becoming popular for consumer awareness of the health risk associated with excessive alcohol consumption. . A higher alcohol content is certainly due to the concentration of soluble sugars present in the must (Chapter 3). In our case, the grapes of the SL site showed the lowest concentration in soluble sugars and the grapes of the CA site the highest concentration (Chapter 3). . The customers are oriented to consume wine with low level of alcohol, obtaining wines with a not very high alcohol content, but in recent years this is becoming a difficult goal to achieve in vintages characterized by excessive thermal and water stress due to climate change. Very often it is difficult to choose the right time for the grape harvest as the excessive thermal and water stress in the summer leads to an imbalance in ripening with an early accumulation of soluble sugars and therefore difficulty in waiting for the accumulation of aromatic precursors necessary to obtain quality wines that proceed more slowly (Kuhn et al., 2014).

Titratable acidity directly affects the color and aroma of the wine and play an important role in in gustatory balance with the sweet and dry flavors of the other components. The total acidity of wines is generally between 4.5 and 9 g / L expressed as tartaric acid (Frost et al., 2017), sweeter wines requiring a slightly higher level to balance the different flavours. Wines with low total acidity can be considered of poor quality since the wine stability against microbes as well as color stability and oxidation processes, thus alters the wine gustative equilibrium (Picariello et al., 2019).

It is known that tartrate/malate ratio at maturity for a given variety in a given region is relatively constant, unless unusual climatic conditions occur during the harvest. Warmer temperatures can infact disrupt the physiological life cycle of grapevine by determining an early onset of flowering and fruit ripening. As a consequence, grapes will be characterized by low acidity (especially malic acid). (Picariello et al., 2019).

The wines obtained showed good total acidity in particular the wine from the SL and AC site, while the wine from the CA and GR sites have a lower total acidity. .

Wines from the SL and AC site have a concentration of malic acid, tartaric acid and citric acid higher than other wines, which explains the high total acidity. Grape berries make respiration actively during the early stages of growth, but the intensity of respiration slows down as they advance in age. Prior to the onset of véraison, L-malic acid is the most abundant organic acid (up to 25 g/L) in the grape berry vacuole, resulting in the low pH of 2.5 of grapes (Ruffner, 1982; Ribéreau-Gayon et al., 2000). But during véraison, the availability of the respiratory substrate, sucrose (via photosynthesis), becomes limited due to the degradation of chlorophyll. The berry is therefore forced to shift its metabolism from sugar to L-malic acid respiration and probably in CA and GR the drought stress accelerates this process leading to a higher degradation of malic acid compared to the vineyards SL and AC.

Another qualitative parameter is the pH, that in white wines usually ranges between 3 and 3.5, and the VA that usually stay in a range of 0.3-0.4 g/l (Chidi et al., 2018). Differences in pH and VA were found but with infinitesimal variationsa. Above the four wines showed a pH in the range between 3-3.5 and VA in the range of 0.3-0.4 g/l.

Usually tartaric acid is a stronger acid than either citric or malic acids, thus implying that for the same molar concentration, the wine pH level will result lower with tartaric acid because of its intrinsic higher tendency to release protons. , Tartaric acid is also less susceptible to climatic conditions during ripening and it is reported by Poni et al. 2018 that varieties with a high tartaric acidcontent are consequently more resistant to climate changes. In our case tartaric acid showed to be inhigher level in SL and AC vineyards compared to the other two vineyards.

Concerning polyphenols, they are responsible for important sensory characteristics of wine such as color, astringency and bitterness. . In the wines analysed, a high concentration of total polyphenols was observed in the wines of the study sites GR and AC. This could depend on the lower vegetative development of the vines which might have exposed the grapes to higher solar radiation thus improving the phenolics accumulation as a defence strategy.

An informal panel test was also performed to evaluate the wine quality, highlighting similarity and differences among the wines produced from the four vineyards. From a visual point of view, the four wines are all showing good clarity, with a pale straw yellow color and greenish reflections for wines of SL, AC, GR while an intense straw yellow color, with golden reflections for CA wine. The olfactory analysis of the wine obtained from SL showed a preponderant herbaceous note with very light floral aromas, similarly to the wine obtained from GR vineyard. In CA wine, very fruity aromas were identified compared to other three wines, with reminiscent of ripe fruit, while AC wine had a well-balanced floral component with delicate fruity aromas. On the palate, the wine of the SL field was characterized by a very marked acidity that gives at the wine a consistent structure. The wine of the GR site as for the sense of smell was similar to SL but differed in a higher alcoholic component and a taste with a less marked acidity but still preponderant on the other sensations perceived. The CA wine had a round taste tending to sweet, a well perceptible alcoholic sensation that gives heat to the palate, a perceptible acid shoulder and overall with a persistent taste. The wine obtained from AC, on the palate showed a good acid shoulder which gives freshness to the palate, well balanced with softness and good flavor.

In conclusion, the four vineyards have proved to give a wine with different characteristics strongly dependent on the must composition, with SL and AC vineyards showing high level of alcohol but lower than wines of CA and GR, and a high value of TA which is important to obtain wine with balanced gustative profile. The lower level of acidity in GR and AC may indicate a more severe drought stress in this two vineyards reflected also by the TA value and lower malic acid.

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Appendix

Table S1. Effects of interaction (F x Y) on Alcohol, TA, AV, pH, Malic acid, of *V. vinifera* subsp. *vinifera* 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

	Alcohol	TA	AV	pH	Malic acid
	Vol %	*g/l*	*g/l*	*g/l*	*g/l*
Field (F)					
SL 2019	11.2±0.071 g	8.53±0.015 a	0.033±0.003 a	3.12±0.008 d	3.73±0.044 a
SL 2020	13.3±0.076 c	7.10±0.009 d	0.336±0.009 cd	3.45±0.015 b	3.25±0.012 cd
SL 2021	12.1±0.017 f	4.37±0.012 i	0.54±0.012 f	3.54±0.01 a	3.25±0.022 cd
CA 2019	13.5±0.024 bc	7.05±0.015 d	0.283±0.009 de	3.34±0.018 c	2.86±0.032 c
CA 2020	14.1±0.076 a	5.43±0.032 g	0.333±0.007 cd	3.32±0.013 c	2.02±0.009 d
CA 2021	12.8±0.032 d	4.33±0.018 i	0.453±0.012 b	3.55±0.012 a	2.02±0.009 d
GR 2019	13.8±0.019 ab	6.93±0.035 e	0.373±0.047 c	3.34±0.017 c	2.85±0.021 c
GR 2020	13.7±0.088 abc	5.74±0.034 f	0.303±0.003 cde	3.41±0.009 b	2.35±0.015 d
GR 2021	12.3±0.02 ef	4.46±0.015 h	0.465±0.035 b	3.54±0.02 a	2.26±0.02 d
AC 2019	12.7±0.048 de	7.22±0.006 c	0.24±0.012 e	3.34±0.007 c	2.91±0.026 c
AC 2020	12.7±0.042 de	7.63±0.006 b	0.243±0.015 e	3.43±0.009 b	3.62±0.012 bc
AC 2021	13.3±0.465 c	4.50±0.013 h	0.55±0.051 a	3.56±0.018 a	2.96±0.405 c
Significance[1]	***	***	***	***	***

[1]NS, *, **, and ***, Not significant or significant at p < 0.05, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests (p ≤ 0.05

Table S2. Effects of interaction (F x Y) on Tartaric acid, Citric acid ANT, PFT, EST, of *V. vinifera* subsp. *vinifera* 'Falanghina' vines at the four study sites: SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquafredde. Different letters within column indicate significant differences according to Duncan's multiple-range test (P≤0.05). Mean values and standard errors are shown.

	Tartaric acid	Citric acid	ANT	PFT	EST
	g/l	*g/l*			
Field (F)					
SL 2019	6.53±0.029 a	0.351±0.006 b	38.0±1.00 d	326±4.66 d	30.9±0.008 b
SL 2020	2.78±0.058 c	0.330±0.012 b	204±26.0 ab	559±2.08 b	26.9±0.005 c
SL 2021	2.23±0.012 e	0.132±0.012 c	150±80.3 abc	232±0.88 f	18.2±0.026 h
CA 2019	2.6±0.009 d	0.337±0.009 b	115±37.3 bc	337±1.73 d	23.5±0.041 f
CA 2020	1.85±0.017 h	0.347±0.009 b	154±38.8 abc	441±0.88 c	26.5±0.031 d
CA 2021	2.03±0.011 fg	0.153±0.003c	90.3±9.10 bc	327±2.96 d	18.7±0.001h
GR 2019	2.3±0.028 e	0.361±0.012 b	145±3.20 abc	441±2.96 c	24.8±0.024 e
GR 2020	1.97±0.017 g	0.341±0.009 b	131±9.00 bc	555±1.67 b	22.8±0.033 g
GR 2021	2.11±0.05 f	0.150±0.020 c	96.0±1.00 bc	282±5.00 e	18.7±0.070 h
AC 2019	4.95±0.041 b	0.363±0.015 b	146±5.20 abc	288±4.09 e	23.5±0.029 f
AC 2020	2.54±0.015 d	0.410±0.006 a	256±59.7a	737±1.73 a	39.1±0.020 a
AC 2021	2±0.044 g	0.142±0.012 c	79.6±17.3 d	228±23.0 f	18.7±0.023 h
Significance[1]	***	***	NS	***	***

[1]NS, *, **, and ***, Not significant or significant at p < 0.05, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple comparison tests (p ≤ 0.05