



Influence of deficit irrigation and canopy structure on water stress management of Touriga Nacional (*Vitis vinifera* L.) in Douro Demarcated Region

Ana Flávia Bonatto Damasceno

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Supervised by:

Prof. Dr. António Castro Ribeiro

Prof^a. Dr^a. Simone Beux

MSc. David Santos Barreales

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"Somewhere, something incredible is waiting to be known."

- *Carl Sagan*

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To be part of the scientific community, even in a tiny portion, is a joy. Not only because my home country is currently suffering from the bankruptcy of democracy and dismantle of education, but also because I do believe that science can make a change.

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Abstract

A worldwide relevant winemaking place, the Douro Demarcated Region, located in Northeast Portugal, is characterized by its Mediterranean climate, with a marked water deficit in the summer period and high temperatures. This aspect may reduce vineyards' productivity and quality. Within the scope of this master's thesis, this study proposed to understand the vineyard relations with the climate changes at the Douro Demarcated Region, establishing three different levels of deficit irrigation (25, 50, and 75% ETc) and two control fields (non-irrigated). Besides the water stress condition, plants were trained in different canopy heights (tall and short) to understand its influence on physiological and agronomical parameters. Evaluation of evapotranspiration (ETc), predawn water potential, NDVI, CCI, leaf gas exchange, and leaf area took place throughout veraison. To control maturation, weight, diameter, and chemical characteristics of berries were measured. At harvest, FTIR preceded chemical characteristics of the must. Pruning weight and Ravaz index completed yield evaluations. The results of this research corroborate with previous studies, indicating that rainfed vines can have a lower yield at harvest than the ones irrigated, due to climatic stress conditions. Moreover, the rainfed vine with the shorter canopy structure could have a higher yield compared to the taller canopy.

Keywords: vine; deficit irrigation; canopy structure; Douro Demarcated Region.

Resumo

A Região Demarcada do Douro, localizada no nordeste de Portugal, caracteriza-se pelo clima Mediterrânico, com um acentuado déficit hídrico no período estival e temperatura elevada. Este aspecto pode reduzir a produtividade e a qualidade das vinhas. No âmbito desta dissertação de mestrado, este estudo propôs compreender as relações da vinha com as alterações climáticas na Região Demarcada do Douro, estabelecendo três níveis diferentes de irrigação por déficit (25, 50 e 75 % ETc) e tratamento controle, não irrigado. Além da condição de estresse hídrico, diferentes alturas de parede de vegetação foram estabelecidas para avaliar sua influência nos parâmetros fisiológicos, químicos e produtivos. A avaliação da evapotranspiração (ETc), potencial hídrico de base, NDVI, CCI, troca de gases e área foliar ocorreu durante todo o período de maturação. Para avaliar a maturação tecnológica foram medidos o peso, diâmetro e características químicas dos frutos. Na colheita, o FTIR processou as características químicas do mosto. O peso de poda e o índice de Ravaz completaram as avaliações agronômicas. Os resultados desta pesquisa corroboram com estudos anteriores, indicando que as videiras de sequeiro podem ter um rendimento menor na colheita do que as irrigadas, devido às condições de estresse climático. Além disso, videiras de sequeiro com a parede de vegetação mais baixa podem ter um rendimento maior em comparação com a parede mais alta.

Palavras-chave: videira; irrigação deficitária; parede de vegetação; Região Demarcada do Douro.

Acronym

ETc - Crop evapotranspiration

BBCH - Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie

GDD - Growing-degree Days

HI - Heliothermal index of Huglin

CI - Cool night index

NDVI - Normalized Difference Vegetation Index

CCI - Chlorophyll Concentration Index

IRGA - Infra-Red Gas Analyzer

A_{net} - Photosynthetic rate

E - Transpiration rate

gs - Stomatal conductance

WUE - Intrinsic Water Use Efficiency

PQA - Point Quadrat Analysis

PG - Percent gaps

LNN - Leaf layer number

PIL - Percentage of interior leaves

PIC - Percentage of interior clusters

TSS - Total Soluble Solids

TA - Titratable acidity

TPI - Total Polyphenols Index

FTIR - Fourier-transform infrared spectroscopy

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Chapter 1

Introduction and objectives

1.1 Introduction

The viticulture and enology are one of the most important sectors of Portuguese agriculture. Historically, it is a significant part of the social and cultural life of Portuguese people, highlighting the country worldwide (Magalhães, 2015).

Economically, in the year of 2018 winemaking represented about 16% of the Portuguese agricultural production, with 693,5 million euros (INE, 2018). In the past decades, the area destined to vineyard production has increased more than 20% around the world, which impacts directly the amount of wine produced (IVV, 2012).

The Douro Demarcated Region (including the Douro and Porto Designations of Origin) is the oldest demarcated region in the world regulated by law, and it is designated by UNESCO as a World Heritage Site since 2001. Furthermore, it is currently the country's main wine-growing region in terms of wine production and vineyard area (1 259 683 hL and 42 thousand hectares) (IVV, 2018).

In the last century, understanding environmental and economic ways of exercising agriculture got an urgent call. Several papers highlight researches for productive harnessing and sustainability in many crops connected to climate change and the use of water in agriculture (Blanco-Ward et al., 2019; Jones, 2013; Miranda et al., 2019).

The main cause of reduced vine growth and productivity in Mediterranean climate regions, such as the Douro Region, is water stress, also affected by light and thermal stress, due to the unbalance between the growth of vegetative and reproductive organs (Chaves et al., 2010). In semi-arid or arid zones with moderate to severe dry season, irrigation can be managed using high-efficiency practices such as regulated deficit irrigation (Costa et al., 2012).

In this way, this study proposes to understand the differences in grape quality and productivity by managing irrigation and vegetative plant structures in a vineyard, aiming to acquire improvement in yield without losing quality.

1.2 Objectives

This study aims to evaluate the influence of the implementation of management techniques in the conduction of a vineyard in the Douro Superior region (Northern Portugal), at the physiological, biochemical, viticultural and enological level, with Touriga Nacional grape variety, through:

1. Vine water stress management by adopting three deficit irrigation strategies with 25, 50, and 75% of crop evapotranspiration (ET_c) and control field (non-irrigated);
2. Comparison of two different canopy heights (tall and short height of the canopy).

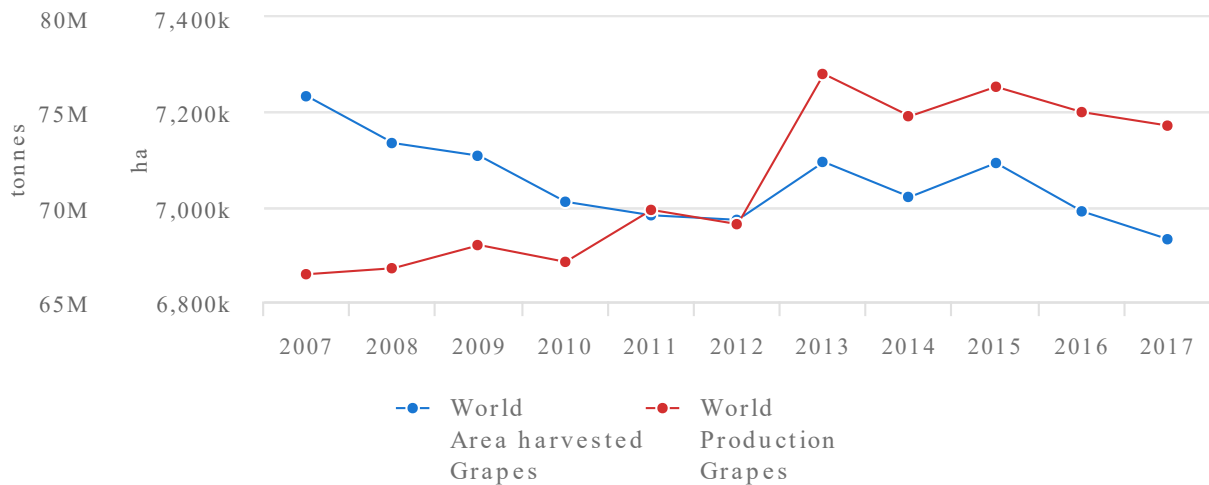
Chapter 2

Climate change: ways to mitigate its impact in viticulture

The weather and climate are crucial factors to viticulture and winemaking, inducing an impact on grapevine yields and berry quality attributes (Fraga, 2019). The recent trends hint to the scenario of future climate change, where it is supposed that the temperature will rise by 1.5 to 2.5° C over the next 50 years, as well as the decrease in precipitation and large variability in the occurrence of wave heat phenomena and intense precipitation, factors that have a direct consequence for the stability of the wine production and the productive sustainability (Jones, 2013, Miranda et al., 2019, Blanco-Ward et al., 2019).

Several negative impacts climate change can produce on viticulture, namely: the advancement of the phenological stages; the acceleration of the technological maturation of the berries, leading to excessive accumulation of sugars (high alcohol content), a low acidity (that can be negative especially for white wines) and a high pH (giving a microbiological instability, which could increase the wine spoilage risk); atypical flavors and aromas in both grape and wine; thermal decoupling between anthocyanins (phenolic maturation delayed) and sugars (advanced technological maturation); and increase in the phenomena of dehydration, clusters sunburn (Fraga, 2019). Productivity and final yield could be threatened by extreme heat combined with water stress (Martinez de Toda, 2018).

Those weather differences could step in on grape and wine production. The IVV, 2012, points to an increase of around 23% in the area of vineyards worldwide in the last 10 years of the analysis (2001-2011), this value is especially linked to the growing incorporation of viticulture in countries such as China, India, New Zealand, and the United States, as well as in countries of the Southern Hemisphere, such as Brazil, Chile, and Argentina. The FAO report from 2007 until 2017 shows the increase in grape production to the detriment of area harvested worldwide in the Figure 2.1, which could be associated to the use of strategies to acquire higher and quality productions.



Source: FAOSTAT (Nov 05, 2019)

Figure 2.1: World grape production and area harvested.

Source: FAO, 2019.

The appropriate plant material united with viticultural techniques in a local climate condition could optimize yield and quality on vineyards (Van Leeuwen et al., 2019). Different strategies have been studied among the viticulture field to mitigate the effects that sorts of stress can cause on vines. Several papers experimented with different types of water management - as deficit irrigation, full irrigation, partial rootzone drying, etc (Baeza et al., 2007, Intrigliolo and Castel, 2009, Conesa et al., 2016, Cancela et al., 2017) -, pruning, canopy structure and training systems (Intrieri et al., 2011, Ribeiro and Andrade, 2013, Mandelli et al., 2008) trying to increase yield without losing quality.

One approach for this cause is at the agricultural practice level. The protection of the leaves to sun and heat waves using Bordeaux mixture and kaolin clay has been studied for grapevines (Coelho et al., 2013, Moutinho-Pereira et al., 2015). Those studies evaluate the capacity of external protection reducing the inner temperature of leaves, finding out that the leaves protected could have a better net production of photoassimilates, having better gas exchange rates. The canopy management, also, influences the volatile compounds on wine. The training system can affect the microclimate conditions, modifying the vine metabolites content, therefore contributing to the final wine composition (Vilanova et al., 2017).

Mediterranean future climate and its impact on wine production

The Mediterranean climate is known for its seasonal droughts, atmospheric and soil water deficits, alongside with high temperatures. Those conditions put major constraints on yield and quality in the vineyards. The main reason for decreasing vine growth and productivity in the Douro Superior region is known to be water stress, which is more or less accentuated by light and heat stress, corresponding to the effect of the balance between the growth of reproductive and vegetative organs of the vine. Along with the loss condition of yield and quality, a significant decrease in carbon assimilation can occur in Mediterranean vineyards due to a large reduction in photosynthesis as a result to high leaf temperature, combined with severe water stress and partial leaf loss in vegetative canopy (Costa et al., 2012).

In a analysis study of the potential effects of climate change on bioclimatic indices for vineyards in the Douro Region, Blanco-Ward et al. (2019) estimated changes in temperature and rainfall accumulation during the vine growth period (between April and October), in the dryness index - DI, among other indices, projected for a medium-term future (year 2049) and long term future (year 2097). In general, it is expected a significant increase in the average temperature during the stipulated period of growth of the vine, as well as a decrease in precipitation in future periods. The estimated dryness index suggests an increase in the water stress condition of the plants in the future, and this index was

already considered high under the recent past climatic conditions. Thus, this scenario express the possibility of increasing the climate stress suffered by the grapevines in the Douro Region, providing support for the need to study vineyard management strategies to obtain quality and sustainable production under adverse dry conditions. Also, Santos et al. (2019) says that increases are expected in the growing-season mean temperatures in all the Portuguese winemaking Denominations of Origin and sub-regions, followed by increasing severe dryness, mainly in south-eastern part of Portugal and along the upper Douro Valley (Douro Superior) in northeastern Portugal.

2.1 Irrigation and water management

Even the water being a fundamental component of vegetable life, grapevines are known for its ability to adapt to water deficits, due to its morphological and anatomical characteristics, such as the xylem vessel caliber, the well-developed root system and the stomatal regulation by physiological processes. Nevertheless, water is a limiting factor of the crop, especially for the Mediterranean region, where the summer period is characteristic hot and dry. Grape production and winemaking are strongly influenced by climate characteristics - which is considered to be the most relevant factor in ecosystem interactions to define the *terroir* (Magalhães, 2015). Projections regarding global warming point to a possible increase in the period of high temperatures, rise in extreme temperatures, a greater amount of stress events, among other factors, which will hinder the management of vineyards in the production of quality wines (Alves et al., 2012). The vineyard is traditionally grown under rainfed conditions and it is not irrigated - except for table grape production, which requires more water. An empirical concept in Europe suggests that watering the vineyard harms grapes for vinification because it is supposed to induce wines with lower alcohol content, higher acidity, and poorer phenolic composition, although, in wine regions of the "New World" - recent winemakers, irrigation is a usual practice, increasing the productivity of these vineyards compared to those of European wine countries, not interfering in the wine quality (Magalhães, 2015).

In South America, countries such as Argentina and Chile use flood irrigation with water from the Andes Mountains. In Australia, the hot and dry climate demands that the vineyard get drip irrigation, developing good price/quality relation in wines, making them competitive in the global market. In California, United States, irrigation is being used in conjunction with studies on its efficient use for each type of vineyard. In Portugal, irrigation is a recent cultural practice in vineyards, being implemented especially in the Alentejo region, and the use is being increased in other regions of the country. However, the need for irrigation is becoming increasingly evident, since that, in recent years the weather has been extremely dry, a factor that limits the profitability of the vineyard

in more arid regions. Further, much is said about future forecasts for global warming, pointing to the expected reduction in rainfall along with increased evapotranspiration in plants (Schultz, 2008). With irrigation, especially in warmer and drier regions, it is expected to prevent production loss due to berry dehydration and increase quality, preventing the interruption of early ripening, which is unfavorable to the concentration of sugars and, phenolic and aromatic components.

The need for irrigation is then understood through the available water, which depends not only on the nature of the soil, but also on the input of water through precipitation and the outflow of water with potential crop evapotranspiration (ETc), which is correlated directly or indirectly with the physical characteristics of the soil, the management of the vineyard, the training system, the atmospheric conditions (regarding temperature, radiation and wind speed). Moreover, soil fertility also influences the availability of water to the plant, as nutritional deficiency limits root hydraulic conductivity. Potassium (K⁺) concentration in stomatal guard cells is directly linked to transpiration by the stomatal opening and closing mechanism (Alves et al., 2012).

Predawn leaf water potential is considered by many researchers to be the best parameter to define the plant water condition at a given time (Lousada, 2016). This analysis makes it possible to make decisions about the need for irrigation of the plant and has been widely used, both scientifically and by wine production companies (Ojeda, 2007).

The literature shows the ideal water potential for grapevine along its vegetative cycle, relating the level of water restriction to the plant, the leaf water potential and the period along the vegetative cycle (Figure 2.2).

Grapevine water status along the vegetative period determines the type of wine obtained, as mentioned by Ojeda (2007), which is a situation of complete water supply (Ψ_b) between 0 and -0,3 MPa can produce herbaceous, dilute and acidic wines, whereas in extreme water shortages (Ψ_b) below -0,8 MPa the wines may be excessively tannic, astringent and alcoholic. Between -0,3 and -0,7 MPa, the wines are more balanced.

The grapevine water requirement is also related to the vineyard training system. Evapotranspiration is influenced by greater or lesser expression of vegetation and plant vigor,

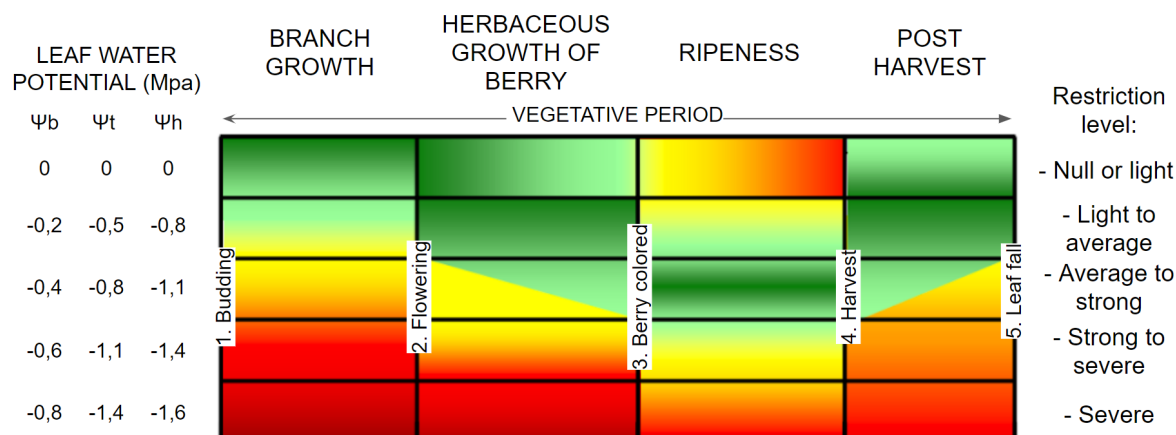


Figure 2.2: Optimum (green zone), unfavorable (yellow zone) and critical (red zone) water states in the vine related to vegetative growing season. Ψ_b : predawn base water potential; Ψ_t : branch water potential at solar noon; Ψ_h : leaf water potential at solar noon.

Source: Adapted from Ojeda, 2007.

along with the manner of training technique, density and disposition of the plantation, canopy management and the variety chosen. Regarding water stress, which is the lack or excess of water for the plants, the varieties respond to this process in different ways. Schultz (2008) classified the varieties according to two behaviors: isohydric varieties, those ones that, when the water potential reach a critical threshold, maintain it through the stomatal closure, (*e.g.*, Grenache and Touriga Franca varieties); and anisohydric varieties, those that, after the critical threshold, continue to absorb available water in the soil, since they keep their stomata open for longer, which allows transpiration and consequent reduction of leaf water potential (*e.g.* Syrah and the Touriga Nacional varieties).

2.1.1 Deficit irrigation

Deficit irrigation has emerged as a potential strategy to allow crops to withstand water stress with little or no yield decrease (Chaves et al., 2010). Vilanova et al., 2018 evaluated the influence of water on yield components, must composition and volatile wine compounds in Verdejo grapevine variety, through different irrigation strategies - no additional irrigation (R0), 25% (R25), and 50% (R50) of reference evapotranspiration (ET_o). It was found that the R50 treatment had the highest productivity, however, the addition of water reduced the concentration of volatile phenolic compounds in wine. Also, the concentration of five volatile compounds individually was higher in the R0 treatment.

Conesa et al. (2016) studied the effect of deficit irrigation after the veraison period to improve berry coloration and bioactive compounds in Crimson Seedless table grapes, where they established four irrigation levels: (i) control, irrigated with 110% of crop evapotranspiration (ET_c); (ii) regulated deficit irrigation (RDI) with irrigation equal to (i) in pre-colored berry and 50% of ET_c in post-colored berry; (iii) partial dry zone in the root region, with irrigation similar to the RDI, however, alternating the sides of the root irrigation every 10 to 14 days; and (iv) without irrigation (NI), only natural precipitation. It was found that the only treatment that had a significant reduction in productivity was the one without irrigation (iv), with the weight of clusters and berries lower than the other treatments. Treatment (iii) had the highest anthocyanin accumulation in the berry skin. The results showed a strong relationship between the total amount of water supplied during the branches growing season and the main parameters related to harvest yield.

In a study carried out in the Douro Demarcated Region, Gonçalves et al. (2016) studied the physiological responses and the effects on yield and quality in the Touriga Nacional grape variety under deficit irrigation, with three irrigation treatments, 1) Not irrigated - NR; 2) Deficit irrigation at 25% of ET_c - R25%; and 3) Deficit irrigation at 50% ET_c - R50%. For this purpose, leaf water potential (Ψ_l), yield and qualitative parameters such as total acidity, pH, probable alcohol and phenolic compounds (tannins, anthocyanins, and polyphenols) were evaluated. It was found an increase in yield at both irrigated

treatments (kg of grape/branch), for modality 2 (25% E_{Tc}) in 20% and 3 (50% E_{Tc}) in 33%, without compromising quality parameters. No significant differences were observed in the reduction of phenolic compounds between treatments.

Studying the crop level, soil, and irrigation management of the cv. Trincadeira in the Mediterranean region, under the influence of different irrigation levels (as rainfed, pre-veraison and post-veraison) and different soil management (tillage and natural cover crops) during berry development, Barroso et al. (2017) found an increase in berry skin due to irrigation and soil tillage of the vineyard.

Much of the impact of water deficit on fruit composition can be measured by reduced plant vigor, leading to increased light interception in the fruiting zone of the vine (Chaves et al., 2010). In this perspective, Keller et al. (2016) studied the behavior of grapevines under water deficit, analyzing physiological parameters, growth, and, microclimate in the cluster zone. Having different irrigation treatments (100%, 70% and 25% of E_{Tc}), they found out that in the treatment with the less amount of water applied to the plant (25% E_{Tc}) had an extremely limited amount of gas exchanges. Besides, the region of the fruiting zone had higher sun exposure and temperature, due to its reduced leaf density, resulting in a significantly smaller size and weight of berries and clusters compared to other treatments.

2.1.2 Effects on yield and quality

The quality of wines has historically been based on water availability in the crop, and irrigation was commonly considered a bad practice for the productive quality of the vineyard. Currently, it is known that both conditions of excess and lack of water can result in a negative impact on the quality of the grapes produced and, consequently, the resulting wines (Lousada, 2016).

Especially regarding water availability, several studies assess the chemical differences of grapes or wine-related to the amount of water supplied to the crop (Basile et al., 2012; Alves et al., 2012; Bucchetti et al., 2011). Water deficit may result in more fruity red wines

with fewer vegetable aromas, higher amounts of anthocyanin pigments, and sometimes less astringency (Matthews, et al., 1990; Chapman, et al., 2005; Castellarin, et al. , 2007, cit by Keller et al. (2016)). Some berry solutes that respond to the water status of the vine are organic acids, sugars, anthocyanins and phenolic compounds (Orts et al., 2005).

Alves et al. (2012) studied the influence of climate and water deficit on vine physiology, yield and quality attributes of Touriga Nacional variety in the Douro region, evaluating parameters such as cluster per vine, cluster weight, Ravaz Index, weight of berry, °Brix, amount of acids (tartaric and malic), pH and phenolic compounds with vines under different water conditions. As a result, it was concluded that plants that received a moderate level of irrigation, compared to rainfed, had a significant impact on yield increase - in number of clusters per vine and berry weight - and on sugar concentration in berries. There was no significant difference in pH and phenolic compounds between treatments.

Bucchetti et al. (2011) studied the effect of water deficit in Merlot variety on the concentration of tannins and anthocyanins present in the must, noting that in the treatments with water deficit the concentration of anthocyanins in the harvest was 53 to 61,6% and tannins were 28,7 to 35,3% higher than in the control treatments (with irrigation).

In Portugal, Chaves et al. (2007) found an improvement in water-use efficiency on plants under deficit irrigation. In a study, two grapevine varieties were evaluated (Moscatel and Castelão) along three years, concluding that the reduction of 50% of ETc, both for deficit irrigation and partial root drying, could control vigor and gain production quality. The vegetative reduction leads to an increase in a light interception in the cluster zone, enriching berry skin anthocyanins and total phenols.

In the South part of Portugal, also studying the cultivar Moscatel, Santos et al. (2007) analyzed different irrigation strategies and its effects on fruit composition. Comparing partial rootzone drying (50% ETc, PRD), deficit irrigation (50% ETc), full irrigation (100% ETc) and non-irrigated plants, the study showed that PRD vines had a higher incident photosynthetic photon flux, denoting a better microclimate at the cluster zone, as a reducing of the vine excessive growth.

In a study conducted in Spain, Orts et al. (2005) studied the effect of moderate watering during the ripening period of the Monastrell variety on grape composition, observing that the irrigation treatments produced higher weight grapes, finding no differences in sugar accumulation compared to the treatment without irrigation. However, the titratable acids and pH quantity were slightly influenced by irrigation, having higher values than in the treatment without irrigation. Also, by sensory analysis, it was found out that wines produced from vines without irrigation generally obtained a higher score for quality and intensity of color and aroma.

Chacón et al. (2009) studied the cv. Merlot in a warm region of Spain, analyzing the water status on the vine's berry and seed phenolic composition. When predawn water potential increases, production, leaf area index (LAI), exposed leaf area (SA), and berry weight significantly decrease. Berry phenolic biosynthesis is impacted by the plant water status. This depends on the water level, duration of the water constraint or stress, and phenological stage when the vine water status is modified.

In Chile, a New World winemaker, Acevedo-Opazo et al. (2010) carried out a study for water potential thresholds and its effects on water consumption, grape quality, and vegetative growth. Three midday stem water potential were analyzed: T1 (-0.8 to -0.95 MPa), T2 (-1.0 to -1.2 MPa), and T3 (-1.25 to -1.4 MPa). A relation of increase in anthocyanins and total soluble solid as a decrease in berry weight was found, as T3 produced the smallest berries with significant increments in quality compounds. No effects on yield per treatment were found.

2.2 Canopy management

The grapevine can be trained and pruned in diverse ways, depending on its productive purpose, the environmental characteristics of the *terroir*, the grape cultivar or variety, among other factors. The management of the vine's canopy can change the composition and quality of grapes and wine. The excess of vegetation makes it difficult to aerate and light the clusters, which leads to an increase in fungal diseases and, directly related, a decrease in productivity and quality (Mandelli et al., 2008).

2.2.1 Training system

Hidalgo (2003), defines the training system as the whole set of decisions made in favor of determining the disposition of the aerial parts of the vine in the space. Those decisions are a result of integrating a set of operations that the vine grower decides before setting up the vineyard, such as the planting distancing (arrangement and planting density), the orientation of the plant lines, and later, when planting, the future geometry of the plant (training system). It also depends on the decisions made annually, such as the pruning load, shoot topping (cut off the apex), elimination of leaflets, defoliation, etc.

Regarding the management in the green/vegetative phase, some activities are necessary for the management of the vine. The shoot topping (cut of the apex) is a practice that aims better light reception by leaves and clusters, favoring the ideal microclimate for plants, ease of traffic of people and machines between plantation lines, furthermore, improving the flowering and leading to a rebalance of energy on the plant, promoting the accumulation of carbohydrates for the clusters (Magalhães, 2015).

The leaf canopy structure can be shaped aiming better grape production, both in quantity and quality. Shoot topping is a common maintenance activity, the plant's height can be chosen depending on what is the purpose of the vineyard. Vasconcelos and Castagnoli (2000), studied the behavior of Pinot noir vines having three shoot topping manages: shoot tipping at full bloom, lateral shoot length, and cluster zone leaf removal. With this study, it was found that shoot tipping at bloom increased not just percent fruit set, but

also berries per cluster, cluster weight, yield per shoot, and yield/pruning ratio. These results were associated with the fact that shoot tip removal increased the main and lateral leaf size and the contribution of lateral leaves to total leaf area, improving, mainly, photosynthetic activity.

Integrating the concepts of the relationship of training systems on water use efficiency and leaf gas exchange, Ribeiro and Andrade (2013) studied the behavior of the Tinta Roriz variety in the traditional free-form system and the bilateral Cordon system under rainfed conditions in the Mediterranean region of Trás-dos-Montes, Northeast of Portugal. Through gas exchange assessments and water use efficiency, they found out that vines conducted in the traditional system had a better water status and a higher rate of net CO₂ assimilation than those of the bilateral cordon system, demonstrating that the traditional system seems best adapted to the hot and dry climate of the study region.

Mandelli et al. (2008) studied the effect of vegetative management on the physico-chemical composition of cv. Merlot, analyzing the effects of summer pruning, sprouting, shoot topping and defoliation, some of them at different times of the vine growing cycle. With the study, it was found that the treatment number 10 (with sprouting, shoot topping, defoliation performed at the beginning of flowering and eliminating all leaves below the clusters) and 9 (of sprouting plus defoliation performed 21 days before harvest and eliminating half of the leaves below the clusters) showed higher synthesis and accumulation of sugar in the grape, expressed by °Brix and lower acidity level, expressed by tartaric and malic acids, pH and titratable acidity. Thus, it is evident that different vegetative interventions on the vine result in different wines.

In a study with semi-minimal-pruned hedge (SMPH) Intrieri et al. (2011), compared plants with 80 and 120 cm heights of vegetative wall with spur-pruned cordon (SPC), finding that SMPH plants had a 35 to 40% increase in yield compared to SPC, but had no differences in most soluble solids, pH, and acidity across treatments.

Vilanova et al. (2017) studied the cv. Albariño behavior in Northwest Spain, in different training systems (Vertical Shoot-Positioned - VSP, Scott-Henry - SH, Geneva

Double-Curtain - GDC, Arch-Cane - AC, and Parral - P). The volatile composition analyzed showed that GDC wines had the highest total volatile composition with the highest concentrations of alcohols, ethyl esters, fatty acids, and lactones families. The results confirm that viticultural practices have a great effect on both grape and wine quality.

2.2.2 Pruning system

Pruning is an extremely important cultural practice in the vine. It consists of the removal of any living part of the plant, whether of branches, shoots or leaves, determining a certain shape and size for the plant, which directly influences the physiological behavior of the vine, such as its growth, yield, and quality of the harvest (Intrieri et al., 2011, Magalhães, 2015).

The pruning system can be short, long, or mixed. In short pruning, having the *Cordon Royat* system as an example, only a two-bud branch is left in the plant (unilateral or bilateral), usually with six to eight shoots growing from that, which depends on the plant vigor, edaphoclimatic aspects, and productive purpose. In the common *Cordon* system, another form of short pruning, the buds are left on the main branch of the vine, which results in the growth of new shoots. In the *Guyot* system, a mixed kind of pruning (short and long branches in the same plant), the vine is left with a two-bud branch for the winter pruning of the following year, and a branch with usually six buds, for the productive shoots of the current harvest, usually (Hidalgo, 2003).

The objectives of pruning are diverse. Pruning is used as a way to control the apical dominance of the vine, which, when not pruned, tends to emit shoots from the buds of the final part of the branches, generating an uncontrolled stretching of the aerial part, which makes plant management difficult and impacts the productivity of the vine. Pruning, indirectly, defines the amount of production and qualitative characteristics of the grape, such as sugars, acids, phenolic compounds, aroma. The good management of the vineyard seeks the optimal balance of the plant, matching great productivity with the desired quality in the final product. Likewise, pruning is a way of guaranteeing the perpetuity of

the vine and regularizing inter-annual production, for the matter of limiting the pruning to the number of buds left in the production unit. When the pruning is severe, vigor is increased; decreasing the total leaf area decreases the number of accumulated reserves in perennial parts such as roots, trunk, and branches. Excessive pruning eventually compresses the vegetation, which can lead to the development of diseases, induced by the provided microclimate. (Magalhães, 2015; Mandelli et al., 2008; Hidalgo, 2003). The proportion between exposed leaf area relative to total vine leaf area is crucial for defining the relationship of plant biophysical processes and atmospheric interactions that relate to vine productivity (Navarrete, 2015).

A strategy for vineyards that need to adapt to extreme environmental weather conditions is the proper choice of training and pruning system. In the Douro Region, the most used pruning system is the unilateral or bilateral cordon, which is a simple system to be installed and adapted to mechanization, being used in many grape varieties. Despite the simplicity and adaptability of the cordon, a debate that a form of conduction with a lower trunk such as *Guyot* may be more appropriate, especially in the drier and warmer regions such as the Douro Superior. Further, *Guyot* grapevines are less susceptible to woody diseases, justified by the lower overlap of pruning cuts, a fact that generally causes longer grapevine longevity (Magalhães, 2015).

Silva et al. (2006) studied the phenological behavior of the cultivar “Patrícia” at different times of fructification pruning in the Southern Hemisphere (Goiás, Brazil), with mixed pruning. With four different pruning times and, consequently, four different cycle lengths, they found no significant difference in the average size and shape of the clusters. However, a significant difference was found in total titratable acidity, where the highest value of tartaric acid (1.44 g L^{-1}) was found in the earliest pruning and the lowest value (0.87 g L^{-1}) in the later pruning.

Chapter 3

Material and methods

3.1 Location and experimental design

The commercial vineyard where this study took place is located in Santa Comba de Vilariga (coordinates 41°20'48.2"N 7°03'47.8"W, altitude of 226 m), subregion of Douro Demarcated Region, Portugal.

The experiment took place in 2019. The Touriga Nacional (*Vitis vinifera* L) vines were 4 years old, planted in 2015 and grafted onto 1103 Paulsen (1103P) rootstock. The plants were trained to a vertically shoot positioned (VSP) trellis system on a *Guyot* system in a north-south orientation, irrigated in a deficit irrigation plan. The experimental design divided the vineyard in four irrigation sectors. In each treatment, a different irrigation level was conducted and among the blocks, the plantation lines were divided into tall (1.1 m) and short (0.8 m) canopy wall height. In each sector, a total 3 blocks with five plants per block were selected to conduct egalitarian measurements (Figure 3.1).



Figure 3.1: Assay vineyard.

Source: Google Earth, July 25, 2019.

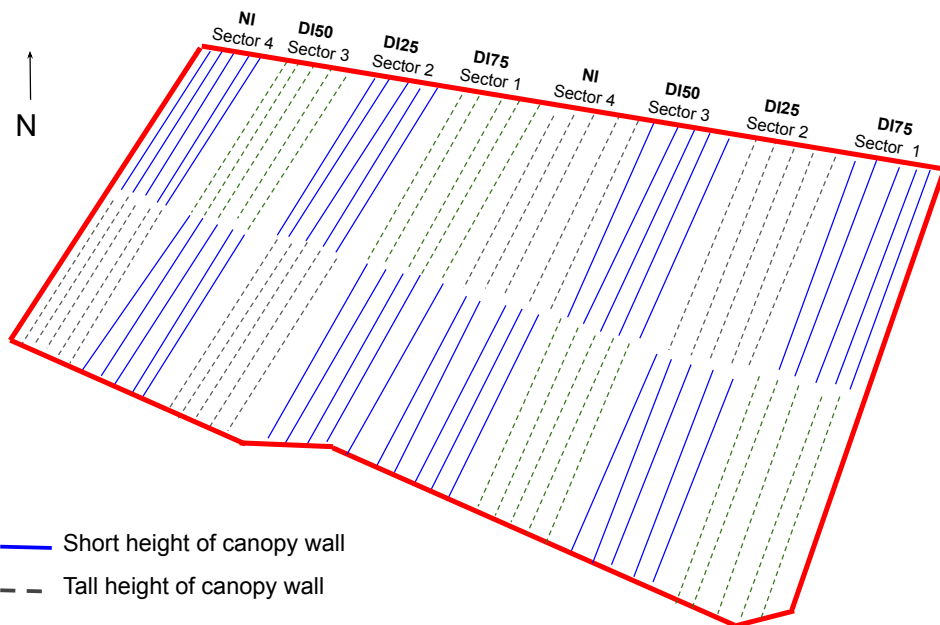


Figure 3.2: Assay sketch: treatments NI, DI25, DI50 and DI75 divided into sectors from 1 to 4 and representation of tall and short canopy wall.

The plantation spacing in the vineyard was 2 x 1.2 meters, that is, 2 between plantation

lines and 1.2 meters between plants, totalizing 4166 plants per hectare.

The vines were managed in three levels of deficit irrigation: 25, 50 and 75% of crop evapotranspiration (ET_c), and a non-irrigated field, shown in Figure 3.1. To calculate the reference evapotranspiration, the FAO Penman-Monteith method (Allen et al., 1998) was used, considering data such as air temperature, air humidity, radiation, and wind speed data from an automatic weather station located near the vineyard.

$$ET_c(t) = K_c \times ET_o(t)$$

Where, t = time step (days), ET_c(t) = potential crop evapotranspiration on t (mm), ET_o(t) = reference evapotranspiration on t (mm), K_c = crop coefficient (Allen et al., 1998).

Table 3.1: Water supplied in each irrigation treatment.

Code	Treatment	% ET _c	Water supply (m ³ /ha)
NIT	Non-irrigated tall canopy	0	-
NIS	Non-irrigated short canopy	0	-
DI25T	25% ET _c irrigation tall canopy	25	605
DI25S	25% ET _c irrigation short canopy	25	605
DI50T	50% ET _c irrigation tall canopy	50	1210
DI50S	50% ET _c irrigation short canopy	50	1210
DI75T	75% ET _c irrigation tall canopy	75	1815
DI75S	75% ET _c irrigation short canopy	75	1815

Still, concerning the irrigation system, the one-meter apart drips had a 1.15 L/h flow. The watering frequency program occurred three times a week, always at night. The irrigation began on June 1st, 2019, the moment most of the plants reached -0.4 MPa of steam water potential. The irrigation ceased on September 9th, 2019, two weeks after the harvest.

The vines were pruned in the beginning of the year, at winter. Hence, the pruning load of six buds per vine was established. Continuing with the management process of the vineyard, on June 4th the plants received the shoot topping. The apex part of the vines

was cut, establishing two treatments: tall canopy wall - in which vines received the shoot topping by the height of the last wire on the vine support structure; and short canopy wall - in which vines received the shoot topping by the height of the wire under tallest one (Figure 3.3).



Figure 3.3: On the right side: tall canopy wall - 1.1 m; On the left side: short canopy wall - 0.8 m.

During the months of March until July the canopy of the vines was managed. The shoot positioning was made whenever required, to place the vines in the right position in the lines, avoiding microclimate conditions to diseases and providing good mechanic transit between the plantation lines.

Along the reproductive (or fruiting) period of the vines, several analyses were made with different qualitative parameters. The samples of the vegetable material, *i.e.*, the berries were collected into plastic bags for each treatment. They were stored in a thermal box until arriving in the laboratory. In the laboratory, after the biometry, the samples were crushed and reserved in plastic recipients to proceed to the rest of the analysis.

Douro Demarcated Region

Located in the Northeast of Portugal, in the Douro river basin, the Douro Demarcated Region (Região Demarcada do Douro - RDD) has great importance, both from a wine and tourism standpoint. It is considered the oldest regulated and demarcated wine region in the world. It was created in 1756 when the General Company of Agriculture of Alto Douro Vineyards (Companhia Geral da Agricultura das Vinhas do Alto Douro) was established by the Marquis of Pombal. The Alto Douro Wine Region was named by UNESCO as a World Heritage Site in 2001. The RDD has a total area of approximately 250 thousand hectares, of which 43 thousand hectares of vineyards are used to produce two types of wines: Porto and Douro (IVV, 2018). The RDD is divided between Baixo Corgo, Cima Corgo, and Douro Superior. In the region of Baixo Corgo, the climate provides milder temperatures and greater rainfall. At Cima Corgo, the climate gradually becomes more Mediterranean, with a decrease in precipitation and an increase in temperature. Finally, in the Upper Douro region, where this study takes place, the climate is typically Mediterranean or arid, often leading to overripening of the grapes (Loureiro, 2017).

By being located in deep valleys, the DDR (Figure 3.4) (Miranda et al., 2019) has general conditions that allow climate suitability for viticulture, due to temperatures between 12 and 22° C and average temperature during the vine growing cycle of 17.8° C, considered a Csa climate in Köppen-Geiger scale for most part of the region. The precipitation in the region is distributed decreasing in the direction of Baixo Corgo - Douro Superior and has an annual median of 950 mm (Jones, 2013).

The geology of the Douro region is characterized by the presence of rocks with shale layers, with some granite outcrops. The average altitude across the region is 443 m, but it ranges from lowlands near 40 m to high zones just over 1400 m (Jones, 2013).

The Douro Demarcated Region had in 2017 a declared production of 1,281,566 hL of total must, corresponding to about 20% of the total production of Portugal (INE, 2017) which shows the relevance of this region for national wine production.

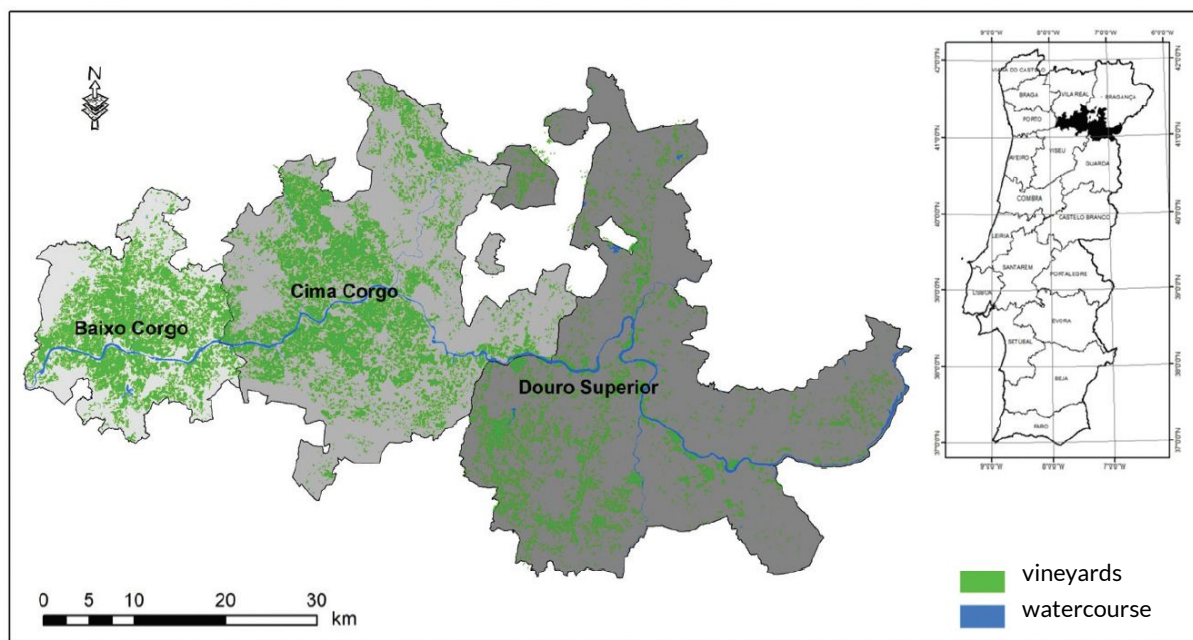


Figure 3.4: Geographic delimitation of the location of the Douro Demarcated Region.

Source: Miranda et al., 2019.

Touriga Nacional variety

The Touriga Nacional variety, pictured in Figure 3.5 (IVV, 2018), is of very old origin in Portugal. The clonal selection of more productive and less sensitive to weed plants have made the grape variety widely used in several wine-growing regions of Portugal, as well as in countries such as Australia and South Africa.

This variety has a small, pentagonal-shaped adult leaf, and may have slightly roughened upper sub-lobes, having wider than long, convex short teeth. It has a slightly open V-shaped petiolar sinus and open U-shaped sinuses. The grape clusters are small, 100 to 150 grams, cylindrical in shape, loose, sometimes compact - because of the fruit displacement. It has a medium-length peduncle, small rounded berry, blue-black in color, of medium thick skin, unstained pulp, juicy and with a specific flavor. In agronomic aspects, the plant has a prostrate growth, which hinders its manage in the green phase, although it has high vigor.



Figure 3.5: Touriga Nacional grape cluster and leaf.

Source: IVV, 2018.

This variety has a long vegetative cycle and requires medium fertility soils and exposures to the South quadrant. Due to the selected polyclonal materials, the variety has acceptable behavior to fruit displacement and uncompleted fruit development. It has relatively moderate productivity, due to the low weight of the cluster, being commonly found 3 clusters per branch. From the enological perspective, it is considered a versatile grape variety, as it produces Port Wines or high-quality table wines. Due to the aromatic complexity, structure and the high quality of phenolic compounds, this variety can produce excellent monovarietal wines or can be used to improve the blend of polyvarietal wines (Magalhães, 2015).

3.2 Phenological stages

To follow the growth process of the vines, the phenological development stages of plants were periodically measured using the BBCH Scale (Lorenz et al., 2008). The main stages are depicted in Table 3.2. This scale works with a decimal code system, which is divided into two growth stages: principal and secondary. This scale was based on a code system (Zadoks scale) developed by Zadoks et al. (1974). Analyzing the phenological stages of the vines gave us the capacity to decide when it would be better to fulfill the duties required for the assay.

Table 3.2: BBCH Scale at vineyard assay.

Date	Code	Description
		Principal growth stage 0: Sprouting/Bud development
11/03/2019	00	Dormancy: winter buds pointed to rounded, light or dark brown according to cultivar; bud scales more or less closed according to cultivar;
26/03/2019	08	Bud burst: green shoot tips clearly visible
		Principal growth stage 1: Leaf development
01/04/2019	11	First leaf unfolded and spread away from shoot
		Principal growth stage 5: Inflorescence emerge
01/04/2019	53	Inflorescences clearly visible
		Principal growth stage 8: Ripening of berries
22/07/2019	81	Beginning of ripening: berries begin to develop variety-specific colour
05/08/2019	85	Softening of berries
01/09/2019	89	Berries ripe for harvest

3.3 Climate conditions and bioclimatic indices

At the vineyard assay site was settle an automatic weather station to register data, such as air temperature ($^{\circ}\text{C}$), solar radiation (W/m^2), air speed (m/s), relative humidity (%), and precipitation (mm). The data was collected the whole year of 2019.

Growing-degree Days (GDD)

For the grapevine crop, Amerine and Winkler (1944) settled a base temperature, which means, a temperature that below that value the vine can't develop, that is 10°C . For this matter, the bioclimatic index of *Growing Degree-days* (GDD) is expressed as the days in which the average temperature exceeds the 10°C threshold, since April 1 through October 31 in the Northern Hemisphere (known to be the growing season), according to the following equation:

$$GDD \approx \sum_{\text{April}}^{\text{Oct.}} \frac{T_{max} + T_{min}}{2} - T_{base}$$

Where T_{max} is the maximum air temperature ($^{\circ}\text{C}$), T_{min} is the minimum air temperature ($^{\circ}\text{C}$) and T_{base} is the base temperature of 10°C . With this equation it is possible to calculate the *Winkler Index* (WI), a classification for the climate of wine growing regions according to the heat summation or growing degree-days.

Heliothermal index of Huglin (HI)

Another bioclimatic index is the *Heliothermal Index of Huglin* (HI), which provides information regarding heliothermal and sugar potential, that is very much correlated with the Thermal Index of Winkler ($r^2 = 0.98$ over 97 grape-growing regions worldwide) but, according to Tonietto and Carbonneau (2004) is more pertinent to the qualitative factors to express the equation as:

$$HI = \sum_{\text{April}}^{\text{Sept.}} \frac{(T_{max} - T_b) + (T_{avg} - T_b)}{2} d$$

Where T_{avg} is the mean air temperature ($^{\circ}\text{C}$), T_{max} is the maximum air temperature ($^{\circ}\text{C}$), d is length of day coefficient ranging from 1.02 to 1.06 between $40(^{\circ})$ and $50(^{\circ})$ of latitude. A value of 1.02 was assumed for a latitude of the assay.

Cool night index (CI)

This index relates the mean minimum night temperature along the month when ripening usually occurs beyond the repining period, purposing to improve the estimate of the qualitative potentials of the wine-growing regions, for this matter, relatively to secondary metabolites as polyphenols and aromas (Tonietto and Carbonneau, 2004). In the following equation, T_{min} is for the minimum temperature in $^{\circ}\text{C}$.

$$CI = \sum_{Sept.} \frac{(T_{min})}{30}$$

3.4 Physiological measurements

3.4.1 Grapevine water potential

The water potential measurement was made at predawn using the Scholanders method of pressured chamber, which consists in subjecting the petiole of an entire leaf without cracks into an hermetically sealed chamber, inserting an increasing pressure (by introducing nitrogen or compressed air into the chamber) until the sap be expelled through the cut section of the stem. The pressure recorded on the pressure gauge (in MPa or bar) corresponds to Ψ_f , which can be read as the lesser free water in the plant, as a result of the lower water availability, in this way, the higher the pressure required for the water output to occur by the petiole the more negative will be the Ψ_f (Deloire, et al., 2005, cit. by Lousada, 2016). This measurement can be better explained in the Figure 3.6 adapted from Ojeda (2007).

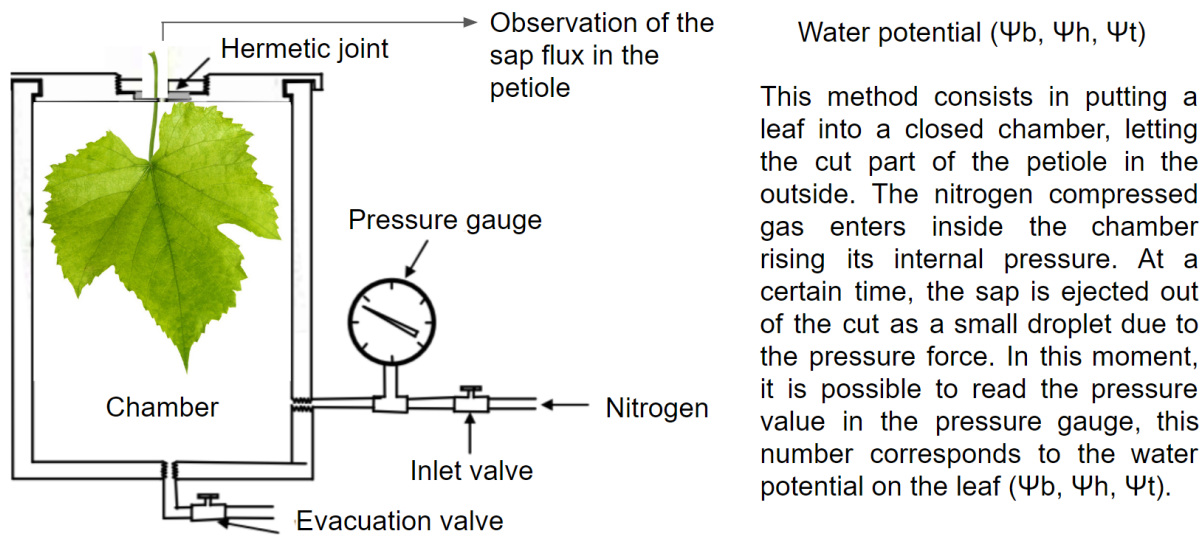


Figure 3.6: Scholanders pressure chamber explained.

Source: Adapted from: Ojeda, 2007.

Eight leaves per treatment/hour/date were put in the chamber to measure the water potential. All grown and healthy leaves, without any cracks. A total of five measurements were done along the fruiting period. Since the month of June the measurements were

conducted in order to control the water status of the plants. The plants started to be irrigated on the 1st of July when the vines reached -0.4 MPa of predawn leaf water potential.

3.4.2 Normalized Difference Vegetation Index (NDVI)

The Normalized Difference Vegetation Index, abbreviated by NDVI is a graphical indicator to assess whether the target being analyzed have live green vegetation or not. It can be used on remote sensing measurements as well as in local sited ones, being obtained by a combination of near-infrared and red reflectances ($(NIR-R / NIR+R)$), the NDVI values are between 0-1, being 1 a representative of grapevines with higher vegetative expression (Carlson and Rypley, 1997). To obtain the NDVI values 3 repetitions were made by treatment. In each, 10 leaves were accounted in the equipment, thus, recording the average value. The measure was made using the NDVI chlorophyll meter (Model, Fieldscout Cm 1000® NDVI, Spectrum Technologies, Plainfield, Illinois, USA).

3.4.3 Chlorophyll Concentration Index (CCI)

The Chlorophyll Concentration Index (CCI) were recorded non-destructively using a portable SPAD (Soil and Plant Analysis Development) chlorophyll meter (Model, SPAD-502 plus, Konica Minolta Sensing Inc., Tokyo, Japan) based on measurements of leaf transmittance at 650 nm (red) and 940 nm (IR) wavelengths, which can be used as an indicator of the nitrogen nutritional state of a plant (Kassoma et al., 2005). In the same manner, as the NDVI, a total of 3 measurements were done. However, the SPAD equipment failed at some points, not being able to have a full analysis.

3.4.4 Leaf gas exchange

The gas exchange is a common way of measure photosynthesis on leaves of a canopy. As being instantaneous, non-destructive to the plant and direct, it can provides direct information of the net rate of photosynthetic carbon assimilation. The portable infra-red gas

analyzer (IRGA - model, LCA-4, Analytical Development Hoddesdon, UK). can measure the reduction in transmission of infrared (IR) wavebands provoked by the presence of CO₂ between the radiation source and a detector (Russell et al., 2019). Employing the IRGA analyses, parameters as net photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs), ratio of intercellular to atmospheric CO₂ concentration (Ci/Ca) and intrinsic Water Use Efficiency (WUE, calculated as the ratio of A/gs) have been generally used to parameterize leaf photosynthesis. With this analyses, prediction models of CO₂ assimilation in natural vegetation and crops are being developed in order to try to predict the consequences of climate change on photosynthesis, and to estimate the influence of different forms of stress, such as extreme temperatures and droughts on the photosynthetic capacity of plants (Flexas et al., 2007).

3.4.5 Canopy density

Point Quadrat Analysis

The Point Quadrat is an analysis to understand the canopy vegetative density. It consists on passing a probe or a stick trough the canopy and record any contact with biomass, such as clusters, leaves or empty spaces. This process needs to be done in samples in the canopy at designated heights, which is usually at the fruit zone, at steady intervals along the plants' row (Smart and Robinson, 1991 cit. by. Palleja and Landers, 2017).

For PQA the Baeza et al. (2001) methodology was followed, with three set of measurements per treatment were made, in reserved blocks. Each one along with a space of 2 meters, in all, 10 measurements in each block.

For this matter, four equations are used:

1. Percent gaps, expressed by the percentage of gaps (% H).

$$\%G = \frac{\text{Number of gaps}}{\text{Number of inserts}} \cdot 100$$

2. Leaf layer number (LLN), refers to the number of leaf layers of the canopy.

$$LLN = \frac{\text{total number of leaves contacted } (H)}{\text{number of inserts}}$$

3. Percentage of interior leaves (%IL). It is a leaves density measure.

$$\%IL = \frac{\text{number of intern leaves}}{\text{total number of leaves}} \cdot 100$$

4. Percentage of interior clusters (%IC).

$$\%IC = \frac{\text{number of intern clusters}}{\text{total number of clusters}} \cdot 100$$

Following the perspective of the Point Quadrat Analysis, to measure the vegetation density (according to Baeza et al., 2001), the height and width were noted using a measuring tape. The width was measured in three height: at the top, the middle and the bottom part of the canopy. A equation of total outer surface (SA) can be calculated from that, as:

$$SA = (2H + A) \cdot \left(\frac{10000}{C}\right)$$

Where, H is the vegetation height, A is the width and C is the distance between rows.

3.5 Yield and berry characteristics

In the maturation, phenological stage BBCH 81 of the vines, many analyses were made to follow maturation control and berry growth track, since beginning of ripening until the harvest.

3.5.1 Berry biometrics characteristics

Since veraison stage, the berries of every treatment were collected to biometric measurements (Sofu et al., 2012). This collection was made randomly, from the central lines of each treatment. In a "zig-zag" distribution, 300 berries were taken out of different plants in the plantation lines, in three repetitions of 100 berries. Those berries were stored in plastic bags inside of a thermal box. Posteriorly, these materials were taken to the laboratory to measure three biometric parameters: equatorial and longitudinal diameter, and weight.

To measure height and diameter a digital caliper with 0.01 mm of precision (Powerfix Profi +, OWIM GmbH Co.KG, Neckarsulm, Germany) was used. The weight was measured with a 0.0001 g precision balance (Kern, ACJ 220-4M, Germany). The samples were analyzed on the very same day as collected, to preserve the biological characteristics.

3.5.2 Control of maturation

The methodology used to measure berry maturation control parameters until harvest was the classical International Organization of Vine and Wine (2015) methods.

Total Soluble Solids (TSS)

Refractometry is an analytical method that measures substances' refractive index, indicating its purity or composition. The refractometer (model Optic Ivymen System, Madrid, Spain) is an equipment used to measure refractive index ("RI") (Son et al., 2009). The Brix measure as the percentage of total solids in a solution, valued in grams of solute/100

g of solution (g/g). It's known that sugar constitutes 95% of the soluble solids in wine or grape juice, and for this reason, Brix is an approximate measure of the real sugar content in wine or juice. For this matter, after the calibration of the digital refractometer, a drop of grape must be added on it, in three repetitions per treatment. The Brix content (% sucrose) is read directly on the refractometer scale, giving the actual temperature of the liquid.

Total Polyphenols Index (TPI)

The polyphenols index was analyzed by the spectrophotometry method. It is based on the quantitative measurement of light absorption by solutions, where the concentration in the solution of the absorbing substance is proportional to the amount of light absorbed (González-Fernández et al., 2012). The absorbance is measured with a UV-Vis spectrophotometer (Genesys 10UV, Thermo Scientific, Madrid, Spain), with must previously dilute in a 1:100 index in distilled water, with an optical path of 1 cm using water as a reference. The following equation is applied to calculate the index: $IPT = A_{280} \times 100$.

Titratable and total acidity

The acidity of the must was measured by the method of titration with sodium hydroxide (0.1 M) and the pH indicator for weak acids and bases bromothymol sulfone phthalein according to OIV-MA-AS313-01 by the International Organization of Vine and Wine (2015).

pH determination

A pH meter was used to measure the pH must of every treatment in five different phenological periods between the colored berry stage until the harvest. The equipment (370 pH meter; Jenway, Essex, UK) was calibrated with a base pH solution. With three repetitions per treatment, the must of a 100 berries was analyzed in the pH meter.

3.5.3 Yield components

The harvest took place on September 10th. Three blocks per treatment with 5 plants each were select to this matter, intending to have the most homogeneous plants per block. To measure the yield capacity of the vines some components were valued, such as clusters per vine (n°) and yield per vine (kg), in the same 6 blocks of selected samples in each treatment. After harvest, in January 2020, the vines were pruned to measure its weight and calculate the Ravaz index.

3.5.4 Must quality at harvest

At harvest, must from grapes collected from each experimental replication were analyzed using FTIR technology (Bauer et al., 2008), Fourier-transform infrared spectroscopy (WineScan FT 120; FOSS, Hillerod, Denmark).

3.6 Statistical analysis

All data were noted and then tabulated at Microsoft Excel ®. The statistics was calculated at Tableau Public ®2019.3 version, and the values were compared by a oneway ANOVA test and mean differences were determined using Tukey's multiple range test.

Chapter 4

Results and Discussion

4.1 Climate conditions and bioclimatic indices

In Figure 4.1 a graphic depicts three important climatic data: air temperature (average, maximum and minimum), relative humidity, and precipitation, from January until October, with monthly average values.

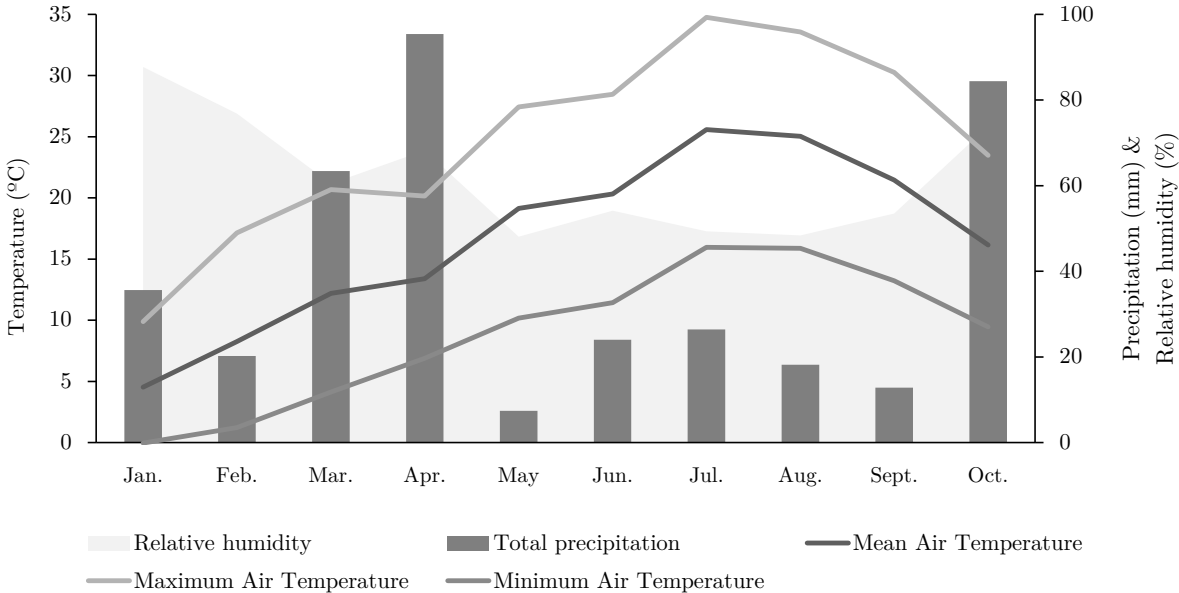


Figure 4.1: Climatic data from 2019 at the vineyard assay site: temperature, precipitation, and relative humidity.

To understand the climate of the region and if the year of 2019 was typical or atypical, Figures 4.2 and 4.3 show the graphics with information from January until October of 2019 and from a 30-year period from 1971 to 2000 with average values for temperature and precipitation from IMPA, 2000.

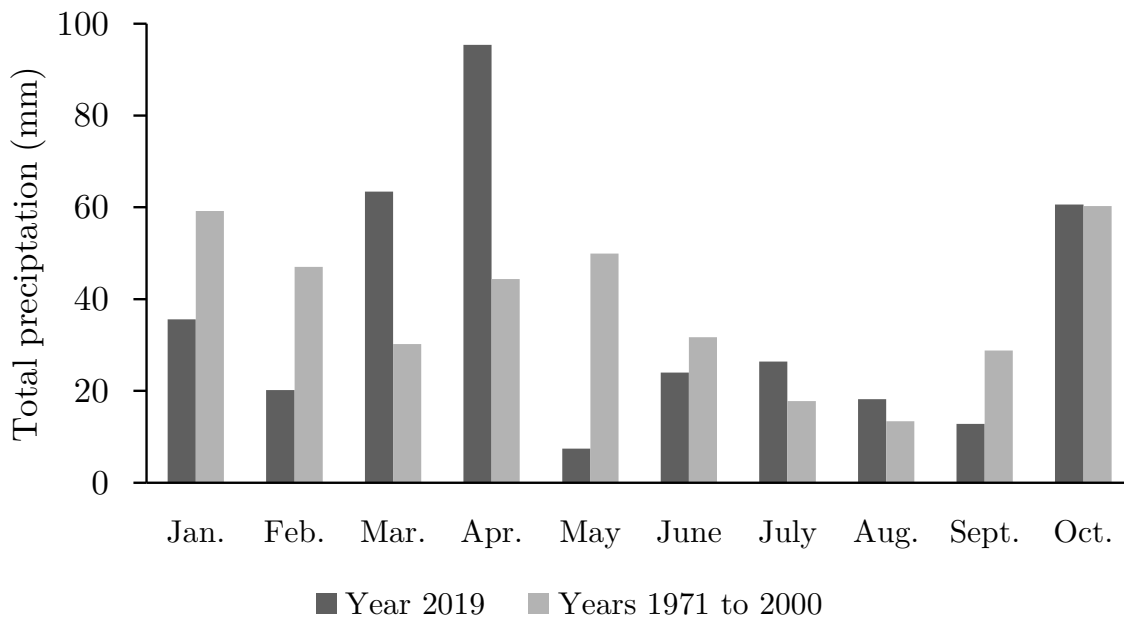


Figure 4.2: Average precipitation in 2019 and in a thirty-year period.

It is observed that the months of March and April of 2019 had higher amounts of precipitation than the regular, compared to the 30-year period. Although, the next month (May) the opposite happens, being a drier month than normal. Further, the temperature was slightly higher in 2019 compared to the other period.

The climate variability have become an agricultural concern in the last few decades. The high temperatures mightly influences viticulture and change wine organoleptic characteristics (Ribeiro, 2013). For this reason, the temperature rise pattern observed in the year 2019 (4.3) could be a problem for the wines in quality compounds and yield.

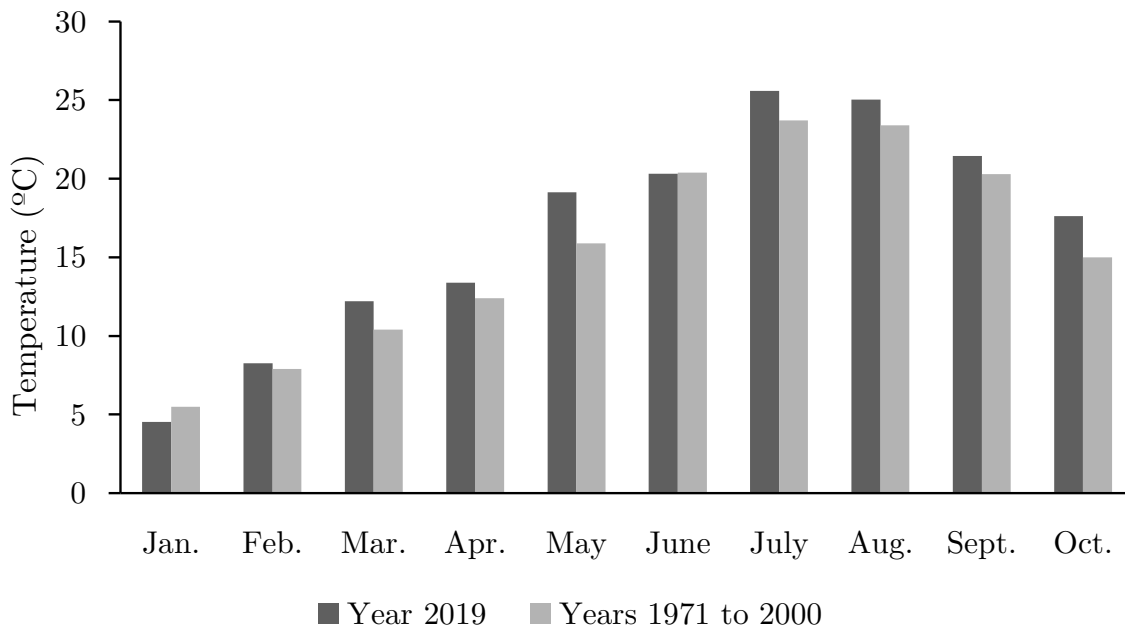


Figure 4.3: Average temperature in 2019 and in a thirty-year period.

Growing-degree Days (GDD)

According to the GDD equation in the Amerine and Winkler (1944) Index (WI), the vineyard assay site is characterized as Region IV, "Favorable for high production, but acceptable table wine quality at best", having 2190,28 °C units of GDD.

Heliothermal Index of Huglin

Following the HI equation, the HI value for the vineyard assay site is 2801, which can be said as a very warm climate class, from Hall and Jones, 2010 (cit. by Birks, 2012).

Cool Night Index

With the CI equation, it was found a value of 13.22 corresponding to a CI 1 classification, which means, *cool nights* viticultural climate class. According to Tonietto and Carbonneau (2004), the threshold concerning berry color and terpenic aroma intensity around 14.2 °C.

4.2 Physiological measurements

Grapevine water status

A total of five predawn leaf water potential measurements were done in the assay (Table 4.1). None of the measurements differed statistically from one another until the last measure, on August 30th. However, an expected pattern is observed: the more amount of water supplied by irrigation, the less water-stressed the plants are, based on its water potential. Analyzing the water restriction of the treatments by its stress, on June 06th, all the treatments were in a zero to mild water restriction level. On July 08th, the treatments were in a light to medium water restriction level. Both on July 29th and August 20th, the treatments were in a medium to strong water restriction level (Ojeda, 2007).

Table 4.1: Predawn leaf water potential (MPa) at different dates throughout maturation.

Treatment	17/06/2019	08/07/2019	29/07/2019	20/08/2019	30/08/2019
Predawn leaf water potential (MPa)					
NIT	-0.15 ± 0.04 a	-0.31 ± 0.04 a	-0.49 ± 0.03 a	-0.66 ± 0.01 a	-0.50 ± 0.02 ab
NIS	-0.15 ± 0.04 a	-0.29 ± 0.02 a	-0.40 ± 0.03 a	-0.60 ± 0.06 a	-0.49 ± 0.05 ab
DI25T	-0.18 ± 0.01 a	-0.43 ± 0.04 a	-0.56 ± 0.05 a	-0.61 ± 0.07 a	-0.53 ± 0.02 ab
DI25S	-0.18 ± 0.01 a	-0.35 ± 0.02 a	-0.46 ± 0.03 a	-0.64 ± 0.05 a	-0.58 ± 0.01 b
DI50T	-0.13 ± 0.02 a	-0.43 ± 0.05 a	-0.49 ± 0.07 a	-0.60 ± 0.06 a	-0.48 ± 0.07 ab
DI50S	-0.13 ± 0.02 a	-0.34 ± 0.01 a	-0.56 ± 0.03 a	-0.45 ± 0.06 a	-0.35 ± 0.03 ab
DI75T	-0.23 ± 0.04 a	-0.35 ± 0.03 a	-0.40 ± 0.06 a	-0.66 ± 0.08 a	-0.31 ± 0.04 a
DI75S	-0.23 ± 0.04 a	-0.28 ± 0.04 a	-0.48 ± 0.04 a	-0.58 ± 0.07 a	-0.39 ± 0.08 ab

Mean values ± standard deviation with different lowercase letters differ significantly ($p \leq 0.05$) for the all treatments studied.

With the water potential of the plants ranging from -0.3 to -0.7 MPa at the period of the harvest, the wines produced from those vines tend to be more balanced, obtaining wines with a greater expression of the fruit to more concentrated wines (Ojeda, 2007).

The last predawn water potential measurement was made on August 30th, eleven days before the harvest. In this measure, the water potentials were in the range of -0,3 to -0,6 MPa, where DI75T is the less water-stressed treatment and DI25S is the more water-stressed treatment. The water restriction levels can be classified in medium to strong (NIT, NIS, DI25T, DI25S, and DI50T) and light to medium (DI50S, DI75T and DI75S) as Ojeda (2007). These changes of restriction level from August 20th to August

30th can be explained to the higher amount of precipitation occurred at the middle of August, which decreased as the month lead to its final days, setting the plants to a water stress level. In a study with similar climate conditions at the Mediterranean Douro Region, Moutinho-Pereira et al. (2007) registered a predawn water potential in Touriga Nacional non-irrigated vines of -0,57 MPa, similar to the water stress level in NIT and NIS treatments. There were no significant differences within canopy height compared to the same irrigation levels for this parameter. Alves et al. (2012) found similar values for predawn water potential for Touriga Nacional in the Douro Region in akin conditions.

Normalized Difference Vegetation Index - (NDVI)

Two measurements were made to obtain the NDVI, one on August 20th and another one on August 30th (Figure 4.4). The levels found for this parameter did not differ statistically from one another between the two measures. The NDVI values tends to decrease with leaf senescence, as the measurement dates are very close, this evolution is not clearly visible. On the other hand, it was expected that the vines of the treatments not irrigated the values would be lower due to the greater leaf senescence due to water stress. Although, this year this difference was not seen due to climatic conditions (rainfall during the summer).

In this way, the values found for NDVI are higher than the ones in the literature. Pinto (2015) found an upper value of 0.556 and a lower value of 0.395 for Touriga Nacional at the center region of Portugal. Dias (2009) found a medium value of 0.48 for Touriga Nacional in the Alentejo region of Portugal. Those results are below the values found in this work. The NDVI values may represent that the plants observed in this study, even on Mediterranean climate, could be healthy for this parameter. However, different physiological analyses may show drought effects thoroughly.

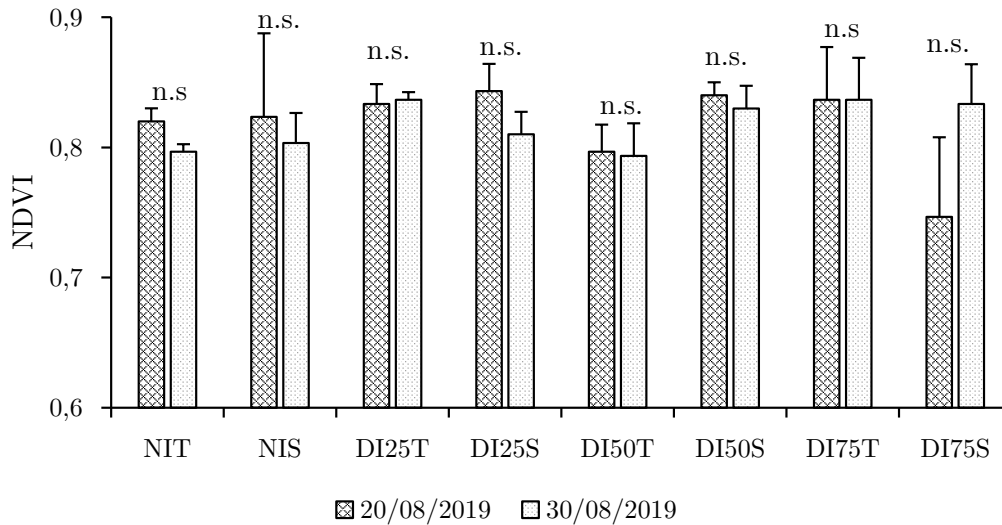


Figure 4.4: Normalized difference vegetation index. Mean values with different letters differ significantly ($p \leq 0.05$) for the all treatments studied and n.s. for non-significance difference.

Chlorophyll Concentration Index (CCI)

The SPAD analyzes is used for understanding the chlorophyll concentration in plants. These measurements occurred twice, in the same dates as NDVI. In situations of water stress after two weeks from the onset of stress, the SPAD value is lower, which can enable the previous detection of the stress condition in the plant (Fanizza et al., 1991). This situation can be seen in Figure 4.5, which correlates better water status (Ψ) and better SPAD for treatment DI75, where SPAD values differed statistically within treatments. Moutinho-Pereira et al. (2007) found fairly similar values for the same non-irrigated vine variety in akin climate conditions.

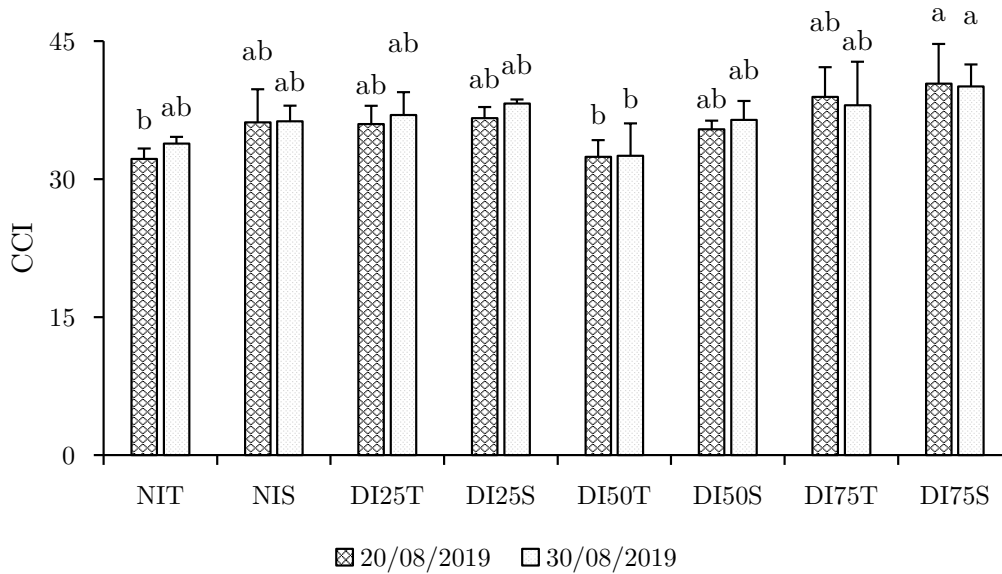


Figure 4.5: Leaf CCI content. Mean values with different letters differ significantly ($p \leq 0.05$) for the all treatments studied and n.s. for non-significance difference.

Leaf gas exchange

The gas exchange results (Table 4.2 and 4.3) showed that vines in these semiarid conditions can struggle severe water and heat stress, which can debilitate its physiological behavior by reducing photosynthetic activity, mostly at a closer-midday time, owing to stomatal and non-stomatal limitations (Moutinho-Pereira et al., 2007). The variation of the photosynthetic rate is directly linked to stomatal conductance, considering the stoma closure at the hottest hours of the day, leading to a lower photosynthesis rate. Furthermore, the positive effect of irrigation can be seen alongside most of gas exchange rates and also in Water Use Efficiency (Alves et al., 2012). The water stress in grapevines can be reflected in g_s , an integrative parameter (Flexas et al., 2002). Therefore, water stress severity can also be classified according to the decrease in g_s (Keller, 2015), for this, in well-watered grapevines the g_s range from 0.2 to 0.5 mol H₂O m⁻² s⁻¹; in mild water stress g_s declines to 0.15 mol H₂O m⁻² s⁻¹; in moderate water stress, g_s range from 0.15 to 0.05 mol H₂O m⁻² s⁻¹, and, finally, in severe stress the g_s values in lower than 0.05 mol H₂O m⁻² s⁻¹.

Regarding the gas exchange at leaves, the usual behavior of the plants was to have

better levels of the average measures at the treatments with higher irrigation, due to plant's water supply being higher and the water stress being lower, both in July 29th (4.2) and August 30th (4.3).

In net rate of CO₂ assimilation the treatments did not have a large range of statistical differences in July, although, the values were higher mostly for treatments with more water available, due to irrigation. The transpiration rate better expresses the drought effects at 14:00 (August) measurement, pointing NIT and DI25T as the lowest values and DI50 and DI75S as the highest ones. Stomatal conductance had a similar result of transpiration rate for previous and post midday measurements in July and August. Finally, intercellular CO₂ concentration also behavior in the same way, as drought being the potential reason for the decrease values in NIT and DI25T.

Comparing the tables, it is possible to observe a decrease in most values of the gas exchange parameters from the first measurement (4.2) to the second on (4.3), and this is a result of the higher water stress in the month of August. For a study in similar conditions Moutinho-Pereira et al. (2007) found similar values for the gas exchange parameters studied.

Table 4.2: Main effects of canopy structure and level of water stress, and their interactions, on the intercellular CO₂ concentration (C_i), transpiration rate (T_r), stomatal conductance (gs) and, average net rate of CO₂ assimilation (A_{net}) in Touriga Nacional cultivar of grapevine on July 29th, 2019 for two periods of the day.

	C_i		T_r		gs		A_{net}	
	(mol mol ⁻¹)		(mmol H ₂ O m ⁻² s ⁻¹)		(mol H ₂ O m ⁻² s ⁻¹)		(mol CO ₂ m ⁻² s ⁻¹)	
	10:00	14:00	10:00	14:00	10:00	14:00	10:00	14:00
NIT	236.16 ± 35.25 ab, ^A	205.66 ± 13.51 bc, ^A	2.89 ± 0.45 bc, ^A	3.13 ± 0.61 cd, ^A	0.09 ± 0.02 c, ^A	0.09 ± 0.02 cd, ^A	6.59 ± 1.91 c, ^B	8.02 ± 1.21 abc, ^A
NIS	215.66 ± 47.42 ab, ^A	218.00 ± 14.81 abc, ^A	2.63 ± 0.37 bcd, ^A	3.30 ± 0.46 bcd, ^A	0.18 ± 0.03 bc, ^A	0.10 ± 0.01 bcd, ^A	13.08 ± 2.47 a, ^A	7.97 ± 0.98 abc, ^A
DI25T	233.83 ± 44.30 b, ^A	195.83 ± 21.60 c, ^A	1.73 ± 0.69 d, ^A	2.71 ± 1.50 cd, ^A	0.11 ± 0.05 c, ^A	0.08 ± 0.05 c, ^A	7.47 ± 2.25 c, ^A	7.01 ± 3.33 bc, ^A
DI25S	192.83 ± 55.61 ab, ^A	196.16 ± 14.98 c, ^A	1.97 ± 0.87 cd, ^A	2.49 ± 0.54 d, ^A	0.10 ± 0.05 c, ^A	0.07 ± 0.02 d, ^B	8.37 ± 3.20 bc, ^A	6.62 ± 1.36 c, ^A
DI50T	257.5 ± 11.84 a, ^A	245.66 ± 12.89 a, ^A	3.49 ± 0.87 ab, ^A	5.19 ± 0.57 a, ^A	0.25 ± 0.07 ab, ^A	0.10 ± 0.03 a, ^A	12.33 ± 2.76 ab, ^A	10.69 ± 1.40 ab, ^A
DI50S	264.16 ± 4.62 a, ^A	237.16 ± 10.59 a, ^A	4.04 ± 0.55 a, ^A	4.95 ± 0.38 ab, ^A	0.31 ± 0.07 a, ^A	0.18 ± 0.02 ab, ^A	13.66 ± 2.08 a, ^A	10.76 ± 0.55 ab, ^A
DI75T	253.66 ± 9.30 ab, ^A	233.16 ± 14.07 ab, ^A	2.95 ± 0.43 bc, ^A	4.35 ± 0.67 abc, ^A	0.24 ± 0.06 ab, ^A	0.18 ± 0.04 ab, ^A	12.53 ± 1.73 ab, ^A	10.91 ± 2.18 a, ^A
DI75S	248.83 ± 13.80 ab, ^A	227.66 ± 22.18 ab, ^A	1.73 ± 0.52 cd, ^A	4.09 ± 1.72 abcd, ^A	0.19 ± 0.06 bc, ^A	0.15 ± 0.08 abc, ^A	11.03 ± 2.61 abc, ^A	9.29 ± 3.42 abc, ^A

Mean values ± standard deviation with different lowercase letters differ significantly ($p \leq 0.05$), for the all treatments studied. Mean values ± standard deviation with different capital letters differ significantly ($p \leq 0.05$) for the canopy structure studied within the same irrigation treatment.

Table 4.3: Main effects of canopy structure and level of water stress, and their interactions, on the intercellular CO₂ concentration (C_i), transpiration rate (T_r), stomatal conductance (gs) and, average net rate of CO₂ assimilation (A_{net}) in Touriga Nacional cultivar of grapevine on August 30th, 2019 for two periods of the day.

	C_i (mol mol ⁻¹)		T_r (mmol H ₂ O m ⁻² s ⁻¹)		gs (mol H ₂ O m ⁻² s ⁻¹)		A_{net} (mol CO ₂ m ⁻² s ⁻¹)	
	10:00	14:00	10:00	14:00	10:00	14:00	10:00	14:00
NIT	253.00 ± 19.80 a, ^A	217.50 ± 15.95 a, ^A	1.63 ± 0.93 cd, ^A	1.36 ± 0.63 c, ^A	0.08 ± 0.06 d, ^A	0.03 ± 0.01 c, ^A	5.34 ± 3.62 cd, ^A	2.32 ± 1.05 d, ^A
NIS	246.00 ± 6.60 a, ^A	226.33 ± 19.12 a, ^A	2.60 ± 0.62 bc, ^A	2.17 ± 0.95 bc, ^A	0.14 ± 0.04 bcd, ^A	0.05 ± 0.02 bc, ^A	9.03 ± 2.21 abc, ^A	3.68 ± 1.78 cd, ^A
DI25T	254.67 ± 37.28 a, ^A	223.33 ± 39.91 a, ^A	1.19 ± 0.83 d, ^A	0.80 ± 0.30 c, ^A	0.05 ± 0.04 d, ^A	0.02 ± 0.01 c, ^A	3.69 ± 3.22 d, ^A	1.16 ± 0.27 d, ^A
DI25S	241.67 ± 21.07 a, ^A	223.17 ± 8.5 a, ^A	1.68 ± 0.62 cd, ^A	2.54 ± 1.37 bc, ^A	0.09 ± 0.04 cd, ^A	0.06 ± 0.04 bc, ^A	6.18 ± 2.45 bcd, ^A	4.45 ± 2.54 bcd, ^A
DI50T	256.00 ± 12.55 a, ^A	223.33 ± 13.69 a, ^A	3.58 ± 0.34 ab, ^A	4.61 ± 0.41 a, ^A	0.21 ± 0.03 ab, ^A	0.11 ± 0.02 a, ^A	11.61 ± 1.42 a, ^A	8.27 ± 0.73 a, ^A
DI50S	258.50 ± 7.06 a, ^A	226.67 ± 8.55 a, ^A	3.87 ± 0.44 a, ^A	4.57 ± 0.78 a, ^A	0.24 ± 0.05 a, ^A	0.11 ± 0.02 a, ^A	12.39 ± 1.87 a, ^A	7.97 ± 1.37 ab, ^A
DI75T	244.83 ± 10.98 a, ^A	211.67 ± 29.82 a, ^A	2.97 ± 0.51 ab, ^A	3.34 ± 1.23 ab, ^A	0.18 ± 0.06 abc, ^A	0.08 ± 0.04 ab, ^A	10.82 ± 2.52 ab, ^A	6.67 ± 2.76 abc, ^A
DI75S	247.83 ± 15.08 a, ^A	228.83 ± 12.56 a, ^A	3.76 ± 0.81 ab, ^A	4.49 ± 1.60 a, ^A	0.24 ± 0.06 a, ^A	0.12 ± 0.05 a, ^A	13.52 ± 2.42 a, ^A	8.10 ± 3.63 ab, ^A

Mean values ± standard deviation with different lowercase letters differ significantly ($p \leq 0.05$), for the all treatments studied. Mean values ± standard deviation with different capital letters differ significantly ($p \leq 0.05$) for the canopy structure studied within the same irrigation treatment.

The Water Use Efficiency (WUE) is a relation between A_{net} and gs . There were no significant differences within canopy height at the same irrigation levels for those parameters (4.4). On July 29th, it is possible to see some significant differences among treatments within irrigation levels for both morning (10:00) and afternoon (14:00) periods. The non-irrigated and 25% ETc treatments had better WUE comparing to the ones with greater levels of irrigation. On August 30th, about one month after, no significant differences appeared between treatments, meanwhile, it is possible to note that the WUE levels itself were way higher than on July 29th. This could be related to the fact that on this date the plants were under lower water stress than they were before. For both different time measurements is possible to observe that the values of WUE increased at 14:00 in comparison with the ones of 10:00. This could be related to the fact that the temperatures tend to be higher before midday, making the plants function on higher physiologic capacity (Moutinho-Pereira et al., 2015, Moutinho-Pereira et al., 2007).

Table 4.4: Main effects of canopy structure and level of water stress, and their interactions on Water Use Efficiency (WUE; A_{net}/gs) in Touriga Nacional cultivar of grapevine on July 29th and August, 30th 2019 for two periods of the day.

	WUE (mol CO ₂ mmol ⁻¹ H ₂ O)			
	July 29th		August 30th	
	10:00	14:00	10:00	14:00
NIT	72.38 ± 22.26 ab, A	84.84 ± 14.05 ab, A	65.13 ± 11.98 a, A	80.58 ± 13.51 a, A
NIS	74.80 ± 25.11 ab, A	77.06 ± 9.22 abc, A	65.73 ± 6.06 a, A	84.55 ± 13.78 a, A
DI25T	72.05 ± 25.99 ab, A	91.92 ± 16.96 ab, A	65.78 ± 18.68 a, A	73.33 ± 19.30 a, A
DI25S	95.26 ± 34.45 a, A	93.09 ± 12.99 a, A	72.23 ± 14.62 a, A	80.82 ± 10.73 a, A
DI50T	50.46 ± 7.52 b, A	55.48 ± 7.14 c, A	56.20 ± 6.98 a, A	73.89 ± 10.44 a, A
DI50S	43.75 ± 4.71 b, A	59.58 ± 7.83 c, A	53.41 ± 5.37 a, A	70.88 ± 3.78 a, A
DI75T	53.09 ± 7.77 b, A	60.73 ± 7.30 c, A	63.78 ± 8.59 a, A	83.07 ± 18.30 a, A
DI75S	57.31 ± 8.92 b, A	68.41 ± 20.04 bc, A	57.75 ± 9.58 a, A	69.55 ± 3.72 a, A

Mean values ± standard deviation with different lowercase letters differ significantly ($p \leq 0.05$), for the all treatments studied. Mean values ± standard deviation with different capital letters differ significantly ($p \leq 0.05$) for the canopy structure studied within the same irrigation treatment.

Canopy density

The Point Quadrat parameters calculated according Baeza et al. (2001) are expressed in the Table 4.5.

Table 4.5: Point quadrat parameters for Touriga Nacional cultivar of grapevine at July 8th and August 5th, 2019.

Treatment	Date	PG(%)	LNN(%)	PIL(%)	PIC(%)
NIT	07/08/2019	48.33 ± 7.92 a	0.98 ± 0.11 b	17.65 ± 4.90 a	54.17 ± 20.83 a
	08/05/2019	50.00 ± 5.77 a	0.93 ± 0.14 a	21.56 ± 5.32 a	66.66 ± 25.45 a
NIS	07/08/2019	33.33 ± 6.66 a	1.17 ± 0.21 ab	15.44 ± 5.32 a	27.78 ± 18.08 a
	08/05/2019	46.66 ± 8.81 a	1.23 ± 0.27 a	20.53 ± 4.21 a	83.33 ± 16.66 a
DI25T	07/08/2019	30.00 ± 7.03 a	1.45 ± 0.14 ab	19.30 ± 1.55 a	77.78 ± 16.48 a
	08/05/2019	53.33 ± 6.66 a	1.00 ± 0.25 a	19.44 ± 10.01 a	61.11 ± 5.55 a
DI25S	07/08/2019	20.02 ± 7.03 a	1.93 ± 0.22 a	24.63 ± 6.19 a	86.11 ± 9.04 a
	08/05/2019	20.00 ± 15.25 a	2.10 ± 0.43 a	33.07 ± 9.04 a	82.22 ± 9.68 a
DI50T	07/08/2019	28.33 ± 10.46 a	1.35 ± 0.20 a	20.73 ± 5.01 a	36.67 ± 16.91 a
	08/05/2019	43.33 ± 14.52 a	1.23 ± 0.27 a	22.61 ± 4.29 a	58.33 ± 30.04 a
DI50S	07/08/2019	25.00 ± 6.70 a	1.35 ± 0.20 ab	21.90 ± 7.74 a	54.17 ± 16.35 a
	08/05/2019	30.00 ± 15.27 a	1.50 ± 0.37 a	29.86 ± 5.68 a	66.66 ± 16.66 a
DI75T	07/08/2019	35.00 ± 5.62 a	1.53 ± 0.17 ab	30.50 ± 1.01 a	36.11 ± 15.16 a
	08/05/2019	30.00 ± 5.77 a	1.37 ± 0.03 a	16.84 ± 4.57 a	66.67 ± 33.33 a
DI75S	07/08/2019	30.00 ± 10.64 a	1.63 ± 0.25 ab	22.27 ± 4.95 a	76.67 ± 10.54 a
	08/05/2019	33.33 ± 18.55 a	1.4 ± 0.35 a	14.45 ± 5.18 a	88.89 ± 11.11 a

PG - Percent gaps; LNN - Leaf layer number; PIL - Percentage of interior leaves, and PIC - Percentage of interior clusters.

In the third manual of Viticare on Farm Trails the Australian Wine Research Institute (2001) relates the optimum values for this measurement, as Percent gaps to 20 to 40%, LLN < 1.0 to 1.5, Percent of interior leaves < 10% and Percent of interior clusters < 40%, also cited by Smart and Robinson (1991). Analyzing this parameter, all treatments despite NIT (both dates), NIS (August, 05), DI25T (August, 05), and DI50T (August, 05) are in the optimum Percent gaps. A hypothesis for this occurrence is the fact that these treatments received less water than the others, enabling more leaves to fall, in detriment of the drought water stress, making more gaps in the canopy. Regarding treatment DI25, the tall canopy had more gaps compared to the short canopy. This could be related, as

well, to the water factor, that in a short wall can be more efficient in a stress condition than in a tall wall, that may require more water to its optimum function. To the LLN values, the treatment DI25S had results upper than the optimum, as well as, DI75T and DI75S at July, 08th. All of the treatments had a level of PIL higher than the optimum, supposing a dense canopy. Only the treatments NIS, DI50T and DI75T on July, 08th had a result at an optimum level of PIC, the rest of the treatments had a superior amount of intern clusters, which could be a good condition in arid climates with high sunlight exposure, such as the Douro Superior region. Smart et al. (1990), considered two different canopies, one as low density and the other as high density. The low density had 26% of gaps. For this matter, only the treatment DI25S had a low-density canopy, as the other treatments have a high-density canopy. The high-density canopy situation can also be in favor of the plants in drought conditions.

4.3 Crop yield and agronomic parameters

The harvest yield obtained different results per amount of water supplied and per canopy wall height. In Figure 4.6 it is possible to see that the highest yield happened at treatment DI50S, followed by DI50T and DI25T, not statistically different from NIS, DI25S, DI75T, and DI75S.

Two inferences can be done in this measurement: (1) the yield is significantly lower in non-irrigated treatments; (2) especially at the two non-irrigated treatments (NIT and NIS) the canopy wall heights has more impact on the yield, where the short canopy wall (NIS) can have a higher production compared to the tall canopy wall. For the reduction of water supply connected to lower yield, Shellie (2014) found similar results when comparing three sustained deficit irrigation systems (90, 70 and 30% of ETc) with Merlot grapevines, where the production (yield/vine) did not differ statistically at the 90 and 70% of ETc treatments, but differed from the 30% treatment, that had lower yield/plant, testifying the increase in yield as the increase of the water supply until some level. Gonçalves et al. (2016) in a study at Douro Region with the Touriga Nacional variety found out results quite similar as well, with three deficit irrigation treatments (0, 25 and 50% of ETc), resulting in the higher yields at 25 and 50% ETc, not differing from each other.

The water productivity (Figure 4.6) shows a particularly interesting situation: when in high amounts of water supply the canopy height does not make such inference in the relation of production and water use, however, in non-irrigated plants (NIT and NIS), the short canopy height plants can use the water in a more efficiently way. That could lead to the understanding of the water stress in non-irrigated grapevines at Mediterranean conditions being managed with vegetation maintenance.

Some of the yield attributes have a range considered to be great (Smart and Robinson, 1991). Considering the Ravaz index, Baeza et al. (2001) says that several studies considered the number of 5 to 10 kg production by kg of pruned shoots is the ideal for grapevines. In Table 4.6 it is possible to see that not all treatments had the ideal number for Ravaz index, exceptionally, NIT, DI25T, DI25S and DI25T, most of that with the tall

height of the canopy.

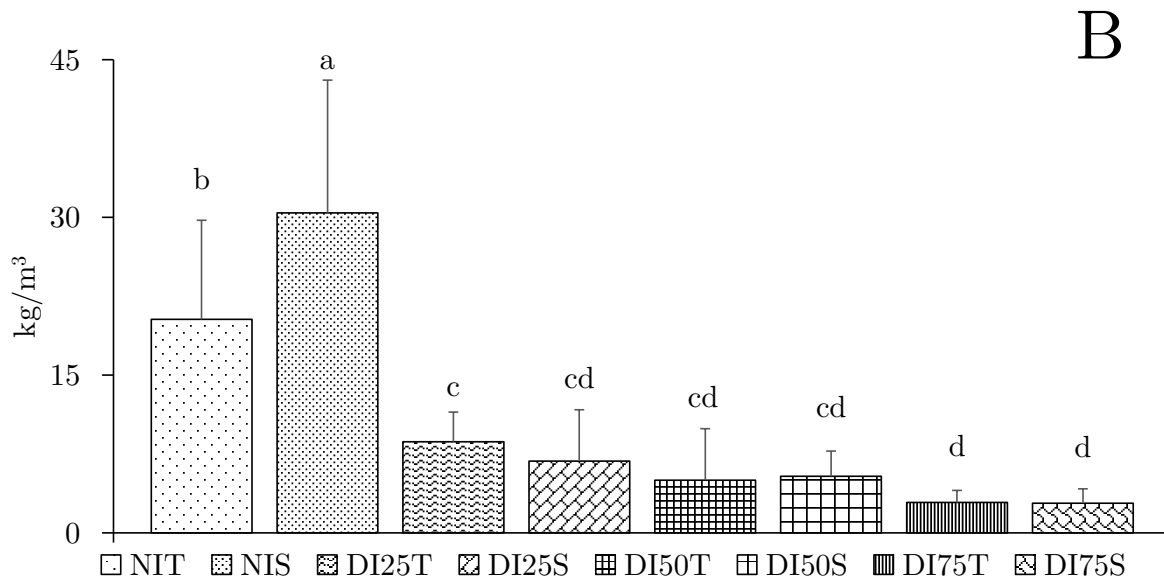
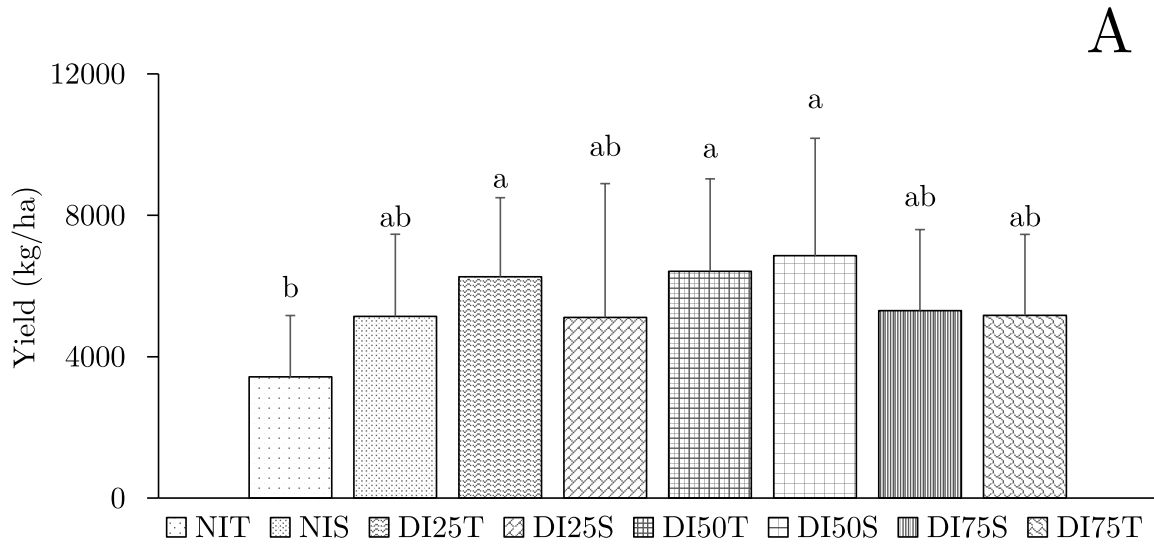


Figure 4.6: Touriga Nacional plants under 25, 50, and 75% ETC deficit irrigation and non-irrigated. Mean values with different letters differ significantly ($p \leq 0.05$) for the all treatments studied. *A*: Total harvest yield per hectare; *B*: Water productivity.

At the production level, regarding the differences among the variables n° of clusters/plant and clusters weight (Table 4.6), the irrigation factor had its impact in the comparison of the treatment NIT - non-irrigated -, and the rest of the treatments, linking the water stress and the low water supply with lower quantitative results. Comparing the canopy structure, it is possible to note that the significant difference among canopy height is shown as well in non-irrigated treatments (NIT and NIS), still having the tall wall height lower levels in the parameters considered than the short wall. Vasconcelos and Castagnoli (2000) found out akin results on a study with plants shoot topping and not topping, where the topping ones had higher values in cluster weight.

Table 4.6: Yield parameters for Touriga Nacional cultivar of grapevine: N° of clusters/-plant, Cluster weight (g), Pruning weight (kg), Shoots/plant, and Ravaz index.

Treatment	N° of clusters/plant	Cluster wt (g)	Pruning wt (kg)	Shoots/plant	Ravaz index
NIT	8.8 ± 0.55 c, B	94.33 ± 30.05 b, B	0.27 ± 0.03 bc, A	4.5 ± 0.20 a	3.94 ± 0.52 ab, A
NIS	9.7 ± 0.60 bc, A	130.33 ± 8.66 a, A	0.25 ± 0.02 c, A	4.7 ± 0.24 a	5.30 ± 0.40 a, A
DI25T	11.6 ± 0.52 abc, A	131.33 ± 5.87 a, A	0.40 ± 0.02 ab, A	4.9 ± 0.22 a	4.24 ± 0.44 ab, A
DI25S	10.3 ± 0.97 abc, A	107.33 ± 7.94 ab, A	0.44 ± 0.03 a, A	4.6 ± 0.26 a	3.02 ± 0.42 b, A
DI50T	12.8 ± 0.50 a, A	118.33 ± 6.70 ab, A	0.34 ± 0.02 abc, A	5.3 ± 0.18 a	5.04 ± 0.45 ab, A
DI50S	11.9 ± 0.79 ab, A	138.66 ± 6.73 a, A	0.37 ± 0.03 abc, A	5.3 ± 0.24 a	5.69 ± 0.69 a, A
DI75T	9.5 ± 0.46 bc, A	135.66 ± 7.25 a, A	0.43 ± 0.04 a, A	4.4 ± 0.24 a	3.56 ± 0.33 ab, A
DI75S	9.7 ± 0.64 bc, A	131.33 ± 9.91 a, A	0.28 ± 0.02 bc, B	4.8 ± 0.18 a	5.30 ± 0.65 a, A

Mean values ± standard deviation with different lowercase letters differ significantly ($p \leq 0.05$), for the all treatments studied. Mean values ± standard deviation with different capital letters differ significantly ($p \leq 0.05$) for the canopy structure studied within the same irrigation treatment.

The pruning weight (Table 4.6) did not differ statistically within treatments, and Shellie (2014), found slightly higher values for this parameter, guiding to higher values of Ravaz index as well.

When comparing the Ravaz index and the water supply (Figure 4.7), no substantial inferences can be induced. In a similar study, Shellie (2014) found similar results in the first year of the experiment, where the treatment without irrigation and the one irrigated at 90% ETc did not differ statistically from one another in this comparison, however, for the third-year evaluation, the outcomes were different. Possibly, this pattern would be achieved in future evaluations of this work.

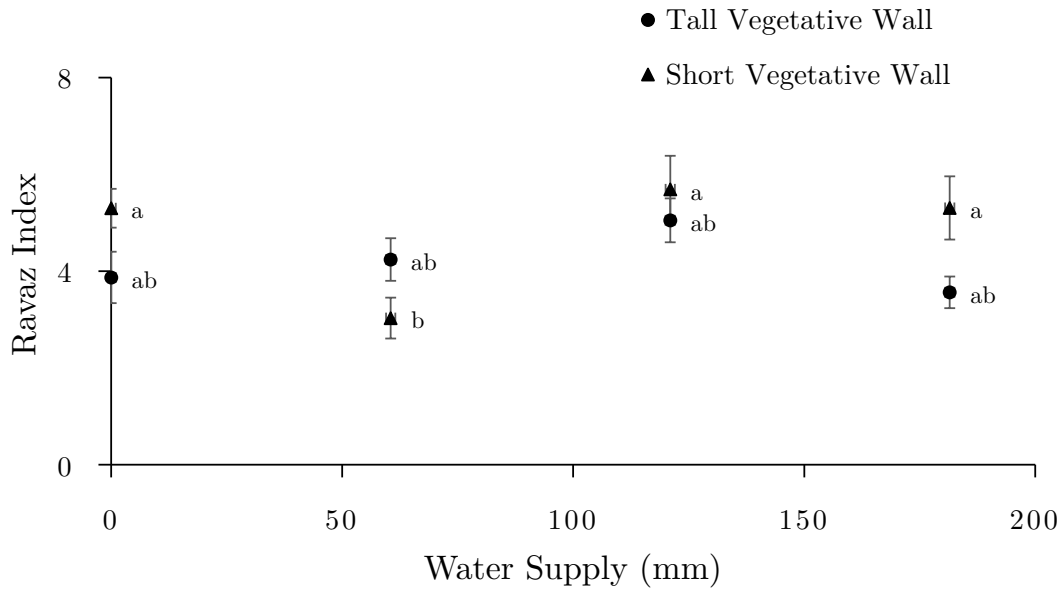


Figure 4.7: Water productivity of Touriga Nacional grapevines: relation of Ravaz index and water supply. Bars represent the standard error of the mean. Mean values with different letters differ significantly ($p \leq 0.05$) for the all treatments studied.

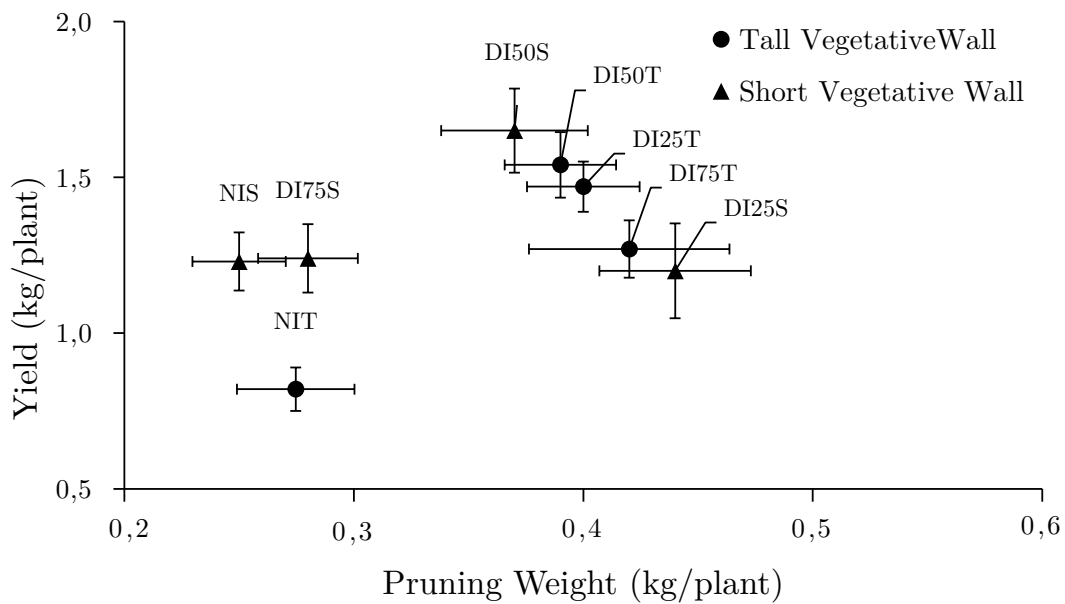


Figure 4.8: Relationship between pruning weight and yield in Touriga Nacional grapevines. Bars represent the standard error of the mean.

Berry characteristics

Considering the berry biometrics on weight evolution since veraison until the harvest, no great differences can be shown between irrigation levels. Although, it is possible to observe in both short (Figure 4.10) and tall (Figure 4.9) canopy heights for all irrigation levels the morphological behavior of the berries to lose weight about the month of August and ahead. At this time the grapevines were close to the technological maturation (Code 89 of BBCH Scale) and the summer climatic conditions had severe high temperatures and less water available, emphasizing the water stress on plants. This behavior of drought-induced berry shrinkage is also observed by Keller et al. (2016). This condition may lead to a loss in yield since the water diminished is equal to less weight at harvest.

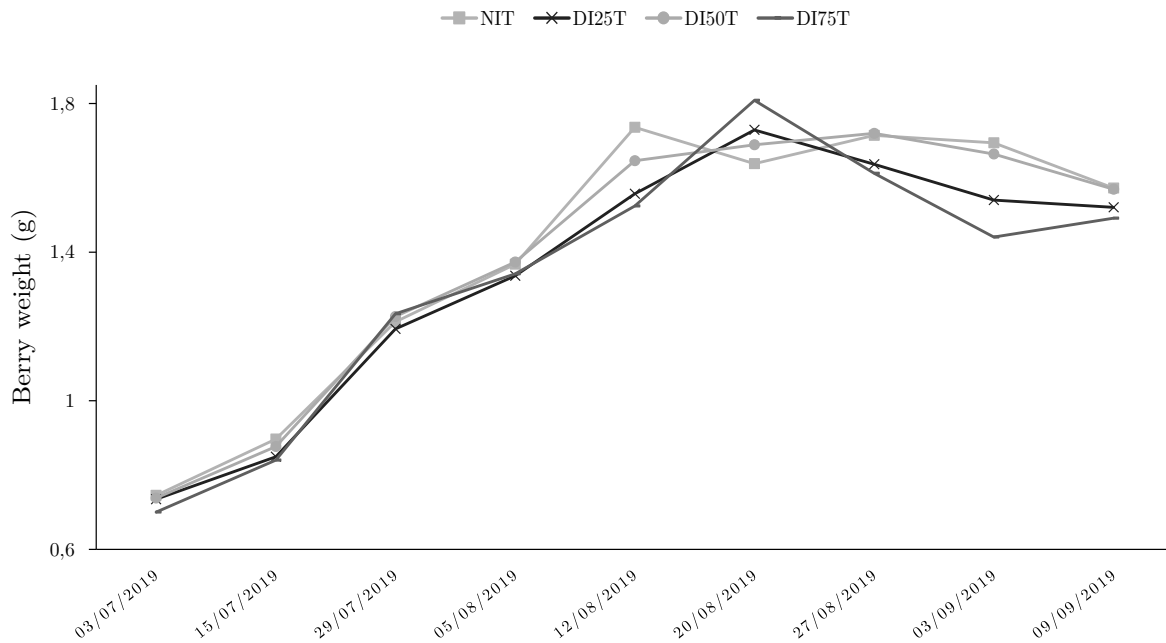


Figure 4.9: Evolution of the berries weight (in grams) for treatments with short canopy. Mean values for the all treatments studied.

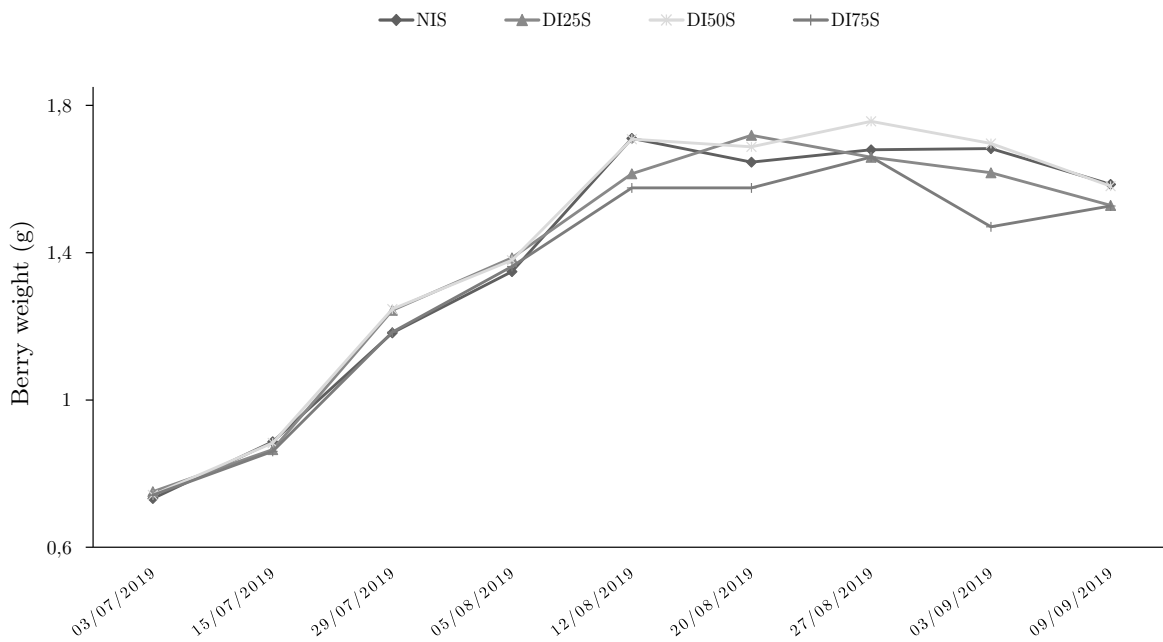


Figure 4.10: Evolution of the berries weight (in grams) for treatments with short canopy. Mean values for the all treatments studied.

4.4 Must quality at harvest

The must quality compounds results analyzed in the *FTIR* are shown in Table 4.7, where it is possible to observe that there were no significant differences within treatments at canopy weight level for the parameters compared at same irrigation levels.

Analyzing the total soluble solids in °Brix (Table 4.7), significant differences are noted within treatments, at different irrigation levels. The lowest level of this parameter was treatment DI75T, followed by DI75S. The highest level is found in DI25T, followed by NIS. This pattern demonstrates the capacity of the water supply increment decreases the sugar content in the berries. Although, the soluble solids for all eight treatments were into normal rates (Alves et al., 2012, Shellie, 2014). A higher sunlight exposure tends to increase TSS, increase pH by having more tartaric acid (g/L) and less malic acid (g/L), as Bowen (2009) mentions, highlighting the importance of timing canopy management to increase the light exposure, especially of young fruit, though also the need to maintain an amount of shading to prevent excess heating of clusters. Moreover, large and dense canopies from bountiful water availability are associated with reduced fruit soluble solids, high acidity, and poor skin color, and, the water deficit, in contrast, typically reduces yield (Keller, 2015). This behavior is seen in Table 4.6.

Still regarding quality aspects, despite total soluble solids, all the parameters analyzed found to be not significantly different among different irrigation levels and canopy heights. However, a tendency is noted, where the higher results on every parameter tend to be at non-irrigated treatments.

Reducing canopy density of vines through leaf thinning can affect aspects of fruit quality, such as pH, total soluble solids, and titratable acidity (Hill et al., 2011). These changes appear when comparing the same irrigation level within different canopy height. In pH, for example, despite NI treatments, DI25, DI50, and DI75 had lower pH in short canopy. The same occurred for titratable acidity, despite in DI50 treatments. Tartaric and malic acids behavior in a similar way, having higher amounts in treatments with short canopy height, despite in DI50. Cancela et al. (2017) found similar results for

cv. "Godello" considering tartaric and malic acids, which had higher values in rainfed treatments than in surface drip irrigation. However, the opposite occurred in cv. Albariño.

Viticulture practices, edaphoclimatic conditions, and post-harvest processing activities can affect the content of total polyphenols compounds in grapes. Although, the varietal or genetic difference is one of the most influential factors (Teixeira et al., 2013). The chemical behavior of polyphenol content within treatments does not follow a clear pattern in this study. While in NI and DI50 treatments the short canopy has a greater amount of polyphenols, in DI25 and DI75 the tall canopy assumes this result. The treatment NIT has the highest value of the total polyphenol index, while DI75T has the lowest value. Yet, those values do not differ statistically from one another. The wine color intensity, as well as concentrations of phenols and anthocyanins, increased in severe water stress (midday stem water potential of -1.4 to -1.5 Mpa) at pre and post veraison for Ferreyra et al. (2004) study. Those results corroborate with the results found in this work for Color Intensity and Anthocyanins. Gonçalves et al. (2016) found similar results in an akin work.

In an overall observation, it is possible to say that quality components on cv. Touriga Nacional were affected but did not differ in a significant way either by different water supplies in deficit irrigation nor by different heights of the canopy in this study.

Table 4.7: Must quality parameters for Touriga Nacional cultivar of grapevine on harvest. *FTIR* analyses for Total Soluble Solids (TSS, °Brix), Titratable acidity (TA, g L⁻¹), pH, Tartaric and Malic Acids (g L⁻¹), Total Polyphenol Index (TPI), Color Intensity and Anthocyanins.

Treatment	TSS (°Brix)	TA (g L ⁻¹)	pH	Tartaric Acid (g L ⁻¹)	Malic Acid (g L ⁻¹)	TPI	Color Intensity	Anthocyanins
NIT	26.47 ± 1.15 ab	4.66 ± 0.21 a	3.35 ± 0.15 a	6.17 ± 0.55 a	0.25 ± 0.07 a	77.57 ± 13.31 a	4.43 ± 1.04 a	93.67 ± 20.26 a
NIS	26.77 ± 1.85 ab	4.87 ± 0.23 a	3.35 ± 0.21 a	6.47 ± 0.31 a	0.50 ± 0.30 a	73.40 ± 14.63 a	4.60 ± 0.50 a	103.0 ± 27.07 a
DI25T	27.47 ± 0.21 a	4.26 ± 0.66 a	3.41 ± 0.06 a	6.10 ± 0.53 a	0.23 ± 0.06 a	69.27 ± 10.70 a	4.40 ± 0.26 a	96.0 ± 18.52 a
DI25S	25.23 ± 0.40 ab	4.90 ± 0.47 a	3.33 ± 0.06 a	6.30 ± 0.20 a	0.60 ± 0.46 a	71.87 ± 7.0 a	4.07 ± 1.94 a	51.67 ± 40.02 a
DI50T	26.0 ± 0.35 ab	5.01 ± 0.56 a	3.34 ± 0.12 a	6.30 ± 0.62 a	0.47 ± 0.29 a	76.70 ± 7.13 a	4.07 ± 0.95 a	52.33 ± 19.66 a
DI50S	26.63 ± 0.59 ab	4.68 ± 0.18 a	3.25 ± 0.02 a	5.97 ± 0.31 a	0.40 ± 0.26 a	70.80 ± 1.93 a	4.27 ± 0.15 a	76.0 ± 16.37 a
DI75T	24.6 ± 0.70 b	4.56 ± 0.03 a	3.34 ± 0.06 a	5.83 ± 0.23 a	0.37 ± 0.21 a	67.53 ± 5.55 a	3.23 ± 3.23 a	38.0 ± 32.91 a
DI75S	25.13 ± 0.90 ab	4.71 ± 0.12 a	3.32 ± 0.08 a	5.97 ± 0.40 a	0.47 ± 0.35 a	70.30 ± 10.0 a	3.80 ± 3.80 a	33.67 ± 18.18 a

Mean values ($p \leq 0.05$) standard deviation with different lowercase letters differ significantly ($p \leq 0.05$), for the all treatments studied. Mean values with different capital letters differ significantly ($p \leq 0.05$) for the canopy structure studied within the same irrigation treatment.

Chapter 5

Conclusions

In an overall analysis, the vines can suffer from warm and water stress, which may harm its physiology, yield, and wine quality.

In the year 2019, the registered temperature at the assay site (Santa Comba de Vilarica, at Douro Demarcated Region) was higher than usual. Also, the precipitation was lower. These are the expected environmental characteristics of climate change. These variations could reduce yield and modify quality components in vines without proper environmental control. This assumption was confirmed when the non-irrigated plants, under rainfed condition, produced statistically less at harvest, comparing to irrigated treatments.

Not only the irrigation modified the harvest yield but, canopy management too. Notably, at the non-irrigated control treatment, the yield was significantly reduced by the most vegetative structure (tall). In physiological aspects, the vines in higher water stress conditions eminently struggle to maintain its correct function in gas exchange processes such as photosynthesis. The different heights of canopy remained unclear at this interference level. The quality compounds were not altered by the different treatments, for both irrigation and vegetative management. Even for TSS, the statistical differences were acceptable and not harmful.

It is worth notice that this study is in the very first year of evaluations. The results found for these parameters may change in future work for the next years, considering the bi-annual reproductive behavior of the vineyard.

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