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RELATIONSHIPS BETWEEN CLIMATE INDICES, PHENOLOGY AND BERRY COMPOSITION OF TOURIGA NACIONAL IN THE DÃO WINE REGION

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Dissertação para obtenção do Grau de Mestre em

Vinifera EuroMaster – European Master of Sciences of Viticulture and Oenology

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- Doutor Carlos Manuel Antunes Lopes, Professor Associado do Instituto Superior de Agronomia da Universidade Técnica de Lisboa.

Lisboa, 2012

Acknowledgements

I would like to express my sincere gratitude and appreciation to:

- Professor Carlos Lopes of the Department of Sciences and Engineering of Biosystems (DCEB) of ISA, UTL, for his help and patience on the supervising of this thesis.

- The Centro de Estudos Vitivinicolas do Dão for providing the Touriga Nacional data and all the team of the research project SIAMVITI (Portuguese Foundation for Science and Technology funded project: PTDC/AAC-AMB/105024/2008) for having invited me to work with them.

- Ricardo Egipto for helping me analyse the large amount of data for this study.

Abstract

Despite the expansion and increasing popularity of Touriga Nacional in Portugal, there has not been extensive research into the variety's response to different climate characteristics. The objective of this research was to test the relationships between different climate indices and berry composition and Phenology in Touriga Nacional in the Dão winegrowing region of Portugal. From the available data we can also explore any evolution of the climate, berry composition and phenology during the recorded years.

Climate and plant data were analysed from records collected at the 'Centro de Estudos Vitivinícolas do Dão' in the Dão wine region of northern Portugal between 1963-2010. Six common climate indices were calculated from the climate data for each year of the recorded period. Berry composition and phenology records of Touriga Nacional planted at the research centre from the same period were also analysed. Any trends or changes in climate or plant data were analysed over the recorded period. All climate indices were correlated against the plant data results to investigate any relationships.

An increase in the Growing Season Temperature Index (GST) during the studied period was observed that agreed with studies over a similar period in other parts of Europe. Various climate indices had some correlations with grape and vine parameters; generally acidity showed a reduction with increases in temperature-based indices. Budburst and harvest dates were confirmed to be hastened by higher temperatures. Also yield had a positive correlation with the Huglin Index (HI). HI had a better correlation with plant parameters because it is calculated over a 6-month (instead of 7-month) period.

Understanding the relationship between Touriga Nacional and climate indices can help producers maximise the potential of this important Portuguese variety, as well as aid its expansion into other Portuguese regions and other regions throughout the world.

Keywords: Touriga Nacional, Climate, phenology, berry composition, yield, rootstock

Resumo

Apesar da expansão e popularidade crescente de Touriga Nacional em Portugal, não foi efectuada ainda uma extensa pesquisa relativamente à resposta da casta a diferentes condições climáticas. O objetivo deste trabalho foi testar as relações entre os índices bioclimáticos, a fenologia e a composição da uva da casta Touriga Nacional na região vitícola do Dão, Portugal. A partir dos dados disponíveis pretende-se ainda estudar a evolução do clima, da fenologia e da composição da uva ao longo de várias anos.

Os dados de clima e planta foram analisadas a partir de dados colhidos no periodo 1963-2010 no 'Centro de Estudos Vitivinícolas do Dão', em Nelas, na região viticola do Dão, Portugal. Seis índices bioclimáticos foram calculados a partir dos dados climáticos em cada ano do periodo considerado. No mesmo período foram analisados as principais datas fenológicas e a composição da uva á vindima. Os índices bioclimáticos foram correlacionados com a composição da uva à vindima. Verificou-se um aumento do índice GST (Growing Season Temperature) ao longo do período estudado o que está de acordo com estudos efectuados noutras regiões da Europa. Verificou-se que diversos índices bioclimáticos se apresentaram correlacionados com os dados da maturação. Em geral a acidez total apresentou uma relação negativa com os índices baseados na temperatura. As datas de abrolhamento e vindima foram antecipadas em situações de elevadas temperaturas. O rendimento também apresentou uma correlação positiva com o Índice de Huglin.

O conhecimento das relações entre os índices bioclimáticos e o comportamento da casta Touriga Nacional permitirá maximizar o potencial desta importante casta portuguesa, bem como, ajudar à sua expansão para outras regiões do Mundo.

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1. Introduction

Touriga Nacional is one of, if not the most important grape varieties in Portugal today. Due to its increasing importance in the wine industry in Portugal and throughout the world it is becoming more and more widely planted. Touriga Nacional was traditionally planted mainly in the Portuguese wine regions of Douro and Dão in the North of the country. There has been little research conducted on the suitability of this variety in various geographical and climatic zones, especially when compared to famous international varieties such as Cabernet Sauvignon, Merlot and Chardonnay (Bohm 2007).

Wine regions worldwide are typically classified in climate-terms using a number of different climate indices, created by several different researchers. Six of the more common climate indices include Growing Season Temperature (GST), Growing Degree Days (GDD), Biologically Effective Degree Days (BEDD), Huglin Index (HI), Cool Night Index (CI) and Dryness Index (DI) (Hall and Jones 2010).

Despite the expansion and increasing popularity of Touriga Nacional in Portugal, there has not been extensive research into the variety's response to different climate characteristics.

The objective of this research was to test the relationships between different climate indices and berry composition and Phenology in Touriga Nacional in the Dão winegrowing region of Portugal. From the available data we can also explore any evolution of the climate, berry composition and phenology during the recorded years.

This research is part of a Portuguese research project on climate changes entitled "SIAMVITI - Climate change in Viticulture: Scenarios, Impacts and Adaptation Measures" funded by the Portuguese Foundation for Science and Technology (Proj. PTDC/AAC-AMB/105024/2008).

1.1 Climate and the grapevine

There are many factors which affect the growth of the grapevine of the Vitis vinifera cultivar and its production of wine grapes. Exterior conditions such as rainfall, wind, sunlight, temperature, soil type, nutrient availability, cultural practices, pests and diseases vary greatly across vine growing regions and countries throughout the world.

These external conditions can have varying effects on wine grape quality and yield. These effects are also dependent on the great variation of internal attributes of a grapevine, such as variety, rootstock and the susceptibility of the plant to pests and diseases.

With such a combination of factors to consider, the grape vine and its main products; grapes and wine, are part of a complicated and diverse system.
When considering the commercial production of wine from grapes, it is necessary to take into account these various and complicated parameters, in order to best meet the requirements of a project in any given location.

1.1.1 Climate

The term 'climate' encompasses several important external influences on the grapevine. Climate can be defined as 'the composite or generally prevailing weather conditions of a region, as temperature, air pressure, humidity, precipitation, sunshine, cloudiness, and winds, throughout the year, averaged over a series of years' as found on the online dictionary (www.dictionary.com).

The effects of climate on viticulture can be separated into temperature, rainfall, sunlight, wind and humidity. These different components can result in varying responses from a grapevine which are being continually studied to aid in the production of quality grapes.

Temperature is the most important factor for vegetative growth and berry maturation. Prescot (1965), when describing areas suitable for grape production reported: 'the mean temperature of the warmest monthly period must be in excess of 19ºC and that of the coldest monthly period must be in excess of 1ºC.' Although this is not a strict rule, it is however suggesting that grapes are native to the warm temperate zone. Phenology describes the processes and timing of a vine's development through dormancy, budburst, flowering, setting, veraison and ripening. The evidence is now clear that vine phenology is controlled by temperature alone (Coombe and Dry 2004). Vines require a dormant period during winter which is initiated when mean temperatures are consistently less than 10ºC. Grapevines can suffer damage during dormancy if exposed to temperatures below –15ºC, and can die below –20ºC (Winkler, 1962). During the vegetative period, warm temperatures promote the growth of shoots and leaves, warmer soils promote root growth which also favours leaf area development. Vine phenological

processes respond more or less linearly from a mean temperature of about 10ºC (below which no growth occurs) to perhaps 16-17ºC, after which the response slows. A large canopy of leaves can provide more potential for photosynthesis, which is also more rapid in warm temperatures. Photosynthesis is vital for vine and berry development, and will determine the ripening of grapes and harvesting time. Warmer temperatures around flowering has proven to provide a greater fruit-set, resulting in higher grape yields. In hotter regions, when water is in short supply and temperatures exceed 35ºC the stomata in the leaves may close in order to prevent further loss of water, in turn photosynthesis is arrested and berry maturity is retarded. This is also dependent on the available water, which we will discuss next. High temperatures can also result in sunburn on berries.

Rainfall is important to replenish soil water. The roots of the vine take up the water via the xylem vessels to the leaves to take part in transpiration and photosynthesis. Photosynthesis will produce carbohydrates for the development of structural and chemical components of the vine and grape berries. When temperatures are high, the cooling effect provided by transpiration keeps the leaves from drying as the water evaporates from the stomata. These are the principle functions that makes rain important for the wellbeing of the grapevine. If rain occurs in excess it can promote favourable conditions for fungal diseases such as downy mildew and botrytis. If the vine is very vigorous, that is, it has many shoots and leaves it will also provide still, humid conditions for fungi and rot. If there is insufficient rain, the vine can suffer water stress. With severe water stress vines will shut down, photosynthesis stops and leaves will senesce. Depending on the time of the season heat stress can affect flowering, berry growth, photosynthesis and sugar accumulation (Greer and Weston, 2009).

Light intensity has a positive correlation with sugar accumulation until a point of saturation, where further increase in light will have no effect (Spayd *et al*., 2002). Although light is related to temperature, for certain functions it plays an exclusive role. Light always has a positive effect on polyphenol production (Spayd *et al.*, 2002). Light can also have some effect on sunburn necrosis of berries (Genovese et al., 2010).

Wind can cause damage to young shoots, as well as destroying inflorescence before fruit-set when too strong. Mild winds can help reduce the risk of fungal disease and frost damage. Wind also has various influences on temperature.

The various climate factors; Temperature, rainfall, hail, wind, and sunlight have different effects on the grapevine and how it could affect wine grape quality.

1.1.2 Overview of vine physiology in a viticultural season

The ripening of the berry is the final physiological process in a series of important biochemical transformations. Although the climate during the ripening period plays an important role. The weather for the whole annual vine cycle has various effects on berry composition. It is important to consider the main phenological stages and how different weather factors can affect them (Riou et al. 1994).

The identification of growth stages is critical to the study of grapevine phenology. For this reason, there have been several descriptive systems developed, such as those by Baggiolini (1952, 1993), Eichhorn and Lorenz (1977), Lorenz et al. (1994), Coombe (1995). However the important general phenological stages are Budburst, Flowering/ Fruit-set, Veraison and harvest.

During winter, buds remain dormant. No growth occurs in the vine and canes are generally pruned back to limit the number of buds per vine. At the end of winter/beginning of spring buds progress from post-dormancy, they begin to swell and leaf tissue emerges. As the buds swell inflorescence primordia growth resumes with further branching, branch elongation and flower formation (May 2000, May 2004).

The shoots begin shooting slowly followed by the ‘grand period of growth’ towards the end of spring that results in a massive growth of vegetative tissues (Coombe et al. 2004). The inflorescences become visible some weeks after budburst. The flowers are clumped into compact groups, before they develop further and separate.

Cap-fall follows, this is when the caps on the flowers loosen as the base of the petals detach and roll backwards. The caps then are released and the stamens are freed, and the flower is more recognisable, this is termed flowering. Pollination of the flower occurs after this. Vitis Vinifera have hermaphrodite flowers, thus they can self-fertilise. The pollen from the male part (stamens) is released into the female part (pistil) to begin the pollen development. When the ovule is fertilised, the stamens will fall and it will develop into a berry. This stage is termed fruitset. Fruitset is in addition a process that quantitatively determines how many of the ovaries become berries (May, 2004).

Berry development occurs after fruitset as the weather generally becomes warmer and is divided into two growth stages, which are separated by a lag phase. The first stage, berry formation, involves an increase in the size of the hard green berries, through cell

division and enlargement. After this is the lag phase where little berry growth occurs and berries reach their highest levels of acidity. Veraison comes next, this is where the berries soften and begin sugar accumulation, veraison is also where red grapes undergo colour change from green to red. Some white varieties also undergo subtle colour changes.

During summer, after the berries have reached the veraison stage, shoot growth slows and eventually the shoot tips abscise, causing shoot lengthening to cease. It is this stage that the latent buds enter a pre-dormant state.

Berries continue to undergo sugar accumulation and flavour development as well as a reduction in organic acids up until the time of harvest that occurs between one to two months after veraison.

Shoots start to lignify in Autumn and some basal leaves are lost. Latent buds enter dormancy completely during this stage. The leaves will fall as winter approaches and the latent buds remain dormant until the following spring, when they will burst.

1.2 Phenological Events

1.2.1 Budburst

There is evidence that temperatures during the latter part of winter influence the budburst date. It is shown in both cases, whether calculated as daily maximum (Antcliff et al. 1955; Baldwin, 1966; McIntyre et al., 1982) or as daily means (Alleweldt and Hofäcker, 1975; Pouget 1967) that higher temperatures hasten the budburst date.
The minimum temperatures during the 10 days prior to budburst accounted to some extent for variation in budburst dates and to a greater extent the average maximum temperature between 1 August and 10 days prior to budburst (Southern Hemisphere) (Due et al., 1993).

The onset of budburst is earlier when soil temperatures at 0.2m depth are warmer (Alleweldt and Hofäcker, 1975). In another study in Germany, growth appeared to start only when soil temperature at a depth of 0.2m exceeded 8ºC, even when air temperatures exceeded 15ºC (Wolfart et al., 1988). This is not conclusive in all areas, for example Blaha (1969) questioned whether the small differences encountered in soil temperature could have important effects on budburst. It could be that soil temperature

was more significant in Germany as the winter temperatures are much more extreme and limiting than more temperate Mediterranean zones.

Grape varieties differ in their timing of budburst, with late-bursting varieties requiring higher temperatures to break dormancy. All budburst in warm, maritime climates is at temperatures above those absolutely needed, but varietal differences in timing appear largely preserved (Coombe et al. 2004).

1.2.2 Flowering

Flowering time is crucial for vine fruitfulness and yield. Flowering coincides in part with bud formation for the next season's cropping shoots, including the primordia of the bunches they will carry (Coombe et al. 2004). There is ample evidence that temperatures during individual bud formation influence their fruitfulness the following season, i.e. the proportion of the shoots developed from them carrying bunches and the number of bunches on each (Baldwin 1965, Buttrose 1970,1974, Sommer et al. 2000, MacGregor 2002). Some evidence (Dunn and Martin 2000) suggests a further influence on bunch size. Fruitfulness and large bunch size result from warmth during bud formation and an ample supply of cytokinins from warm, well aerated roots. High temperatures (25ºC, Pouget 1981 or 28ºC, Ezzeli 1993) have been shown to have a reduction in the number of flowers produced when compared to vines exposed to 12ºC. Grape varieties differ greatly in their fruitfulness and the temperatures during bud initiation needed to achieve it (Buttrose 1970).

Weather around fruit set in the current season also has an important influence on yield. Poor fruit set is generally attributed to weather conditions. Vines need warmth and sunshine during and around flowering for best fruit set. Wet, cold and cloudy weather results (to varying degrees according to grape variety) in either or both of two conditions: poor set or coulure, where many of the berries fall off when small, and 'hen and chicken' or millerandage, in which small, seedless berries or berries with incompletely formed seeds accompany normally-seeded larger berries on the same bunch (Coombe, 1988). Coulure appears to be caused by lack of assimilate within the bunch, often from competition by vigorous growth under ample water and nitrogen supply as well as low sunshine. Millerandage, by contrast, results directly from low air temperature just before

and during flowering. This interferes with ovule development and pollen function, and with normal development of the resulting seeds and hence berries (Ebadi *et al*. 1996).
Most cell divisions of a grape's fleshy tissue (pericarp) occur before flowering, with just one to two more generations in the three weeks or so after (Coombe, 1976) during which the berries are sensitive to environmental stresses. High temperatures (Hale and Buttrose 1974) and water stress (McCarthy, 2000) at this time both irrevocably reduce potential for final berry size. Ojeda *et al.* (2001) present evidence that this is due to reduced extensibility of the newly forming cell walls, rather than to curtailment of the final cell divisions.

1.2.3 Post setting to Veraison

After the fruit set, the berries of grapes enlarge very rapidly. Before Veraison, berries proceed through two periods. The first stage of berry growth is rapid, the pericarp and seed increase in size and weight while the embryos remain small (Winkler, 1962). In the pericarp there is a period of rapid cell division lasting 3 to 4 weeks after anthesis, which is followed by rapid cell enlargement (Harris et al., 1968). The berries remain green and firm and are characterized by rapid acid accumulation, a high rate of respiration and a respiratory quotient value of 1 or less. This period usually last 5 to 7 weeks in most grape varieties.

In the second period the overall growth rate of the berry has slowed down, in this stage the seeds develop but the berries remain small and hard. The berries reach their highest level of acidity and begin to accumulate sugar during this period. The berries lose their chlorophyll and begin to undergo a colour change. This period generally lasts 2 to 4 weeks (Winkler, 1968).

Environment and management can also influence the duration of this stage. Both low and especially very high temperatures tend to prolong the second stage (Hale and Buttrose 1974), as do a heavy crop load or factors leading to it. During this stage the berries and vine are very tolerant to heat and water stress.
Unfortunately records of veraison date are rather rare and subject to uncertainty because of differences between recorders in the method of its determination (Coombe, 2004).

Previous recordings suggest that veraison dates are not as focused as are dates of flowering. Season-to-season comparisons of juice ºBrix curves extrapolated back in time suggest that veraison date greatly influences harvest date (Coombe and Iland 1987).

1.2.4 Veraison to Harvest

As with most stages of grapevine phenology, temperature again is a major factor influencing grape ripening and harvest dates. Harvest dates are less clear-cut phenological indicators because the timing is decided by humans. Therefore, adaption for different end-uses, eg. wine-style, can alter the timing of harvest. Within regions where varieties are used for more uniform styles of wine from year to year, harvest dates can still be considered important in terms of phenological records.

Some results from research between the effects of temperature functions and harvest dates from Coombe (2004) are summarized as follows:

• mean January (southern hemisphere) temperature correlated with the harvest date of 4 varieties (Smart, 1979)

• mean November-December (southern hemisphere) temperatures predicted veraison date and hence harvest date of Grenache in 4 years (Coombe and Iland, 1987);

• temperature predicted harvest date for late varieties but not for early varieties;

• cumulative daily air temperature difference was the most reliable predictor of the interval flowering to harvest (McIntyre, 1982);

• the greater the number of days above 25ºC and 30ºC for the intervals flowering to veraison and veraison to harvest, respectively , the earlier the harvest (Jones and Davis 2000);

• variety, year and region accounted for 57% of the variation in harvest date; the inclusion of the minimum temperatures for the epochs, (1) 1 January to 11 of January prior to harvest, (2) 10 days prior to harvest and (3) flowering to harvest, and the maximum temperature 10 days prior to harvest, accounted for a further 32% of the variation in harvest date (Due et al. 1993).

Grape composition and the concentration and balance between sugars and acids are fundamental indicators for harvesting times. To better understand the climates influence

on harvest times, sugar and acid development needs to be considered with berry ripening.

1.3 Berry Development

Berry Development is generally described by a double-sigmoidal growth pattern (see Fig. 1) (Kanellis and Roubelakis-Angelakis, 1996) characterised by three successive phases: Phase I is the green or herbaceous stage immediately after flowering. The berries are hard and green, and undergo a short period of cell division and cell enlargement resulting in rapid expansion of the berry (Kanellis and Roubelakis-Angelakis, 1996; Terrier *et al*., 2001). Characteristic of Stage I is the increase in vacuolar size of the grape berry cells due to the rapid storage of L-malic and L-tartaric acid (Fillion *et al*.,1999; Pratelli *et al*.,2002). Stage II comprises a short lag phase during which berry growth ceases and berry acidity reaches a maximum due to the continued accumulation of L-malic and L-tartaric acid. Following the lag phase, there is a second period of 'berry growth'(Stage III). The entry into Stage III begins with the sudden onset of ripening or "*véraison*", which generally starts between 6 to 8 weeks after flowering and lasts for 35 to 55 days depending on the grape cultivar (Coombe, 1992; Pratelli *et al*., 2002; Ribéreau-Gayon *et al*., 2000).
During the ripening period berry size increases significantly and the accumulation of sugar, aroma and flavour compounds occurs as well as anthocyanins in coloured varieties, at the same time acid levels decline.

1.3.1 Sugar accumulation

From the initiation of ripening until harvest (stage III), the berries become the primary sink for sucrose transported from the leaves as a result of photosynthesis. When sucrose reaches the berry it is separated into its constituent sugars glucose and fructose.

Along with berry enlargement is the termination of water flow into the berry via the xylem vessels (Coombe 2004). The supply of water and nutrients is then limited to the sugary sap of the phloem, obviously having a significant effect on berry composition. As the berries grow from this stage, sugar concentration shows a steady rise. Calculations of

sap flow rates based on rates of sugar input per berry, and the per cent sucrose estimated to be in such sap, show that the idea is sustainable provided allowance is made for the daily loss of water transpired through the skin of berries (Coombe and McCarthy, 1999). Higher temperatures around the ripening period can result in faster sugar accumulation leading to higher sugar levels at harvest and consequently higher alcohol levels in wine.

1.3.2 Acidity

The most prominent organic acids in grapes and wine are L-malic acid and tartaric acid (Boulton et al., 1996). Although L-malic and L-tartaric acids have similar chemical structures, they are synthesised from glucose via different metabolic pathways in grape berries. L-Malic acid is formed via glycolysis and the TCA cycle, while ascorbic acid is the principle intermediary product of L-tartaric acid biosynthesis. Slight differences in grape acidity among different grape varieties are usually found, affecting especially the ratio between L-tartaric acid and L-malic acid in different grape cultivars (Kliewer *et al*., 1967). L-Tartaric acid is usually present in grapes at average concentrations of 5 to 10 g/L (Ruffner, 1982), while mature grapes contain between 2 and 6.5 g/L L-malic acid (Boulton *et al*., 1996; Ribéreau-Gayon et al., 2000). Excessive amounts of malic acid (15 to 16 g/L) may be present in grapes harvested during exceptionally cold summers in the cool-climate viticultural regions of the world (Gallander, 1977). Although tartaric acid is often found at higher concentrations than L-malic acid and is the stronger acid of the two, its concentration is relatively constant. It is the fluctuating concentration of L-malic acid that usually poses problems to winemakers (Margalit, 1997).

In a particular year, a must's total acidity and acid composition depend mainly on geography, soil conditions, and climate, including soil humidity and permeability, as well as rainfall patterns, and, above all, temperature. Temperature determines the respiration rate, i.e. the combustion of tartaric and, especially, malic acid in grape flesh cells. The predominance of malic acid in must from cool-climate vineyards is directly related to temperature, while malic acid is eliminated from grapes in hotter regions by combustion. The rate of respiration of L-malic acid is significantly slower in cold climates, resulting in "immature grapes" at harvesting, containing a high titratable acidity (TA) content and low pH.

Independently of climate, grape growers and winemakers have some control over total

acidity and even the acid composition of the grape juice during ripening. Leaf-thinning and trimming the vine shoots restrict biosynthesis and, above all, combustion, by reducing the greenhouse effect of the leaf canopy. Another way of controlling total acidity levels is by choosing the harvesting date (Ribéreau-Gayon et al., 2000).

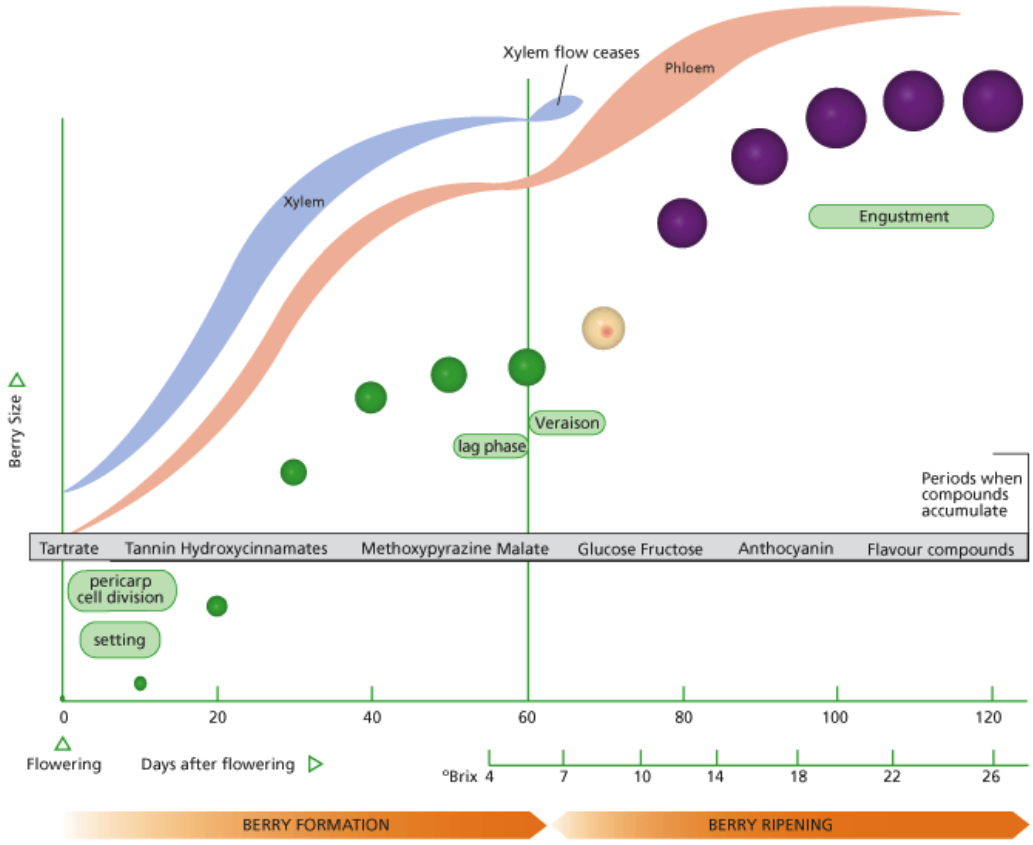


Figure 1. Diagram showing relative size and colour of berries at 10-day intervals after flowering, passing through major developmental events (rounded boxes). Also shown are the periods when compounds accumulate, the levels of juice ºbrix, and an indication of the rate of inflow of xylem and phloem vascular saps into the berry. Illustration by Jordan Koutroumanidis, Winetitles. (Kennedy 2002).

1.4 Wine and Must Parameters

1.4.1 Titratable Acidity

The titratable acidity is basically the total acidity in the must or wine, determined by neutralisation with a basic solution of sodium hydroxide of known normality.

The titratable acidity takes into account all types of acid i.e inorganic acids such as phosphoric acid, organic acids such as tartaric, malic, citric, succinic etc. as well as amino acids whose contribution to titratable acidity is not very well known.

Titratable acidity is usually measured in grams of tartaric acid per litre, although in France it is measured in grams of sulphuric acid per litre. As mentioned in the previous section (1.3.2), titratable acidity is likely to maintain higher values in grapes grown with a cooler ripening period. Because titratable acidiy is a measure of the total acidity, it generally has an inverse relationship with temperature, as higher temperatures increase the respiration rate of organic acids during berry ripening.

1.4.2 pH

pH is the measure of the concentration of hydrogen ions (H+) in a solution. Numerically it is the negative logarithm of that concentration expressed in moles per litre (M). Defined mathematically as negative log subscript ten of the concentration of hydronium ions in a solution (Nelson et al. 2005).

$PH = -\log_{10}[H_3O^+]$ **(equation 1)**

The importance of pH in must and wine is made more confusing because it is an abstract, theoretical measurement with no units. However it plays an important role in the microbiological properties of must and wine (Ribéreau-Gayon et al., 2000).

The total acid of a wine consists of two portions, the ‘free acid’ which is measured as pH and a ‘bound’ form. Only a small proportion of the total acid is present as ‘free acid’ (about 0.2-2%), but it is the latter form which is important to wine stability. Should the small amount of free acid be consumed, say be titrated with alkali, then some of the bound acid releases some more ‘free acid’ to restore roughly the same ratios of ‘free acid’ to ‘bound acid’ as existed previously. This action is known as buffering and acts to keep wine pH fairly stable (Birks et al., 1978). It is this buffer capacity of wine that makes it hard to relate directly with titratable acidity, and thus it’s relationship with temperature may also be less predictable. The pH of wine is usually between 3.0 and 4.0. White wines usually have lower pH values than red wines.

1.4.3 Alcohol

Besides water, ethanol (ethyl alcohol) is the most plentiful compound in wine. A wine's strength is expressed in terms of alcohol content, or the percentage of alcohol by volume. As ethanol has a density of 0.79, a wine with an alcohol content of 10% vol contains 79 g/l of ethanol by weight. The alcoholic strength of wine is generally 100 g/l (12.6% vol), although it may exceptionally be as high as 136 g/l (e.g. an alcohol content of 16% vol).

Due to the low density of ethanol, dry wines, containing negligible amounts of sugar, have densities below that of water (1.00), ranging from 0.91 to 0.94. This value decreases as the alcohol content increases. Ethanol in wine is mainly produced by the alcoholic fermentation of sugar in must. As approximately 18 g/l of sugar is required to produce 1% vol of ethanol during alcoholic fermentation, grape must has to contain 180, 226 and 288 g/l of sugar to produce wines with 10, 12.6 and 16% ethanol by volume. The latter is considered to be the maximum ethanol content yeast can survive, although, under certain laboratory conditions, some strains have been found capable of resisting up to 18% vol. Some types of wine may, of course, have even higher alcoholic strengths, but this results from the addition of ethanol. Temperature can effect alcohol levels because higher temperatures around the ripening period can result in faster sugar accumulation leading to higher sugar levels at harvest and consequently higher alcohol levels in wine.

1.5 Climate Indices

Much work has gone into developing various climatic indices in order to better classify viticultural regions in terms of climate. The objective of this is to better understand the potential of current viticultural areas for varietal suitability, as well as having a tool to predict potential viticultural areas that are not yet planted. These indices can also be used with climate change models to predict future changes in viticultural areas (Hall and Jones, 2008).

There are many different climate indices, some of the more common ones include 'Growing Season Temperature' (GST), 'Growing Degree Days' (GDD), 'Huglin Index' (HI), 'Biologically Effective Degree Days' (BEDD), 'Cool Night Index' (CI) and 'Dryness Index' (DI). These Indices are then used to classify regions in terms of climate. The

classification of regions makes it easier to compare conditions between areas in different parts of the world.

Climate indices are developed using different indicators. Several indicators have been used to try and predict grape quality such as heat accumulation, length of the growing season, sunshine hours, rainfall, diurnal temperature range, evapotranspiration and humidity. There are numerous different climatic indices that focus on each, or several of such indicators, in an effort to better explain grapevine and grape development.

Temperature is widely accepted as being the primary climatic factor affecting the quality of viticultural production (Winkler et al.. 1974, Gladstones 2004). Because temperature is considered such an important indicator, most of the climate indices are temperature based. However, due to the relative simplicity of universal indices they are unable to take into account more complicated phenomena such as 'terroir' factors and cultural practices (Hall and Jones 2010).

Some site characteristics such as slope and aspect can change solar-radiation-loading at a particular site, also there are different cultural practices that evolve in regions to deal with hot or cool climate conditions.

1.5.1 Growing season temperature (GST)

Growing season temperature (GST) is the most simple of the temperature based climatic indices. GST is established by calculating the sum of the mean air temperature of all days between 1st April and 31st October (northern hemisphere) (Winkler et al. 1974). Mean daily air temperature is calculated as an average of the minimum and maximum daily temperatures. This may vary slightly from the average of a constantly recorded temperature (Hall and Jones 2010) which may skew results. The simplicity of GST, means that it is easy to understand and requires little data to be calculated. However because of its simplicity it doesn't have any adjustments to emphasise parameters that may have more important effects on plant physiology, as do some of the more complex indices.

$$\text{Average GST} = \sum_{1/04}^{31/10} [T\max + T\min] \quad \textbf{(equation 2)}$$

Table 1. Growing Season Temperature class limits taken from Hall and Jones (2010).

Too cool	*<13ºC*
Cool	*13-15ºC*
Intermediate	*15-17ºC*
Warm	*17-19ºC*
Hot	*19-21ºC*
Very hot	*21-24ºC*
Too hot	*>24ºC*

1.5.2 Growing degree days (GDD)

Growing degree days (GDD) is a measure of heat accumulation. It is calculated by subtracting a base temperature (10ºC for wine grapes) from the average temperature recorded each day from 1st of April to 31st October (Northern Hemisphere). This heat unit was first extensively used by Winkler et al. (1974). While the degree days index has been commonly used, it has significant limitations. Reliance on GDD may have been responsible for the late arrival of grapes in such cool areas as Tasmania, Australia (Coombe and Dry 1988).

The base temperature of 10ºC has been chosen because little to no growth occurs below this temperature, however other authors believe other temperatures are possible, also depending on the growth period (Boehm 1970). Some upper and lower boundaries for this index were set by Hall et al. (2010) as follows: if GDD totals are below 850ºC-days they fall in the too cold class for viticulture, a too hot class was suggested for GDD totals above 2700ºC-days.

$$\mathrm{GDD}=\sum_{1/04}^{31/10}\max\left[\left(T_{\max}+T_{\min}\right)/2-10,0\right] \qquad \textbf{(equation 3)}$$

Table 2. GDD limits defined by Winkler (1974).

Too cool	*<850*
(Region I)	*850-1389*
(Region II)	*1389-1667*
(Region III)	*1667-1944*
(Region IV)	*1944-2222*
(Region V)	*2222-2700*
Too hot	*>2700*

1.5.3 Huglin Index (HI)

Huglin Index (HI) is an altered version of GDD. The HI takes the temperature accumulated during the daylight rather than a whole 24 hour period. This is because, it is the daylight period where photosynthesis occurs. There is also a length of day coefficient, 'k', that varies between 1.02 and 1.06 at 40º to 50º latitude. Unlike most of the other climatic indices the HI is measured over a 6 month period instead of 7 months. In areas where there is a large annual temperature variation, the 7th month will be the coolest and can distort the results to give a lower than expected index value for the region when compared with a region that is considered cooler but has less variation between summer and winter temperatures. For example, a highly continental area may have a very hot growing season and early harvest, followed by a significantly cooler 7th month at the end of the season. If this 7th month is included in the summation of a climate index, the area may show a similar climate index value to a region that had a much cooler growing season but maintained higher temperatures over the 7th month than the other more variable region. Sometimes (in warmer regions) this 6 month period may more closely match the growing season of the grapevine, hence being more relevant for obtaining the information required, such as viticultural potential of the region and varietal suitability.

$$\text{HI} = \sum_{1/04}^{30/09} \left[(T_{mean} - 10) + (T_{max} - 10)/2 \right] K \qquad \textbf{(equation 4)}$$

Where *K* is an adjustment for latitude/day length.

Table 3. Huglin Index climate class limits (Hall and Jones, 2010).

Too cool	*<1200*
Very cool	*1200-1500*
Cool	*1500-1800*
Temperate	*1800-2100*
Warm temperate	*2100-2400*
Warm	*2400-2700*
Very warm	*2700-3000*
Too hot	*>3000*

1.5.4 Biologically effective degree-days (BEDD)

Biologically effective degree-days (BEDD) uses similar adjustments as the Huglin Index, however with a different emphasis. For example it uses an adjustment to estimate diurnal temperature range as well as adjusting for day length. The factor for diurnal temperature is adjusted upward if the range is greater than 13ºC and adjusted downward if the range is less than 10ºC. The day length coefficient ranges from 1.000 to 1.045 at 40º to 50º latitude. The interval of effective heat summation is considered between 10ºC (base temperature) and 19ºC (upper threshold) i.e all further increases in mean temperature above 19ºC are disregarded). Unlike the Huglin Index, BEDD maintains the 7 month growing season model, instead of 6 months. As discussed, if there is a large temperature range in an area, this can distort values.

$$\text{BEDD} = \sum_{1/04}^{31/10} \min\left[\left(\max\left[\left(\left[T_{\max} + T_{\min}\right]/2\right) - 10, 0\right]\right), 9\right] . DTR_{adj} . K \qquad \textbf{(equation 5)}$$

Where

$$DTR_{adj} = \begin{cases} 0.25\,[DTR-13], & [DTR] > 13 \\ 0, & 10 < [DTR] < 13 \\ 0.25\,[DTR-10], & [DTR] < 10 \end{cases} \qquad \textbf{(equation 6)}$$

DTR_{adj} is the diurnal temperature range adjustment and *K* is an adjustment for latitude/day length.

Table 4. Too cool and too hot BEDD class limits for viticulture (Jones and Hall, 2010).

Too cool	*<1000*
Too hot	*>2000*

1.5.5 Cool night index (CI)

Cool night index (CI) is a night coolness variable which takes into account the mean minimum night temperature during the month when ripening usually occurs.
In the northern hemisphere it is the mean minimum temperature of the month of September (March in the southern hemisphere). This variable has been used to give a better indication of potential quality of a region in terms of secondary metabolites such as aroma and polyphenols as well as colour development (Kliewer and Torres, 1972; Kliewer, 1973; Tomana et al., 1979).

In the Northern Hemisphere:

CI = minimum air temperature in the month of September (mean of minima), ºC. **(eq. 7)**

In the Southern Hemisphere:

CI = minimum air temperature in the month of March (mean of minima), ºC. **(eq. 8)**

1.5.6 Dryness Index (DI)

Dryness index (DI) is based on the water balance model of Riou (Riou *et al.*, 1994, eq. 9). This model can predict the potential water available to the vines in an area by taking into account soil reserves, the transpiration of the vine, soil evaporation, runoff and the rainfall of an area. The Dryness index is calculated using the same 6 month period as the Huglin index (April -September).

$$W = \sum_{01.04}^{30.09} Wo + P - Tv - Es \qquad \textbf{(equation 9)}$$

Where, W is the estimate of soil water reserve at the end of a given period, Wo the initial available soil water reserve, which can be accessed by the roots, P the precipitation, Tv the potential transpiration in the vineyard, Es the direct evaporation from the soil.

In Eq. 9 Tv and Es are calculated month by month using:

Tv = ETP*k* **(equation 10)**

ETP is the potential evapotranspiration (montly total), using the Penman method (Penman, 1948), *k* the coefficient of radiation absorption by vine plant (which is in relation to transpiration and depends on vine architecture)

Es = [ETP/N] (1-*k*)JPm **(equation 11)**

N is the number of days in the month, JPm is the estimated number of days of rainfall per month.
K values adopted were as follows:

• In the Northern Hemisphere, k = 0.1, for April, 0.3 for May and 0.5 for the months from June to September. **(equation 12)**

• In the Southern Hemisphere, *k* = 0.1 for October, 0.3 for November and 0.5 for the months from December to March. **(equation 13)**

W can be negative, for expressing the potential water deficit, but should not be greater than Wo. The index is calculated month by month, based on monthly values of P, ETP, Tv and Es. DI is called the value of *W* obtained at the final moment following the rules above and adopting Wo = 200mm.

Climate indices that focus on other indicators other than temperature, can complement temperature indices and provide a more thorough explanation of grapevine development. For example Carbonneau and Tonietto (2004) combined three climatic indices (DI, HI and CI) to create a multi-criteria climatic classification.

1.6 Climate Change

Climate change is arguably one of the most studied and debated scientific issues of our day. Observed trends and potential future changes in temperatures have and will continue to exert a strong influence on virtually every form of agriculture where production viability may be altered due to changes in winter hardiness potential, frost occurrence, growing season lengths, and heat accumulation for ripening potential.

Viticulture is included as one of these forms of agriculture that is effected by such changes. Extreme weather events such as hard winter freezes, spring or fall frosts, and hail can result in major losses in a given vintage, while long-term changes in climate can result in changes in production viability and wine styles.

Throughout the history of wine production, it has been a very regional product, with the adaption of different varieties to different regions where the conditions match closely with the requirements of the variety. This means that many varieties are planted in regions where conditions are within quite a narrow range for them. These scenarios make viticulture very sensitive to even small changes in climate.

Over the last 50 years research has shown that many of the world's wine regions have experienced a decline in frost frequency and timing and warmer growing seasons with greater heat accumulation (Jones 2006).

An examination of climate and phenology trends over the last 30–50 years for nine locations across a range a climate types in Europe (cool to warm) and for 16 varieties shows that warming has occurred across most seasons, but is strongest in the spring and summer (Jones 2006). Growing seasons have warming across Europe by 1.7°C on average with most of the warming coming at night. Heat accumulation has increased as well with degree days rising by 250–300 units (in °C units) while precipitation frequency and amounts have not changed significantly. The grapevine's phenological timing has showed strong relationships with the observed warming with trends ranging six to 25 days earlier over numerous varieties and locations. Changes are greatest for veraison and harvest dates which typically show a stronger, integrated effect of a warmer growing season. Interval lengths between the main phenological events have also declined with bud break to bloom, veraison, or harvest dates shortening by 14, 15, and 17 days, respectively. Averaged over all locations and varieties, grapevine phenology shows a three to six-day response per 1°C of warming over the last 30–50 years (Jones 2006).

1.7 The Dão Wine Region

The region of Dão is located in central Portugal. A research centre for viticulture is located in the region within the smaller demarcation of Nelas. The vineyards of Dão can usually produce about 500 000 hectolitres of wine depending on the seasonal conditions. The designated area of IG 'Terras do Dão' is divided into two DO areas; DO Dão and DO 'Lafões'. The council of Nelas is located in the sub-region of Terras de Senhorim within the DO Dão demarcation. There are a range of varieties (Table 1.) that are grown within the area, and must be adhered to when producing DO Dão wines (IVV, 2011).

Table 5. Main vine varieties grown in the Nelas area, used to create wines with DO 'Dão' status. (IVV, 2011).

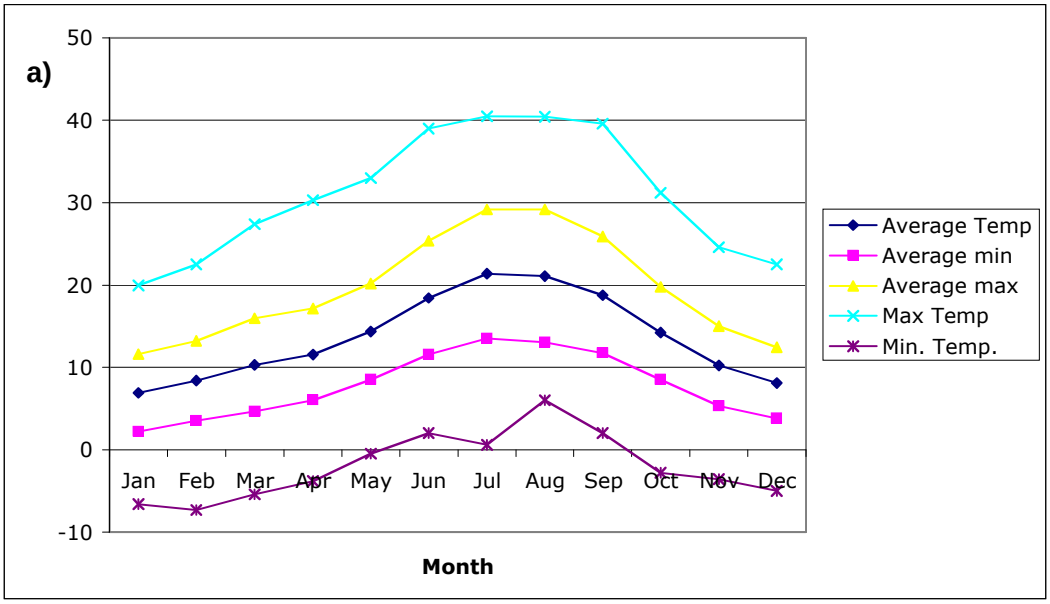
Red:	White:
Alfrocheiro, Alvarelhão, Aragonez (Tinta Roriz), Bastardo, Jaen, Rufete, Tinto Cão, Touriga Nacional and Trincadeira (Tinta Amarela).	Barcelo, Bical, Cercial, Encruzado, Malvasia Fina, Rabo de Ovelha, Terrantez, Uva Cão and Verdelho.

The wine region is located primarily on a plateau that is sheltered on three sides by the granite mountain ranges of Serra da Estrela, Serra do Caramulo and Serra da Nave. This helps the area maintain its temperate climate away from the effects of the nearby Atlantic Ocean. The region experiences abundant rainfall in the winter months and long, warm dry summers leading up to harvest. The region's vineyards are planted on sandy well-drained soil on top of granite rock (Robinson, 2006).

From the weather data collected at Viseu between 1971 to 2000 by the 'Instituto de meteorologia, IP Portugal' (Fig. 2), it is possible to obtain a reasonable understanding of the climate in the region. The temperatures are generally warm in summer with average maximum temperatures around July and August of 29.2ºC and average temperatures of 21.4ºC. Temperatures have also on occasions reached extremes of up to 40ºC in the months of June to September. The average minimum temperatures in the two hottest months are 13.5ºC and 13ºC in July and August respectively.

The winters are cool with a monthly average temperature falling to the lowest in January (6.9ºC). There have been occasions of temperatures falling below 0ºC at night, the lowest recorded temperature between 1971-2000 was –7.3ºC which occurred during February.

The region has a winter-dominant rainfall with the average monthly rainfall peaking in December (195.4mm). The summers are relatively dry with low average precipitation (July-19.2mm, August-17.9mm).



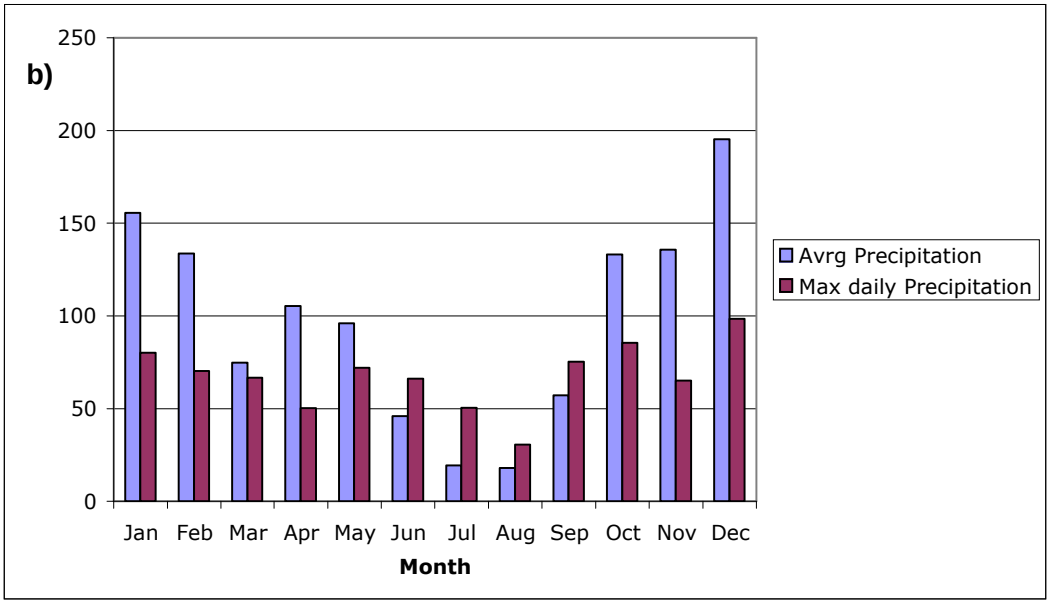


Figure 2. Monthly averages of observed values from 1971 to 2000 a) Average monthly temperatures b) Average monthly precipitation levels. Source: *www.meteo.pt*.

Records from the meteorological database of Viseu and Nelas between 1961-1990 show that December and January are the months where frost occurrence is higher. In some years there is risk of spring frost in April and May, the highest risk time for frost-damage of vines in the region.

1.8 Touriga Nacional

Touriga Nacional has been recognised for its capability of producing high quality Port and table wines suitable for ageing. Due to the recognition of Touriga Nacional's potential for quality, there has been an increase in the planting of the variety throughout the country, as well as internationally; in countries such as Spain, South Africa, Australia, USA and Brazil (Magalhhães, 2008).

Over the last twenty-five years in the Dão region alone, the planted area of Touriga Nacional has increased by 300%. From 422 hectares in 1983 to 1300 hectares in 2008 (CVR Dão 2008).

Reported oenological characteristics express that Touriga Nacional has the potential to produce quality wines. The variety can produce wines with a very high potential alcohol and high acidity but very balanced (Brites and Pedroso, 2000). Touriga is a very versatile variety (Magalhães, 2008).

The variety is capable of producing wines of intense colour, with violet tones when young. The aroma is equally intense, expressing very ripe black fruits, with something wild such as; blackberries, rosemary, lavender and pine needles. The palate is full, balanced and persistent with robust tannins and lots of fruit when young. Wines produced can have a good ageing potential. Magalhães (2008) and Bohm (2007) add that because of its aromatic complexity, structure and quality of phenolic compounds it is an excellent variety for the production of single varietal wines as well as blends. It is also highly recommended for quality port wines.

Despite the reported big expansion of Touriga Nacional to other terroirs, to our knowledge, very little is known about its responses to climate variability.

2. Materials and Methods

2.1 Touriga Nacional (Technical Description)

Touriga Nacional is a vigorous variety with a tendency for downward shoot growth. The variety is susceptible to water-stress and is prone to the loss of leaves around the fruit-zone in such conditions. Budburst is moderate, but with high fertility, including from basal buds. Production levels are medium to high with selected plant material and adequate conditions, however without such conditions, Touriga is typically a low-yielding variety.

Touriga Nacional has small to medium sized bunches containing small, lightly compacted berries.

For further ampelographical and phenological information on Touriga Nacional See *appendix 1*.

2.2 The Vineyard Site

All data was collected in three vineyard plots from the 'Centro de Estudos Vitivinícolas do Dão', Quinta da Cale, Nelas (Latitude 40 ° 31 'N, longitude 7 ° 51'W, Elevation 440 m).

The viticultural data from 1961 to 1987 was collected from a vineyard plot labeled 'Folha 3'. This vineyard was planted in 1946 with 26 varieties and 15 different rootstocks for a study of the affinity between different rootstocks and varieties. The experimental design is a factorial design with two factors: variety x rootstock but in this work we have used only data from one variety. The elemental plot comprises 28 vines distributed over four adjacent rows of seven vines each. The rootstocks used were: 420A, 161-49, 3309 Couderc, 110 Richter, 99 Richter, 420 A and 161-49.

The density of planting is 5050 plants per hectare (row-spacing 1.80m x 1.10m).

The vines were trained to a vertical shoot-positioned trellis and pruned to a double 'Guyot' system. The height of the cordon is 0.6m from the ground.

The soil is porphyritic granite of coarse texture. It is an acidic soil with low organic matter content. The soil has a high hydraulic conductivity and a low water retention capacity.

Records were taken from all 28 vines. Qualitative parameters were measured from juice samples made from each strain of rootstock.

Data collected from 1995 to 2008 was collected from a vineyard plot labeled ‘Folha 1C’ from the same research centre. The vineyard has a slightly different row-spacing (1.80m x 1.00m) and was spur pruned in a bilateral Royat cordon. The rootstocks used here were 1103P, SO4 and 99R.
Data collected from 2009 to 2010 was collected from ‘Folha 5’. The rootstocks used in this plot were 110R.

2.3 Climate Data

Climate data was collected from a weather station located in the research centre. Daily minimum and maximum temperatures and rainfall were collected for the period, with some periods of missing data as discussed below.

2.4 Missing Data

There were 39 years of complete data available over the recorded time period from 1963 to 2010 to calculate the various climate indices. In some years, where there was only partial missing data, it was still possible to calculate some of the indices; for example CI only requires the average minimum temperature values for September. However, these exceptions were excluded and only years where all indices were available were included in the final results for better comparability between indices. In total there were eight years where there was insufficient data to calculate all of the climate indices.

There was data between 1961 and 2010 that was available for phenology, yield and berry composition. However, all yield and berry composition information was missing from 1988 to 1994. In addition to these missing values Alcohol and pH values were also missing from 1998. TA was missing from 1999 to 2003, and yield values were missing from 1998 and 2001.

All veraison phenological dates were missing until 1982. Veraison dates from 1961 to 1982 were estimated based on the correlation between available Veraison date observations (1983-2010) and observations of flowering dates.

Budburst, Flowering and Veraison dates are missing in 1989, while only Budburst and Flowering dates are missing from 1990. Budburst dates are missing in 1993 and there

were no phenological results for 1998. The date of Veraison is missing from the 2005 records.

Phenological data from 1995 was removed because it was considered to be an outlier, as it showed a harvest date 20 days earlier than the next earliest year.

All data until 1987 was collected from the 'folha 3' vineyard plot. Because this plot was set-up originally as a study between rootstocks and varieties, there are results collected from five, sometimes seven different rootstocks (420A, 161-49, 3309 Couderc, 110 Richter, 99 Richter, 5BB and Rupestris du Lot). Although all of the vines are of the Touriga Nacional variety, the rootstock variation can lead to varying responses of the vine under different conditions. The yield and berry composition data varies between rootstocks, however phenological dates were all uniformly assigned for the whole vineyard, except for budburst dates which were recorded separately in different rootstocks from the years 1983 to 1988, the median values of these were used for these six years for better comparability.

When measuring the evolution of the plant characteristics it would be ideal to take data from vines of the same rootstock, as well as when measuring correlations with climate. Accepting this inconvenience, an attempt to display any variations in data due to differences in rootstocks in the samples will be made. Allowing the inclusion of all of the rootstocks provides a considerably larger sample size for a more thorough evaluation.

The data available after 1987 was taken from 1995 to 2008 from an adjacent vineyard plot labeled 'Folha 1C'. The rootstocks used here were 1103P, SO4 and 99R.

Finally, results from 2009 and 2010 were collected from 'Folha 5' from only one rootstock (110 Richter).

The database has the following missing values:

• Alcohol: 1988-1994 and 1998;

• TA: 1988 to 1994 and 1999 to 2003;

• pH: 1988 to 1994, as well as 1998;

• Yield: 1988 to 1994, as well as 1998.

2.5 Analysis of Data

A database was created in Microsoft Excel (Microsoft Excel for Mac, version x, Microsoft corporation 2001) with daily minimum and maximum temperatures and rainfall from which the different climate indices were calculated. All climate indices were calculated according to the formulas in section 1.5. All relationships were analysed by Statistix 9 (version 9, Analytical software, Tellhassee, Fl).

A database was taken from the research centre with annual agronomic data including; budburst, flowering, veraison and harvest dates, yield data, potential alcohol, titratable acidity, pH and the rootstock from which the results were taken.

Budburst dates were taken from when 50% of the buds reached Baggiolini's B stage (Baggiolini, 1952). Flowering dates were taken from when 50% of flowers were open. Veraison dates were taken from when 50% of the berries changed colour. Maturity is a phenological stage that cannot be defined with great precision. Harvest dates were taken from when grapes reached what was considered sufficient sugar and flavour ripeness for red wines of the region.

Several Linear regression analyses were made for each of the berry composition parameters (dependent variables) and each of the climate indices (independent variables) as well as correlation coefficients. Statistical significance of the data was tested using the statistics program, Statistix 9 (version 9, Analytical software, Tellhassee, Fl). Three different levels of significance were labeled (* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$).

3. Results And Discussion

3.1 Climate Variables

3.1.1 Evolution of Climate Indices

This chapter explores the evolution of the climate indices, investigating any changes or trends in the index values over the 47-year time period. We also have a good foundation with which to investigate and quantify differences between the four temperature-based indices under varying conditions.

3.1.2 Growing Season Temperature-derived Indices

Most of the observed values of the Growing Season Temperature index (GST) are between 16ºC and 19ºC (Fig. 3.). There is only one value below this range (1972, 15.9ºC) and one above (2010, 19.6ºC). According to the GST categories established by Jones et al. (2005) the values classify the data as being in the range of the 'intermediate'(15-17ºC) and 'warm' (17-19ºC) categories. The average GST is 17.5ºC and the median value is 17.3ºC. Therefore the average of the seasons have been in the very lower limit of the 'warm' category.

When GST is correlated against the years from 1963 to 2010 an increase can be observed in the GST of 0.28ºC every ten years. This increase agrees with research in other European wine regions and could be attributed to climate change. Many wine regions have experienced increases in average growing season temperatures to this scale. It was found that between 1950-1999 growing season average temperatures had increased in some of the world's high quality wine producing regions by 1.26ºC. This was established by collecting average temperature data during the growing season (April-October in the Northern Hemisphere and October-April in the Southern Hemisphere) from weather stations in 27 wine regions. Temperature trends were significant in the majority of the U.S. and European wine regions while the majority of the southern hemisphere temperature records showed insignificant trends (Jones et al. 2003).

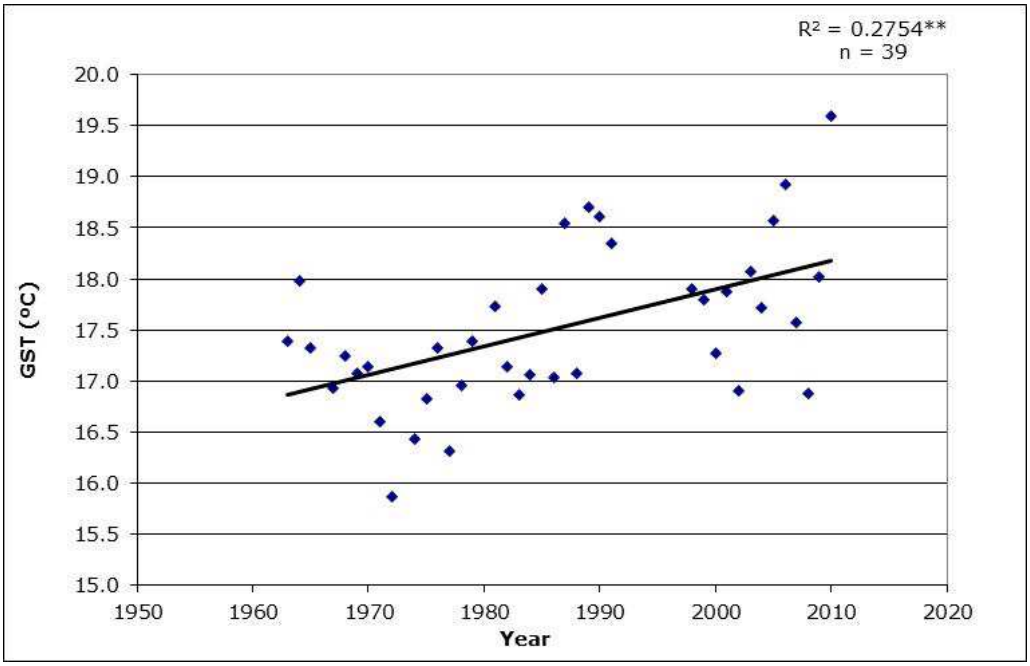


Figure 3. Evolution of Growing Season Temperature (GST) over 39 years between 1963-2010.

The relationships between the Growing Degree Day index (GDD) and the recorded years between 1963-2010 shows a very similar behaviour to GST as expected. GDD and GST are shown to be functionally very similar. The coefficient of determination that describes the relationship between these two indices is close to unity (fig. 4a and table 3). Therefore there is effectively no difference between these indices except in terms of their magnitudes. GDD maybe useful for determining stages of annual phenological development at time steps within a season, but in terms of describing a region's growing season temperature, the GST is a simpler index with fewer methodological issues that produces a similar comparative result when considering the conditions over the whole season (Jones 2006, Hall *et al.* 2010).

Linear relationships occur between all four temperature-indices GST, GDD, the Huglin index (HI) and the Biologically Effective Degree Days index (BEDD), however, as expected there is less correlation between HI and GST, and much less between BEDD and GST (fig. 4 and table 3). The HI shows a functional difference to GST unlike GDD. This is because HI, as explained in the introduction of this paper, uses an estimation of the day-time temperature rather than a mean diurnal temperature, it also includes a latitudinal day-length correction (*K*) and is based on a slightly different period (6-months instead of 7-months).

The BEDD index is the most different to the other three indices. Again, the most suitable description for this index was a simple linear regression, however values of this index have a weaker relationship with GST and this is reflected in the lower correlation coefficient (Fig. 4 and table 6). This variation in the data points shows that a given GST value can sometime relate to a relatively lower BEDD value due to the capping of BEDD degree day accumulation to maximum of 9ºC per day. If there is a high average GST for the year, there may have been many days with average temperatures exceeding the 19ºC cap, thus not being counted in the cumulative degree days for BEDD. This cap is responsible for the variation rather than the base temperature 'cut-off' at 10ºC. GDD has the same lower limit (10ºC), but as discussed earlier, this index has almost a perfect fit with the linear regression for GST (Fig. 4a and table 6).

Table 6. Sample Size, coefficient of determination (R^2) and formula for each model describing relationships between the different indices. Growing Degree Days, Huglin Index and Biologically Effective Degree Days (dependent variables) with Growing Season Temperature (independent variable).

Function	**n**	**R^2**	**Formula**
GDD = f(GST)	39	0.99**	GDD = 206.07GST - 1996.9
HI = f(GST)	39	0.91**	HI = 229.88GST –1978.9
BEDD = f(GST)	39	0.72**	BEDD = 90.617GST – 187.09

The Cool night index (CI) is simply the average of the minimum temperatures for the month of September. This variable has been used to give a better indication of potential quality of a region in terms of secondary metabolites such as aroma and polyphenols as well as colour development (Kliewer and Torres, 1972; Kliewer, 1973; Tomana *et al.* 1979). The CI values range from 9.26ºC to 14.77ºC, with the average over the recorded period being 12.68ºC (Fig. 5). There is a trend of increasing CI values over the period 1963-2010. An increase of 0.31ºC for every ten years is observed. This increase agrees with research in other European wine regions and could be attributed to climate change, it was found that an increase in the average growing season temperatures of several regions was influenced a lot by increases in night time temperatures (Jones 2006).

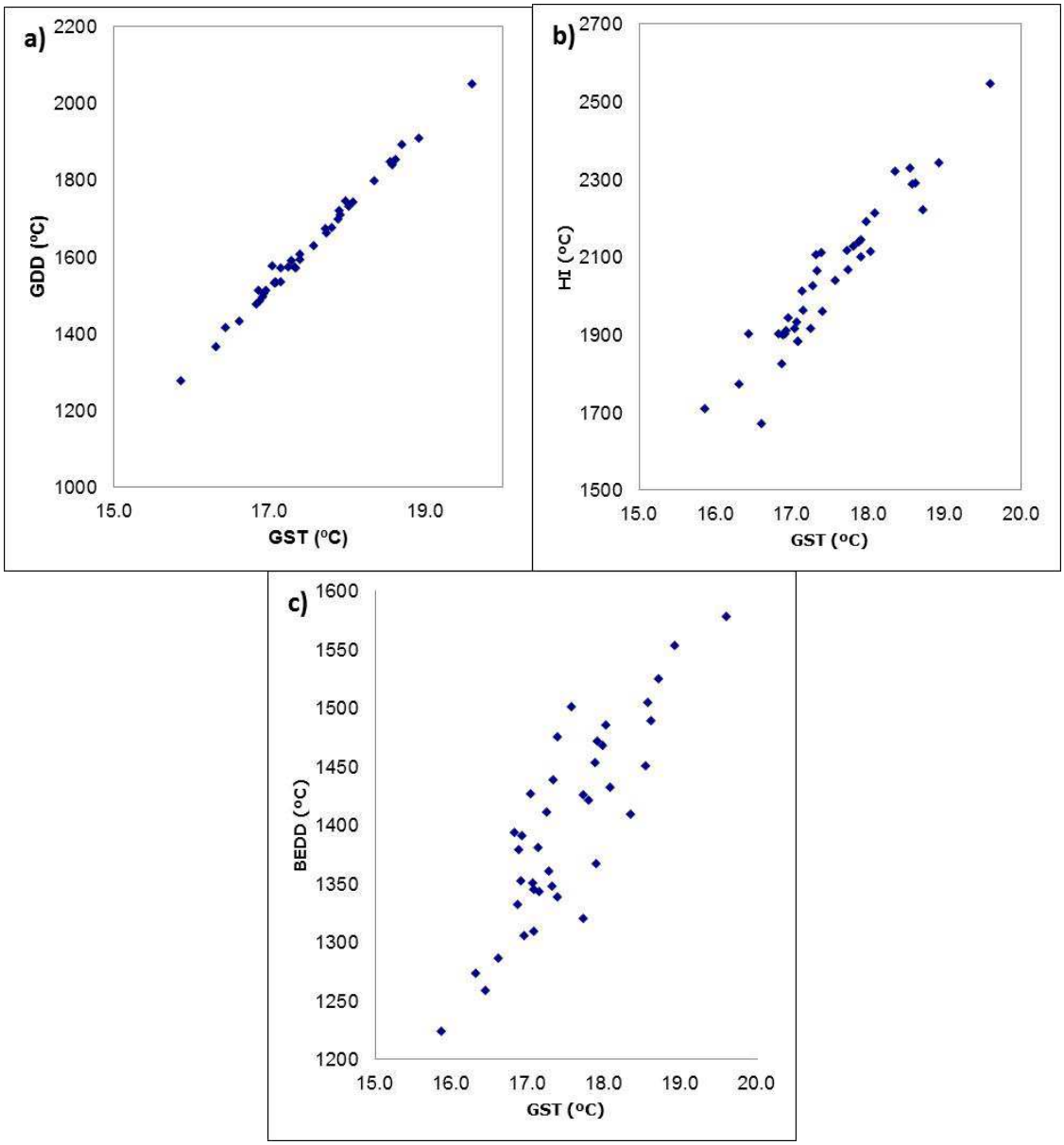


Figure 4. Relationships between different growing season temperature based climate indices. a) Linear relationship between Growing Degree Days (GDD) and Growing Season Temperature (GST). b) Linear Relationship between Huglin Index (HI) plotted against Growing Season Temperature (GST). c) Linear Relationship between Biologically Effective Degree Days (BEDD) against Growing Season Temperature (GST).

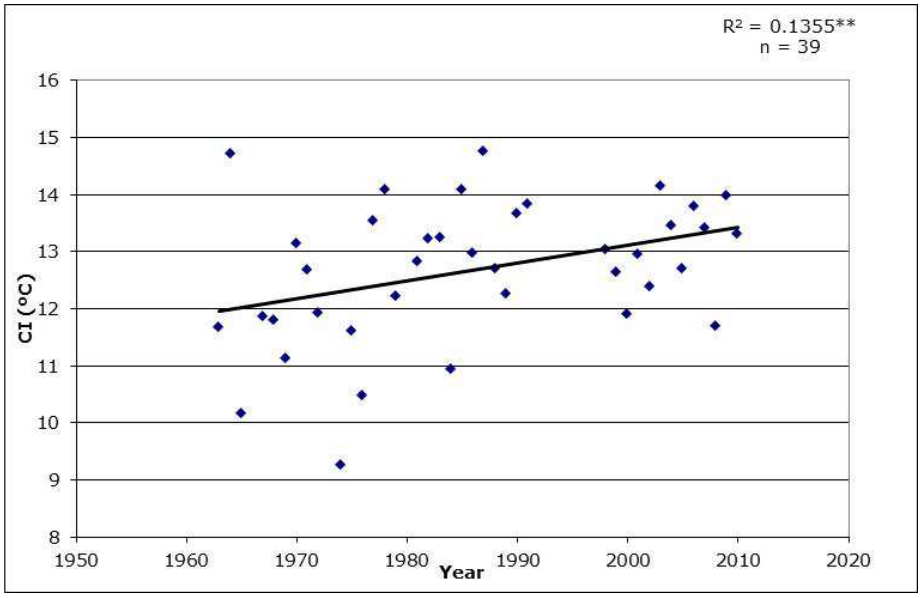


Figure 5. Evolution of Cool Night Index (CI) (Tonietto, 1999) over 39 years between 1963-2010.

The Dryness index (DI) had an average value of –25.6 mm over the recorded period. This DI value corresponds to the DI class 'DI + 1' including it in regions that are described as having 'large presence of dryness'.

This 'moderately dry' class of viticultural climate presents climatic conditions where the vine will potentially face a certain level of dryness. This situation, in which there is significant stomatal regulation of the plant, is usually favorable for berry maturation (for example: Montpellier, France; Funchal, Madeira). Irrigation is practiced in certain cases. At around DI < 50 mm we begin to find regions classified as Mediterranean-type climates, with water deficits in the summer (Riou *et al.* 1994).

Two-thirds of the calculated seasons have had negative soil water reserves at the end of the viticultural season (26 out of the 39 calculated seasons) when using a value of 200 mm available soil water at the beginning of the viticultural season.

There was no significant trend for DI for years between 1963-2010 (Fig. 6). The DI is very variable over the recorded period.

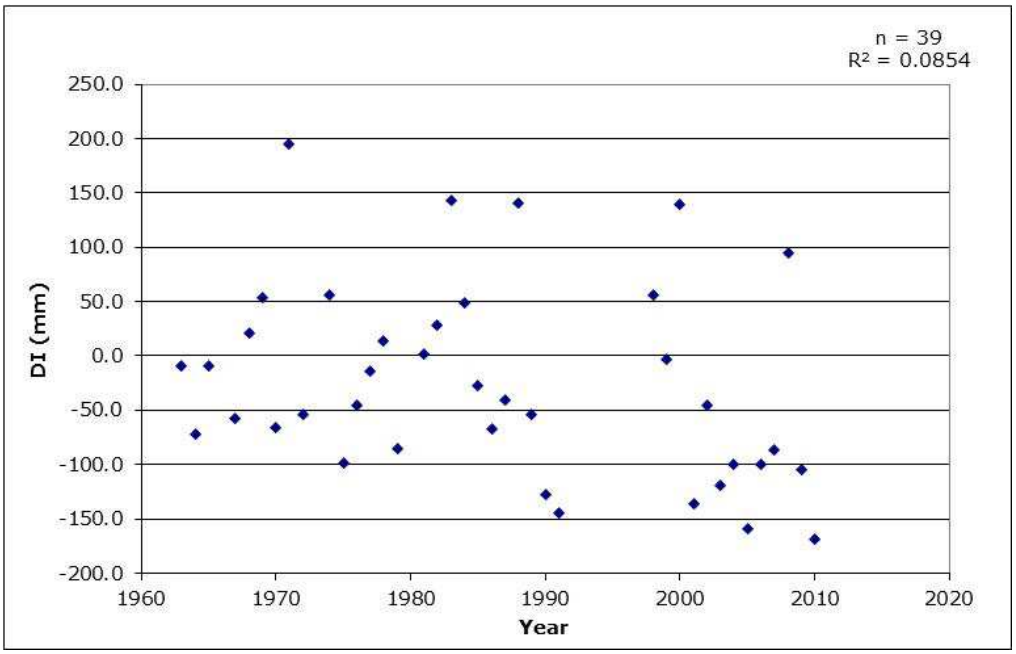


Figure 6. Evolution of the estimated soil water at the end of each viticultural season (considering 200mm as the available soil water reserves at budburst) over 39 years between 1963-2010.

Table 7. Sample size, coefficient of determination (R^2) and formula for each model describing relationships between annual values of each climate index; Growing Season Temperature, Growing Degree Days, Huglin Index, Biologically Effective Degree Days, Cool night Index, Dryness Index (dependent variables) and year (independent variable).

Function	**n**	**R^2**	**Formula**
GST = f(year)	39	0.28**	GST = 0.0279year – 37.902
GDD = f(year)	39	0.27**	GDD = 5.6918year – 9678.5
HI = f(year)	39	0.26**	HI = 6.4791year - 10823
BEDD = f(year)	39	0.23**	BEDD = 2.6894year – 3942.3
CI = f(year)	39	0.14**	CI = 0.031year – 48.875
DI = f(year)	39	ns	ns

3.2 Phenology, Yield and Berry Composition

3.2.1 Phenology

The average date of budburst over the observed period was 1 April. The earliest recorded budburst date was 8 March, while the latest was 21 April. From 1961 until 1979 there were 11 years where budburst occurred after 8 April. After 1979 budburst only occurred after 8 April once (9 April, 2006) in the 31 remaining years of data (Fig. 7).

The budburst data shows the strongest relationship with years of recorded data of the four phenological events (Fig. 7, table 4). It shows a trend of budburst dates occurring earlier by four days each ten years (table 4).

As discussed in the introduction of this paper there is evidence that temperatures during the latter part of winter influence the budburst date. It is shown in both cases, whether calculated as daily maximum (Antcliff *et al.* 1955; Baldwin, 1966; McIntyre *et al*.1982) or as daily means (Alleweldt and Hofäcker, 1975; Pouget 1967) that higher temperatures hasten the budburst date. In the previous chapter, there were results to suggest an increase in the average growing season temperatures, this could help explain the trend towards earlier budburst dates as well. The presence of this relationship could be tested by calculating a heat summation by cumulative GDD up until each respective budburst date of each year.

The mean date of flowering was 7 June and ranged from 2 May to 1 July. There is a weak correlation between the dates of flowering over the recorded period and the relationship between flowering date and the recorded years between 1961-2010 was not considered statistically significant (table 4).

Because the values of Veraison initiation dates until 1982 have been estimated from the flowering date observations, the correlation between values are identical to flowering dates as discussed above. The relationship between observed veraison dates after 1982 and the number of years of recorded data were not considered statistically significant (table 4).

Harvest dates showed the most variation of the phenological dates between years. The harvest was as early as 22 September and as late as 3 November. The mean date of harvest was 9 October. The Harvest dates have stronger correlation between years compared to the other phenological events with the exception of budburst. As with the

dates of budburst, the harvest records show a trend that they are becoming earlier by almost four days per 10 years.

Harvest dates are mostly earlier in the latter part of the record after 1987. There were only two occasions of harvest occurring earlier than 7 October before 1987 (1961, 1962). Since 1987 harvest has taken place in 18 out of the 21 observed dates before 7 October. Similar to budburst, Harvest dates have also been well associated with temperature (Smart 1979, Coombe and Iland 1987, McIntyre 1982). Therefore, the increases in average growing season temperatures could also explain the trend towards earlier harvest dates.

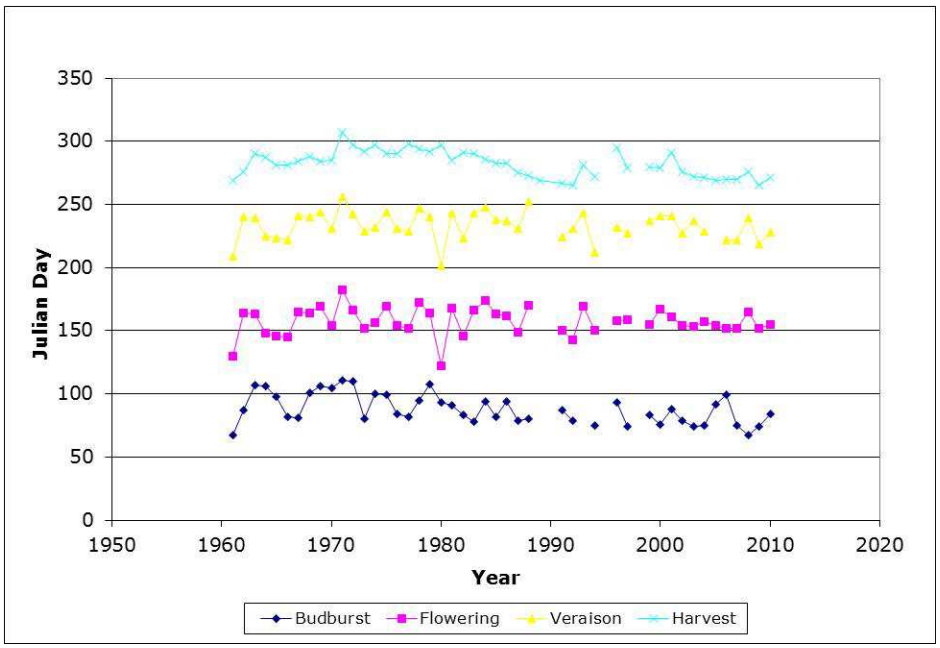


Figure 7. Annual dates of the main phenological events between 1961-2010; budburst dates, flowering dates, veraison dates, harvest dates.

Often more important than the actual date of each phenological event is the interval between events, which gives an indication of the overall climate during those periods. Short intervals are associated with optimum conditions that facilitate rapid physiological growth and differentiation (Coombe, 1988; McIntyre et al., 1982). Long intervals between events indicate less than ideal climate conditions and a delay in growth and maturation (Caló et al., 1996; Gladstones, 1992).

Table 8. Degrees of freedom, coefficient of determination (R^2) and formula for each model describing linear relationships between annual Phenological dates (Julian day) (dependent variable) and year (independent variable). NS indicates not significant trends and *, **, and *** indicate significance at the 0.05, 0.01 and 0.005 levels respectively.

Function	**n**	**R^2**	**Formula**
Harvest date = f(year)	47	0.28**	Harvest date = -0.3656year +1007.8
Veraison date = f(year)	45	0.01 ns	Veraison date = -0.0837year + 399.15
Flowering date = f(year)	46	ns	ns
Budburst date = f(year)	45	0.25**	Budburst date = -0.3952year + 872.32

An important interval is the length of the growing season (number of days from budburst to harvest) which ranged from 171 to 216 days. The average length of the growing season was 195 days. There is a good correlation between budburst and harvest dates (Fig. 8). When the budburst date is delayed often the harvest date is also delayed, and vice versa. This means that the timing of the ripening period could also be affected by budburst dates. This could be useful information when considering the timing of pruning, as it can influence the budburst date (Martin and Dunn, 2000). Also with warmer seasons, earlier budburst dates make vines more susceptible to late spring frost events. Earlier harvest dates could be an advantage in the region, so as to miss the common late September rain events.

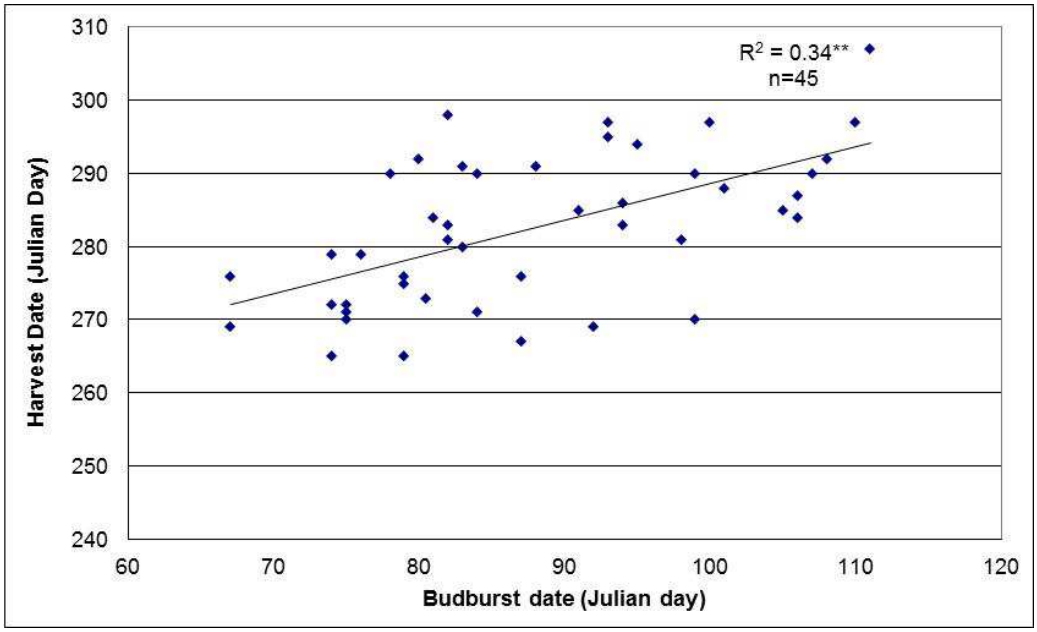


Figure 8. Relationship between budburst date (independent variable) and harvest date (dependent variable).

3.2.2 Yield and Berry Composition

As the data available from 1961 to 1987 was from the rootstock study in the plot ‘folha 3’, there is separate berry composition data available from each rootstock. Therefore, for each year in this period there are multiple and varied berry composition observations.
The data set between 1961 and 1987 is complete with data for all years in this interval available.
The average alcohol percentage (v/v) for the data collected between 1961 and 1987 was 13.8% and the average of the data collected after this (1995 - 2010) was only 12.1%. It appears that plots ‘folha 1C’ and ‘folha 5’ reached lower sugar levels generally, compared with ‘folha 3’ (Fig. 9). Because of this variation, if a trendline is fitted to the alcohol data from 1961 to 2010 there is a trend indicating a reduction in alcohol over time although with weak correlation (Fig. 9a), however if the two periods are considered separately there is no trend in alcohol content from year-to-year, with no statistical significance in the separate periods (Fig. 9b and 9c). These varied responses of the different vineyard plots highlight different plant-responses with variations in planting material and vineyard-age. These differences should be considered when making conclusions after exploring effects of climate on berry composition.
The average variation of potential alcohol between rootstocks for each year was 1.33% v/v (Fig. 10). This ranged from a minimum variation of 0.3%v/v in 1974 to a maximum of a 2.7% v/v range in 1969 (13.7% v/v – 16.4% v/v). This range of results for given years highlight the different plant responses when grafted with different rootstocks.

These variable results for each year indicate that climate cannot simply be correlated with a variety, that there are also other factors affecting plant behaviour and berry composition; such as selected rootstocks.

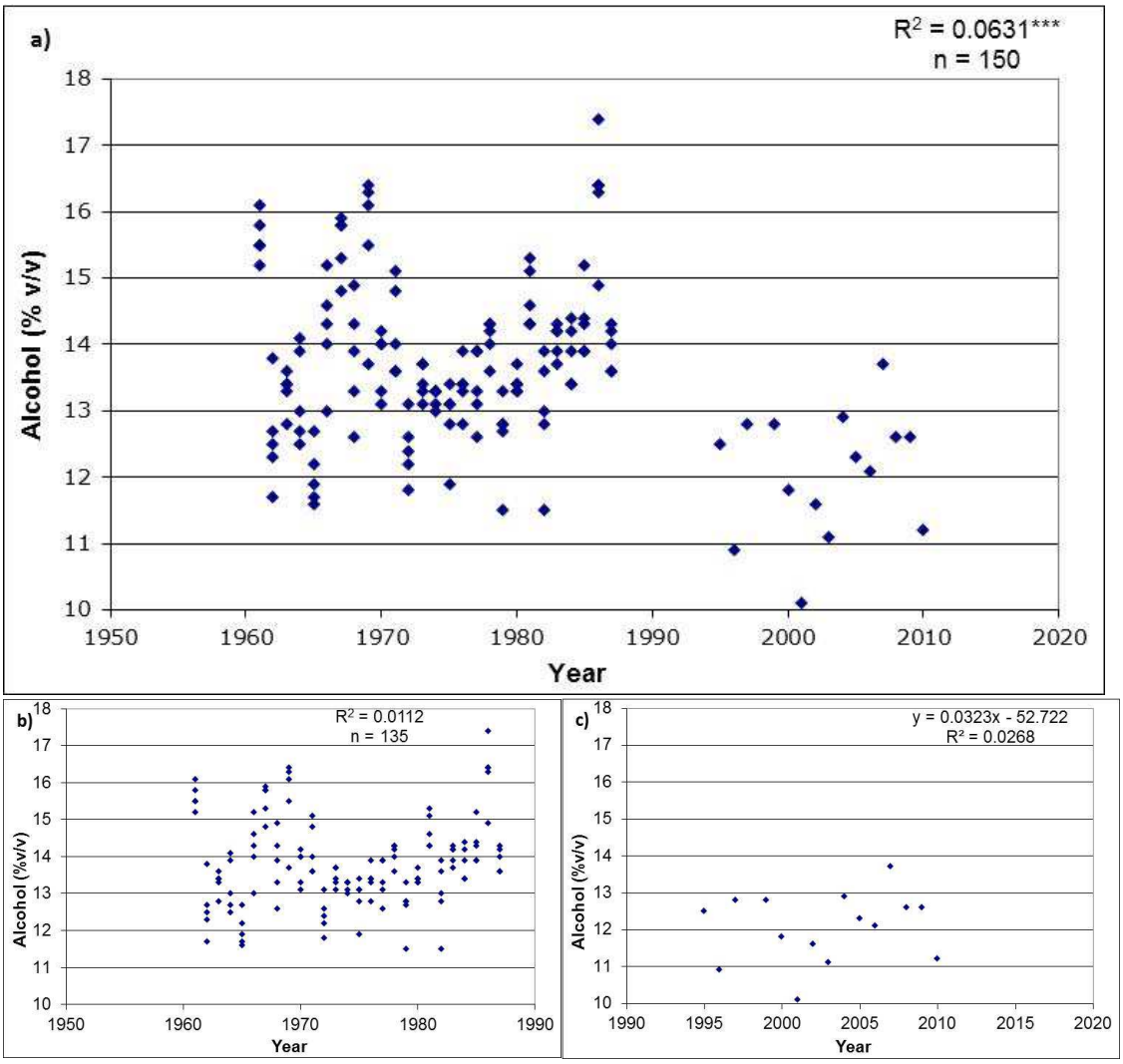


Figure 9. a) Potential alcohol (% v/v) plotted against year (1961-2010). b) Potential alcohol (% v/v) plotted against year (1961-1987). c) Potential alcohol (% v/v) plotted against year (1995-2010).

TA values were missing from 1988 to 1994 and 1999 to 2003, this makes it difficult to assess recent TA data as there are 12 out of the last 23 years where there are no TA values available. The average TA for the data collected between 1961 and 1987 was 7.86 and the average of the data collected after this was 6.7. It appears that plots 'folha 1C' and 'folha 5' obtained lower TA levels generally, compared with 'folha 3'. There is no significant evolution of TA over the recorded years (table 5).

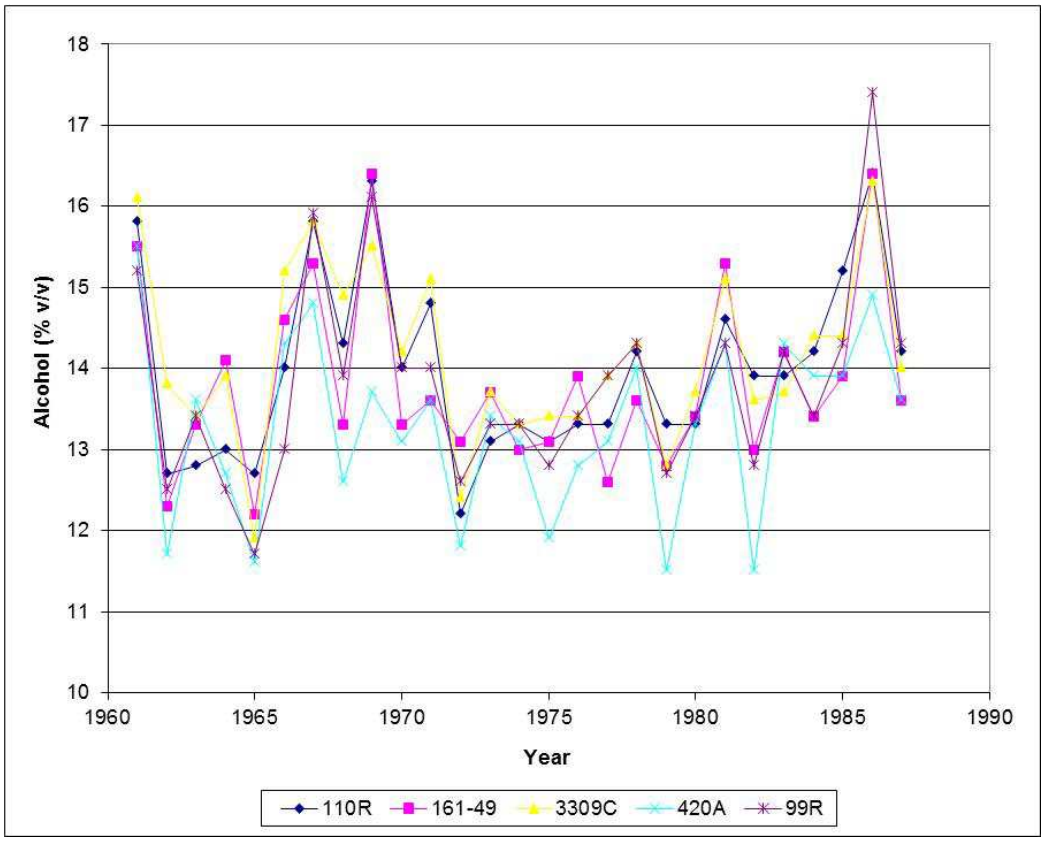


Figure 10. Potential alcohol (% v/v) per year for different rootstocks in vineyard plot 'folha 3' – 110R, 161-49, 3309C, 420A, 99R.

The average variation of TA between rootstocks for each year was 1.34 (Fig.12a). This ranged from a minimum variation of 0.52 in 1965 to a maximum of a 2.78 range in 1977 (7.05 – 9.83). As with the alcohol levels, the difference between vineyard plots and rootstocks should be considered when making conclusions about these results. These differences in results could be related with the age of the vines and the quality of plant material as well as yield differences because 'folha 3' was a much older vineyard in comparison with the others.

There was no significant evolution of pH over the recorded years (table 9). pH values varied between 3.07 in 1968 and 1972, to 3.80 in 2004. The average variation of pH between rootstocks for each year was 0.12 (Fig. 12b). This ranged from a minimum variation of 0.03 in 1972 to a maximum of a 0.28 range in 1981 (3.32 – 3.6). Generally there appeared to be less variation in pH values between rootstocks than with other berry composition parameters (alcohol and TA). However these differences of pH values

between rootstocks should be taken into account when making conclusions about results.

The average yield for the data collected between 1961 and 1987 was 1.42 kg/vine and the average of the data collected after this (1995-2010) was 2.5 kg/vine. It appears that plots 'folha 1C' and 'folha 5' had higher yield levels generally, when compared with 'folha 3'. This variation of crop yields between plots could also explain the differences in potential alcohol found between plots. High crop yields can sometimes disrupt the balance between vegetative growth and yield, delaying berry ripening (Gladstones, 1992). Due to the yield variations between plots, if a trendline is fitted to the yield data from 1961 to 2010 there is a statistically significant trend towards an increase in yield, but with a weak correlation coefficient (Fig. 11a), however if the two periods are considered separately it is evident that the yield of 'folha 3' shows no significant evolution (Fig. 11b), and plots 'folha 1C' and 'folha 5' have higher yields, but no statistically significant trend (Fig. 11c). This variation of yields between the different vineyard plots should be considered when making conclusions about the effect of climate on yield in this data set.

The average variation of yields between rootstocks for each year was 0.83 kg/vine (Fig.12). This ranged from a minimum variation of 0.09 kg/vine in 1977 to a maximum of a 1.71 kg/vine range in 1975 (1.13– 2.85 kg/vine). This range of results for given years highlights the different plant responses when grafted with different rootstocks.

Table 9. Relationships between different berry composition parameters; potential alcohol (%v/v), TA (g tartaric/l), pH and yield (kg/vine) (dependent variables) and the years of recorded data (independent variable). NS indicates trends that are not significant and *, **, and *** indicate significance at the 0.05, 0.01 and 0.005 levels respectively.

Function	**n**	**R^2**	**Formula**
Alcohol (%v/v) = f(year)	150	0.06***	Alcohol (%v/v) = -0.0273year + 67.616
TA (g tartaric/l) = f(year)	156	ns	ns
pH = f(year)	150	ns	ns
Yield (kg/vine) = f(year)	159	0.06***	Yield (kg/vine) = 0.0215year - 40.951

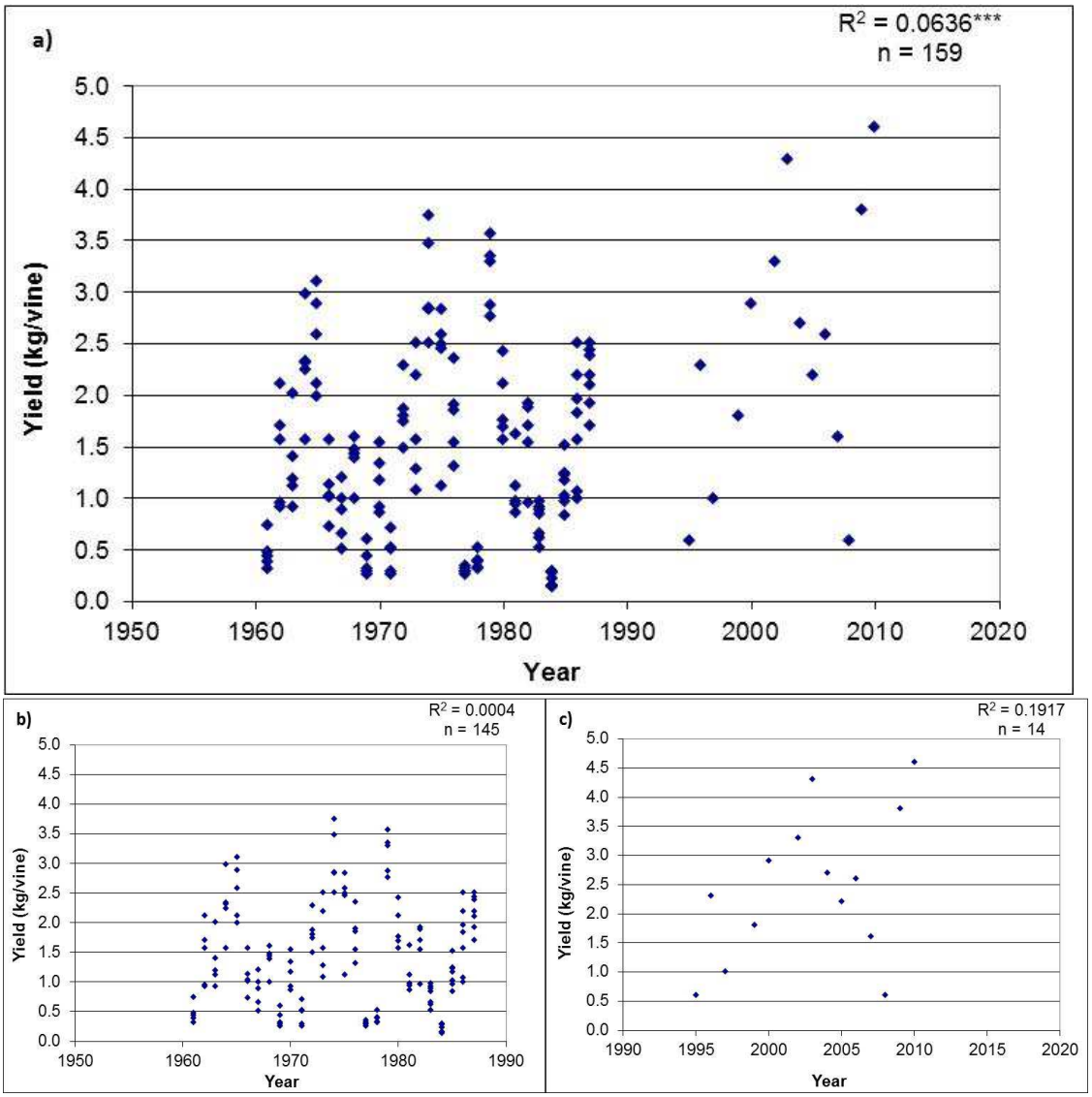


Figure 11. a) Yield (kg/vine) plotted against year (1961-2010). b) Yield (kg/vine) plotted against year (1961-1987). c) Yield (kg/vine) plotted against year (1995-2010).

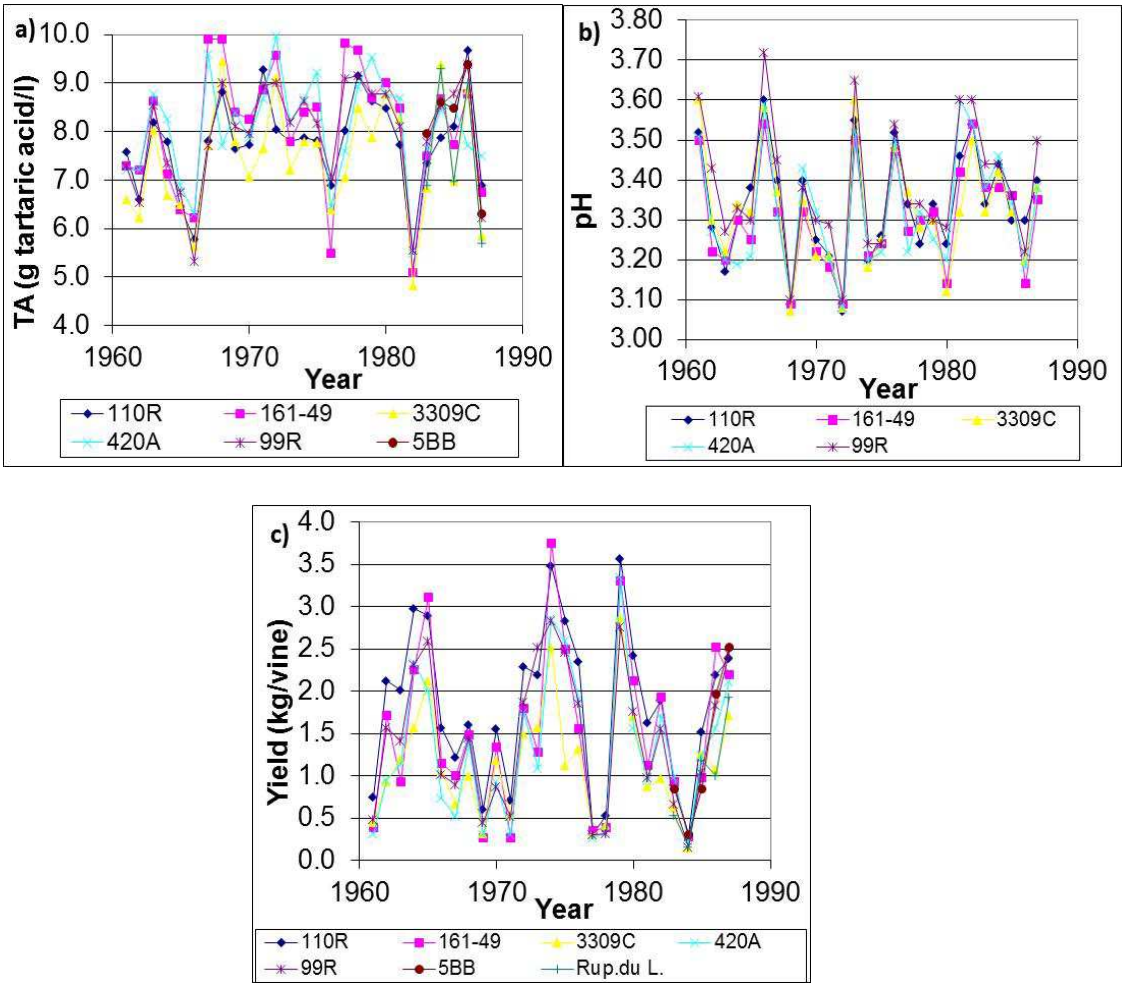


Figure 12. Effect of Rootstock on: TA (g tartaric/l) (a) pH (b) and Yield (kg/vine) (c) in the vineyard plot 'folha 3'.

3.3 Relationships between climate indices and agronomic variables

This chapter explores relationships between the various climate indices presented earlier with alcohol, titratable acidity, pH and yield.

HI and DI were the only indices that had any relationship with alcohol over the recorded period (Table 10). What was surprising is that HI had a negative relationship with alcohol. Suggesting that an increase in HI is likely to result in a reduction of alcohol or sugar content in the berries (Fig. 13). This relationship is not supported by general knowledge and research on the topic. It is common knowledge that higher temperatures (up to a limit) lead to higher sugar levels and consequently higher alcohol levels in the

Table 10. Sample size (n), coefficient of determination (R^2) and formula for each model describing relationships between Alcohol (%v/v) (dependent variable) and different indices (independent variables): Growing Season Temperature (GST), Growing Degree Days (GDD), Biologically Effective Degree Days (BEDD), Huglin Index (HI), Cool Night Index (CI) and Dryness Index (DI). NS indicates trends that are not significant and *, **, and *** indicate significance at the 0.05, 0.01 and 0.005 levels respectively.

Function	**n**	**R^2**	**Formula**
Alcohol = f(GST)	122	ns	Ns
Alcohol (%v/v) = f(GDD)	122	ns	Ns
Alcohol (%v/v) = f(BEDD)	122	ns	Ns
Alcohol (%v/v) = f(HI)	122	0.05**	Alcohol = -0.0018HI + 17.178
Alcohol (%v/v) = f(CI)	132	ns	Ns
Alcohol (%v/v) = f(DI)	132	0.12***	Alcohol = -4E-05DI^2 + 0.0062DI + 13.938

resulting wine. Sometimes when temperatures become excessive (eg. Above 35ºC) the vine will close its stomata in an effort to limit water loss, by doing so photosynthesis is also limited (Hsiao 1973). It is not uncommon in hotter regions to have periods where berry maturation is stopped due to excessive temperatures. An excess of temperature could be a reason for the negative relationship between Alcohol and HI, however there is such a weak correlation with this relationship, that HI is only explaining a small amount of the variation in the Alcohol levels. There was also a general difference in the alcohol levels between the vineyards that was investigated in the previous chapter (Chapter 3.2), this may have resulted in the unusual relationship between alcohol and HI.

The relationship between alcohol and DI is better described with a polynomial trend line (Fig. 14). It indicates that years of higher sugar levels occurred more so when there was an intermediate soil water content and that extreme highs or lows of available soil moisture resulted in lower alcohol yields. However, the coefficient of correlation is relatively weak, making this a very general conclusion. The effect of water deficit stress on berry sugar content has been shown to be yield-dependent; for low yields, vine water deficit enhances berry sugar content and for high yields, it depresses berry sugar content (Tregoat et al., 2002). When water stress is excessive, photosynthesis is overly restricted and fruit ripening may be delayed, particularly when the yield is high.

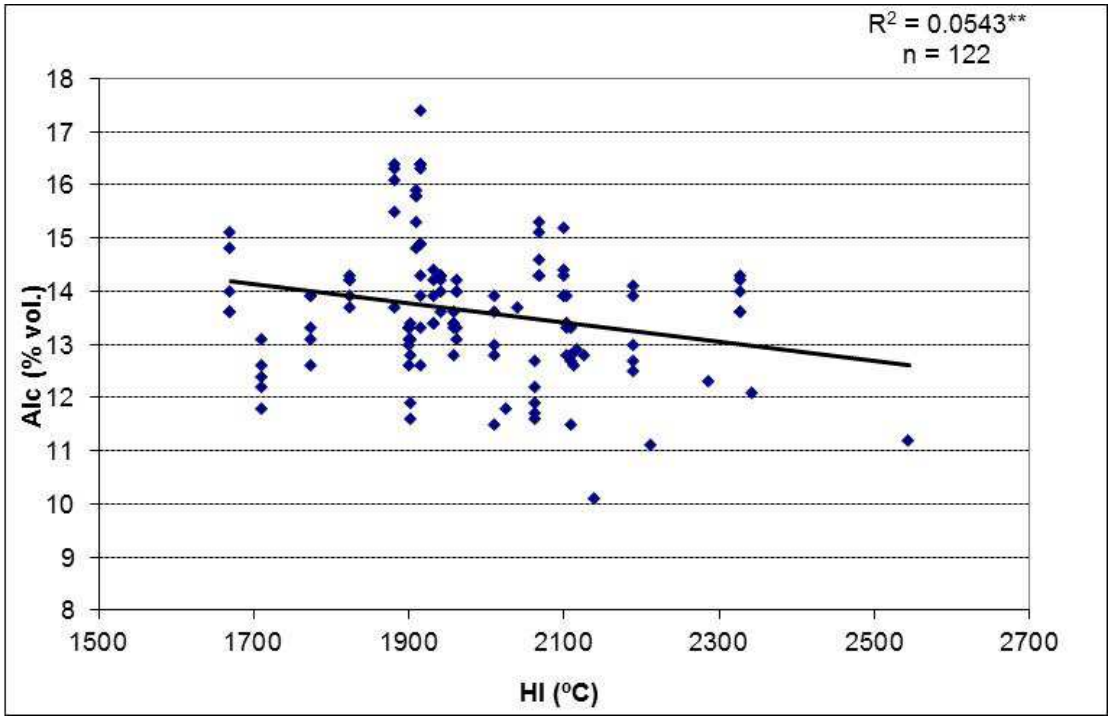


Figure 13. Relationship between Huglin Index (independent variable) and Alcohol (dependent variable).

The main problem with the DI modeling approach is in estimating the total transpirable soil water at field capacity which is extremely difficult due to varying conditions in vine cultivation (rooting depth, amount of stones in the ground, variations in soil texture, variations in soil bulk density, etc.). Hence, application of the DI at plot level provides inconsistent results (Pellegrino et al., 2006). However, these models are useful for describing the climatic and plant-related aspects of vine water deficits at a regional scale and they are powerful tools for classifying vintages according to their dryness.

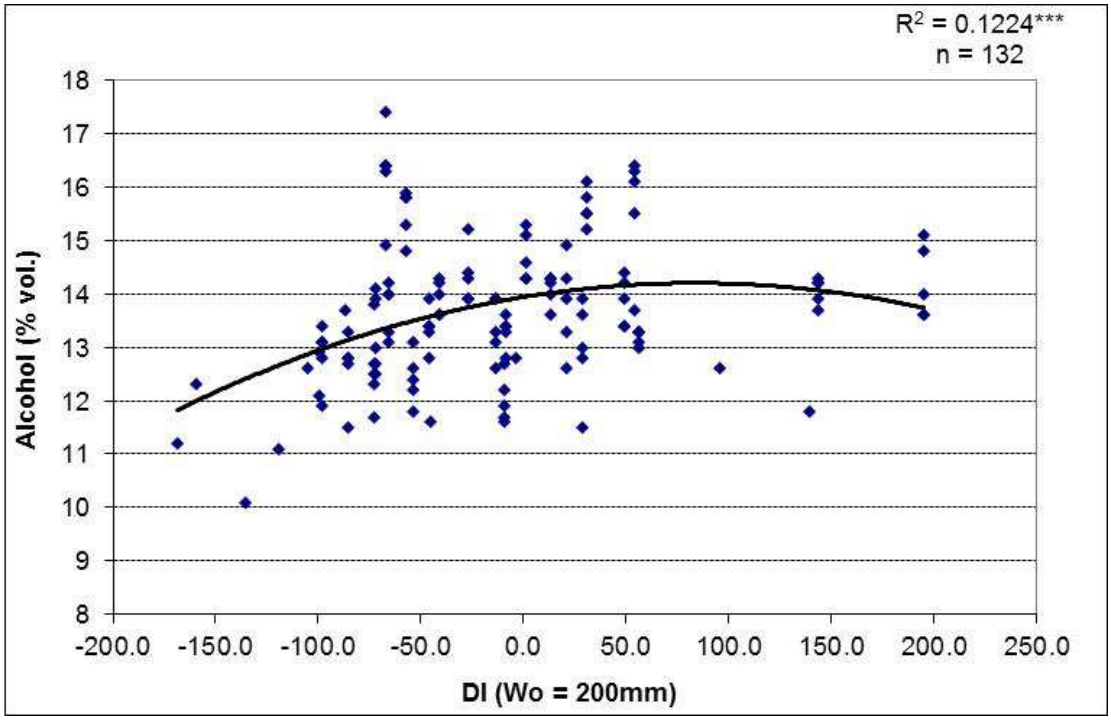


Figure 14. Relationship between Dryness Index (independent variable) and potential alcohol (dependent variable).

TA has the best correlation with the climate indices of the berry composition parameters. HI had the best correlation with TA from the recorded data (Fig. 15) GST had the next strongest correlation followed by GDD and BEDD all of these correlations were highly significant. The relationships between CI and DI with TA were not statistically significant (table 11).

The indices HI, GST, GDD and BEDD all had negative relationships with TA (Table 11). This is expected as it is generally considered that warmer temperatures increase the rate of L-malic acid respiration resulting in lower organic acid levels at maturation (Volschenk et al. 2006). However it was surprising that CI had no relationship with TA, as this is a measure of the minimum temperature during September. September is the month where the grapes were generally ripening, hence the period when this acid respiration was occurring, perhaps the minimum temperature alone during this period is limiting when relating the index with acid depletion.

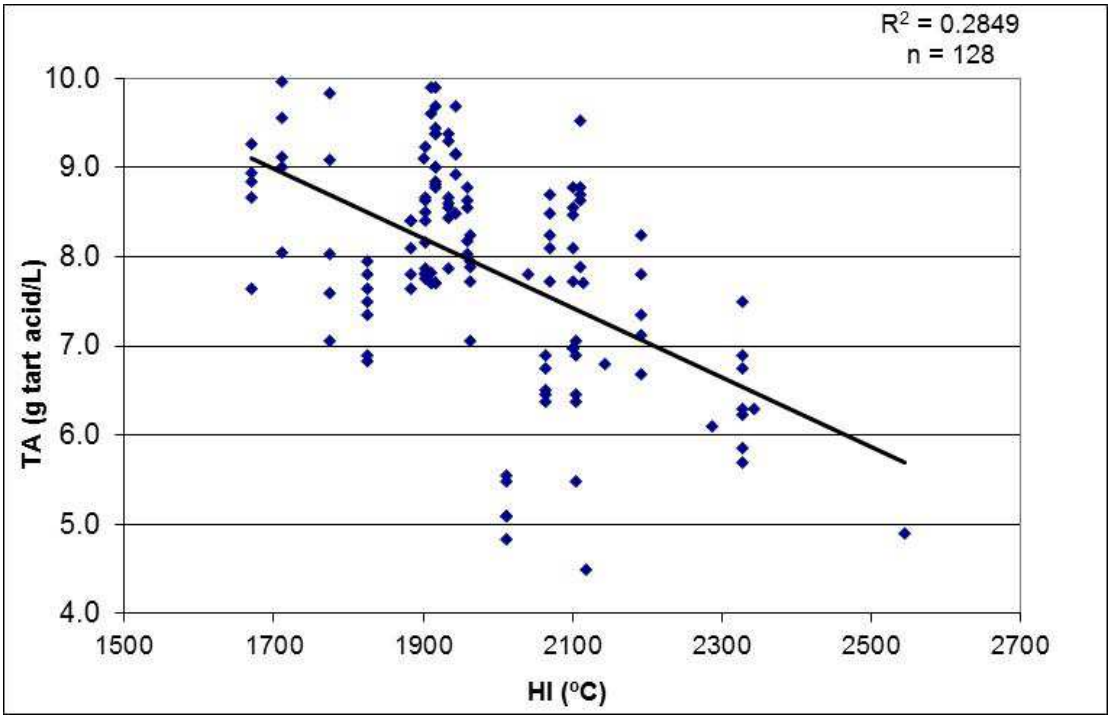


Figure 15. Relationship between Huglin index (HI) (independent variable) and titratable acidity (TA) (dependent variable).

Table 11. Relationships between Titratable Acidity (TA) (g of tartaric acid/l) (dependent variable) and different climate indices (independent variables): Growing Season Temperature (GST), Growing Degree Days (GDD), Biologically Effective Degree Days (BEDD), Huglin Index (HI), Cool Night Index (CI) and Dryness Index (DI). Sample size (n), coefficient of determination (R^2) and formula for each model describing the relationship. NS indicates trends that are not significant and *, **, and *** indicate significance at the 0.05, 0.01 and 0.005 levels respectively.

Function	**n**	**R^2**	**Formula**
TA = f(GST)	128	0.22***	TA = -0.8793GST + 23.046
TA = f(GDD)	128	0.20***	TA = -0.0039GDD + 14.085
TA = f(BEDD)	128	0.12***	TA = -0.0055BEDD + 15.393
TA = f(HI)	128	0.28***	TA = -0.0039HI + 15.612
TA = f(CI)	128	ns	ns
TA = f(DI)	128	ns	ns

GST is the simplest index to describe seasonal temperature conditions. It is useful to observe this as a base and then investigate what adjustments in the HI gave a better correlation with acidity. There are several adjustments that have been mentioned previously in this paper for HI. GST measures the average daily temperature over 24-

hours, whereas HI tries to best estimate the average temperature for the daylight period by taking the mean of the average and maximum temperatures into calculation and using a length of day coefficient, k (eq. 4). HI is a modification of GDD, so it also has a base temperature limit, which means it doesn't accumulate average temperatures if they are lower than 10ºC (eq 3, eq. 4). Importantly HI is measured over a 6-month period instead of 7-months like most other season temperature indices. The correlation coefficient is reduced when TA is correlated with GDD, and even more so with BEDD.

HI, GDD and BEDD all take out any temperatures below the base temperature (10ºC) (eq. 3). This difference could possibly weaken any correlation with TA. Temperatures below 10ºC may not be important for vine function and berry ripening, however when we are measuring acidity in the berries after ripening, it is merely the acidity that was retained, thus these lower temperatures are still relevant as they slow the respiration of acids.

Probably the most important factor in explaining the better correlation of HI compared with the other temperature climate indices is that HI is measured over a period of 6-months compared to the other indices that are measured over 7-months. To investigate this effect, the length of time over which the indices are calculated were swapped. GST, GDD and BEDD were all calculated for a 6-month growing season (instead of the usual 7-months) and HI was calculated over 7-months. These new values were then correlated against the same values of TA (table 12). The correlation coefficient of HI was reduced when it was measured over 7-months from 0.2849 to 0.1883. The correlation coefficient of GST, GDD and BEDD were improved when they were measured over 6-months instead of 7-months (table 12). Thus it is a reasonable conclusion to suggest that the 6-month growing season is the most significant of the indicators that makes HI a more suitable index for explaining TA in this region. It more closely matches the length of the growing season of Touriga Nacional in these vineyards with an average harvest date of 9 October. However, these climate indices have a set growing season length in order to be used as a climate comparison tool for wine regions around the world.

Changing the season length in the calculation would no longer make it useful for simple and direct comparisons with other wine regions.

Table 12. Relationships between Titratable Acidity (TA) (g tartaric acid/l) (dependent variable) and different indices with adjusted growing season length (independent variables): Growing Season Temperature (GST, 6-months), Growing Degree Days (GDD, 6-months), Biologically Effective Degree Days (BEDD, 6-months), Huglin Index (HI, 7-months). Sample size (n), coefficient of determination (R2) and formula for each model describing the relationship. NS indicates trends that are not significant and *, **, and *** indicate significance at the 0.05, 0.01 and 0.005 levels respectively.

Function	**n**	**R^2**	**Formula**
TA = f(GST)	128	0.31***	TA = -0.8385GST + 22.65
TA = f(GDD)	128	0.28***	TA = -0.0045GDD + 14.213
TA = f(BEDD)	128	0.30***	TA = -0.0082BEDD + 17.578
TA = f(HI)	128	0.19***	TA = -0.0034HI + 15.4

pH had a positive relationship with GST, GDD and HI. There was no significant relationship between pH and BEDD, CI and DI (table 13). pH had the best correlation with HI of all the climate indices, a positive correlation (Fig. 16) with high statistical significance. This relationship between HI and pH, although only explaining a small proportion of the variation in pH agrees with the concept that higher temperatures generally result in a reduced berry acidity.

Table 13. Relationships between pH (dependent variable) and different indices (independent variables): Growing Season Temperature (GST), Growing Degree Days (GDD), Biologically Effective Degree Days (BEDD), Huglin Index (HI), Cool Night Index (CI) and Dryness Index (DI). Sample size (n), coefficient of determination (R^2) and formula for each model describing the relationship. NS indicates trends that are not significant and *, **, and *** indicate significance at the 0.05, 0.01 and 0.005 levels respectively.

Function	**n**	**R^2**	**Formula**
pH(GST)	122	0.10***	pH = 0.0635GST + 2.2185
pH(GDD)	122	0.08***	pH = 0.0003GDD + 2.8793
pH(BEDD)	122	ns	ns
pH(HI)	122	0.12***	pH = 0.0003HI + 2.758
pH(CI)	122	ns	ns
pH(DI)	122	ns	ns

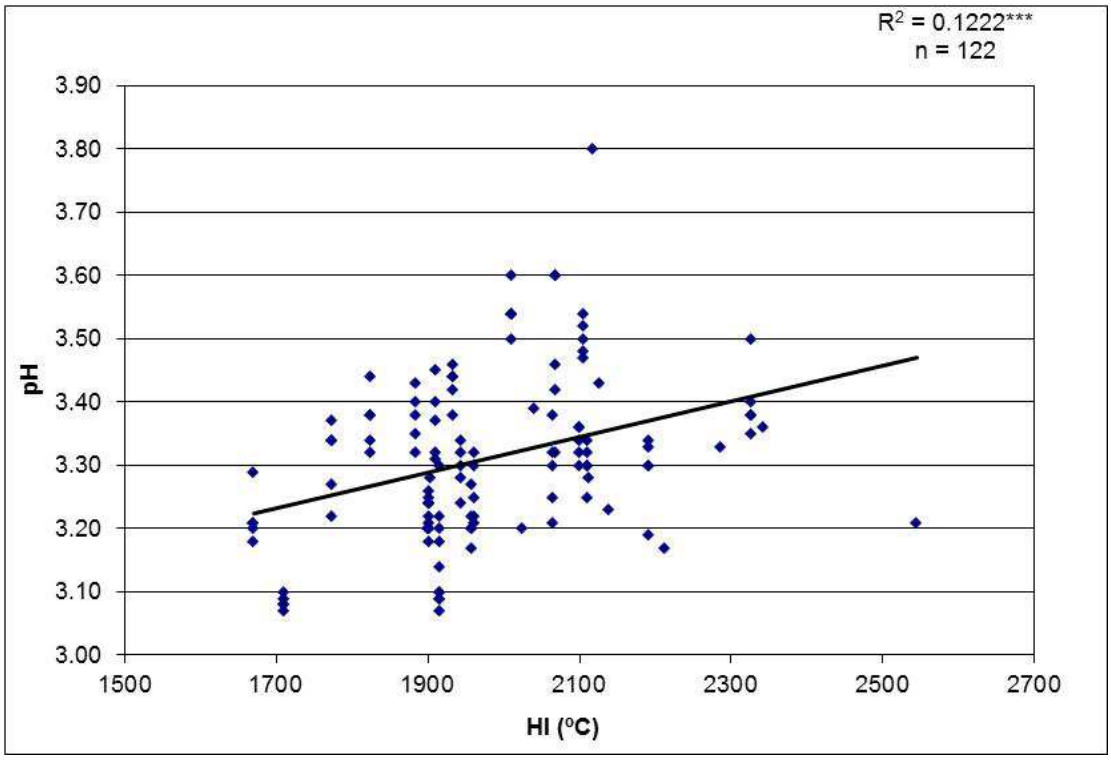


Figure 16. Relationship between Huglin Index (independent variable) and pH (dependent variable).

This relationship is more complicated than discussing TA, as TA is a measurement of the quantity of organic acids measured in units grams of tartaric acid per litre. Temperature determines the respiration rate i.e the combustion of tartaric and malic acid in grape flesh cells. However, this is difficult to directly apply to pH. The practical usefulness of pH in relation to climate is difficult to explain, because grapes and wine are 'buffer' solutions i.e. a modification in the chemical composition produces only a limited variation in pH.

Yield had a positive relationship with GST, GDD, BEDD and HI. There was a positive relationship between yield and DI, while there was no significant relationship between yield and CI (Table 14).

Positive relationships between yield and the temperature based climate indices indicates that yields were higher in warmer seasons. As discussed in the introduction of this paper, the time around flowering has a large influence on yield. Vines need warmth and sunshine during and around flowering for best fruit set. Wet, cold and cloudy weather results (to varying degrees according to grape variety) in either or both of two conditions: poor set or coulure, where many of the berries fall off when small, and 'hen and chicken'

or millerandage, in which small, seedless berries or berries with incompletely formed seeds accompany normally-seeded larger berries on the same bunch (Coombe, 1988). The relationship of yield with the temperature indices supports this situation. HI had the highest correlation with yield. A high HI value means there has been a high accumulation in daytime temperatures over a 6 month period and less average temperatures below 10ºC. It is arguable that in years with higher HI values, it could be more likely there have been more favourable conditions around the flowering period, resulting in higher yields.

Table 14. Relationships between Yield (kg/vine) (dependent variable) and different indices (independent variables): Growing Season Temperature (GST), Growing Degree Days (GDD), Biologically Effective Degree Days (BEDD), Huglin Index (HI), Cool Night Index (CI) and Dryness Index (DI). Sample size (n), coefficient of determination (R^2) and formula for each model describing the relationship. NS indicates not significant trends and *, **, and *** indicate significance at the 0.05, 0.01 and 0.005 levels respectively.

Function	**n**	**R^2**	**Formula**
Yield = f(GST)	131	0.11***	Yield = 0.5235GST - 7.4682
Yield = f(GDD)	131	0.12***	Yield = 0.0025GDD - 2.4376
Yield = f(BEDD)	131	0.10***	Yield = 0.0042BEDD - 4.1613
Yield = f(HI)	131	0.26***	Yield = 0.0031HI - 4.5708
Yield = f(CI)	131	ns	ns
Yield = F(DI)	131	0.25***	Yield = -0.0066DI + 1.5043

These higher temperatures (provided they didn't coincide with rain) could also create drier conditions around ripening, decreasing the risk of rot (eg. Botrytis) that could also reduce the potential yield.

The trend in the linear regression between yield and DI shows a negative relationship between the two variables (Fig. 17). When there has been more available soil water, yields have been lower. This is an interesting result which doesn't immediately make clear biological sense. Usually higher available soil water, results in higher yields, especially towards the end of the growth cycle where excess water results in the swelling of berries around ripening. This all depends very much on the timing of the rainfall, information which the standard DI does not provide. However, in years where there is a higher DI, there has been more rainfall during the growing season. Thus, this negative relationship with yield could fit the theory discussed above for the positive relationship with temperature indices. That is to say, it could be more likely that there

has been rainfall or poorer conditions around the crucial period of fruit-set that ultimately leads to a reduction in yield. Higher rainfall also increases the risk of disease throughout the growing season. The Dryness index is being broken down into smaller increments by the project SIAMVITI to better understand these relationships.

Because all indices are measurements of the entire growing season, these explanations rely on a lot of assumptions so are far from conclusive.

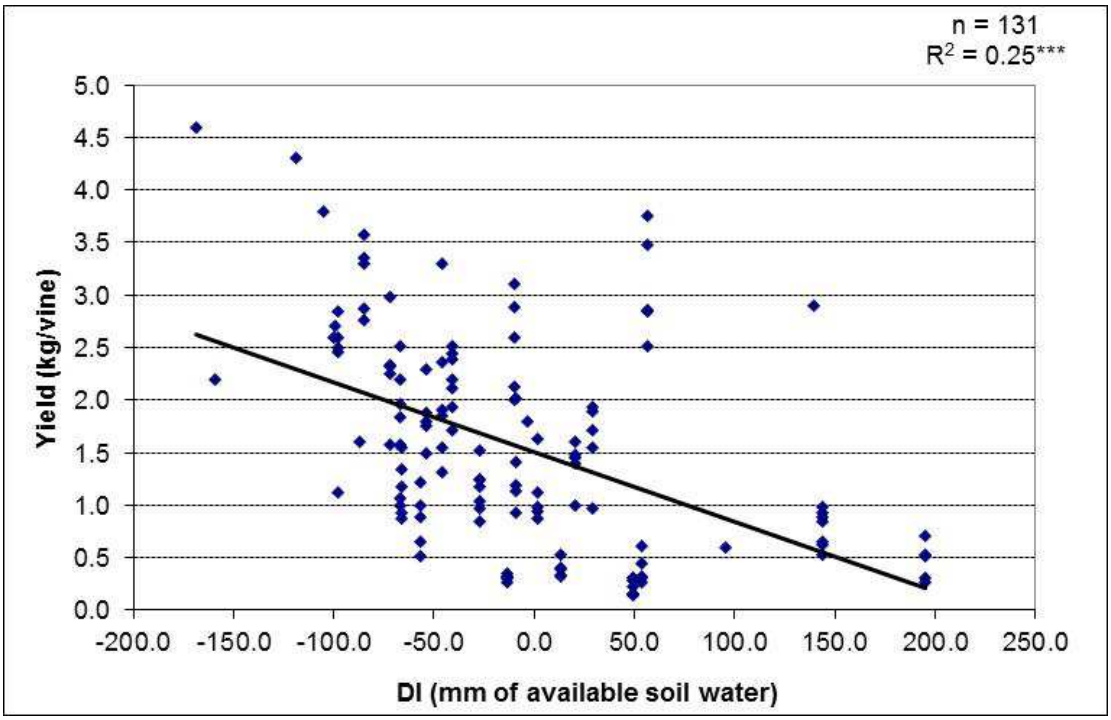


Figure 17. Relationship between Dryness Index (independent variable) and Yield (dependent variable).

4. Conclusions

Relationships between some common climate indices and the properties of must and wine were found from Touriga Nacional planted at the 'Centro de Estudos Vitivinícolas do Dão' research centre in the Dão winegrowing region. Whilst analysing the data, trends in some of these indices suggested a changing climate that agreed with climate change models created from research conducted in other wine regions of Europe.

According to the results, there is a trend towards increasing growing season temperatures of the region, with an increasing GST of 0.28ºC every ten years, as well as an increasing CI of 0.31ºC every ten years. This agrees with previous research that has found growing seasons have shown warming across Europe by 1.7ºC per 50 years on average with most of the warming coming at night (Jones 2006). These observed trends and potential future changes in temperatures may have a strong influence on future viticulture in the region.

In terms of describing the temperature characteristics of a wine region, it was found that GST provided the simplest and most practical summary of the indices. GDD, BEDD and HI gave very similar results in terms of describing the general temperature conditions. However, GDD, BEDD and HI require much more manipulation, that make both calculating and understanding the indices more complicated than GST which is simply the average daily temperature throughout the period 1 April - 31 October (Northern Hemisphere).

Some important Phenological dates such as budburst and harvest were observed to be affected by temperature variations. As expected Budburst and Harvest dates were hastened by higher temperatures. If there is a continuing trend in climate change this could result in these events occurring earlier and earlier. Risks involved with this are that vines may be more susceptible to spring frost events, due to budburst occurring earlier in the season. Earlier harvest dates could be an advantage so as to miss the common late September rain events in the region.

HI generally had the best correlations with berry composition and yield. This was mainly due to the fact that HI is calculated over a 6-month period (1 April – 30 September), rather than the other climate indices that are calculated over a 7-month period (1 April – 31 October). This is most likely due to the fact that the 6-month period more closely

matched the growing season of Touriga Nacional in the Dão region over the recorded period.

It was observed that TA had a negative relationship with all of the temperature indices (GST, GDD, BEDD and HI). This is reinforced by the knowledge that warmer temperatures increase the rate of L-malic acid respiration in the berries resulting in lower organic acid levels at berry maturation.

pH had a positive relationship with GST, GDD and HI. This agreed with the TA relationship and the faster respiration of acid in higher temperatures described previously.

Alcohol, and thus sugar accumulation was not so well explained by the indices. There was a slight negative relationship with HI that could only be explained by possible years where excessive temperatures limited photosynthesis and berry sugar accumulation. However, this relationship was very weak.

Yield showed good correlation with temperature indices as well as with DI. These relationships could best be explained by the fact that good weather around the time of flowering determines better yields, however because these indices are measured over such a large period it is difficult to speculate about such specific time-frames. The correlation between yield and DI should be treated with caution as it doesn't make clear biological sense, this index is being broken into smaller time incraments in the SIAMVITI project to better understand this correlation.

The fact that there were certain differences found between results obtained in different vineyard plots should be considered in terms of the validity of the relationships and trends mentioned above.

Due to trends of increasing temperatures in the recorded data from the Dão winegrowing region and higher temperatures correlating with a decrease in acidity of Touriga Nacional, perhaps the future production of the variety in a warming climate could involve a venture towards plantings in cooler latitudes.

Some of the climate indices such as GST require limited climate information. If simple research into relationships between such climate indices and Touriga Nacional could be made in other regions within Portugal, and in parts of the new world, it could help producers and the market to optimise the potential of this important grape variety.

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Annex I: Touriga Nacional ampelographical and phenological descriptions. (IVV. 2011)

Touriga Nacional

Tradicionalmente cultivada no Douro e no Dão, donde presumivelmente será originária.

Sinonímia		
Abrolhamento	Época de rebentação	Precoce
Pâmpano Jovem (extremidade)	Forma	Completamente aberta
	Distribuição da pigmentação antociânica	Na orla
	Intensidade da pigmentação antociânica	Média
	Densidade dos pêlos prostrados	Baixa
Folha Jovem	Cor da página superior	Bronze
Pâmpano	Porte	Horizontal
	Cor da face dorsal dos entrenós	Verde
	Cor da face ventral dos entrenós	Verde
	Intensidade da pigmentação antociânica dos gomos	Média
	Fertilidade dos gomos basais	Elevada
	Vigor	Médio
Gavinhas	Número de gavinhas consecutivas	2 ou menos
Inflorescência	Flor	Hermafrodita
Folha Adulta	Tamanho	Pequeno
	Forma do limbo	Pentagonal
	Número de lóbulos	Cinco
	Distribuição da pigmentação antocânica das nervuras principais da página superior	Até à 1.ª bifurcação
	Enrugamento do limbo	Ausente ou muito fraco
	Perfil	Irregular
	Bolhosidade da página superior	Média
	Forma dos dentes	Rectilínea
	Comprimento dos dentes	Curto
	Grau de abertura do seio pecíolar	Lóbulos sobrepostos
	Forma da base do seio pecíolar	Em chaveta
	Particularidades do seio pecíolar	Nenhuma
	Densidade de pêlos prostrados entre nervuras principais (pág. inf.)	Média
	Densidade de pêlos erectos entre as nervuras principais (pág. inf.)	Média
	Densidade de pêlos prostrados nas nervuras principais (pág. inf.)	Baixa
	Densidade dos pêlos erectos das nervuras principais (pág. inf.)	Baixa
Sarmento	Cor	Violeta avermelhada

Touriga Nacional

Cacho	Comprimento	Médio
	Compacidade	Baixa
	Comprimento do pedúnculo	Curto
	Forma	Cónica
	Número de asas	1-2 asas
	Peso	Baixo
Bago	Tamanho	Médio
	Forma	Esférica
	Coloração da polpa	Não corada
	Consistência da polpa	Mole
	Sabores particulares	Ausentes
	Grainhas	Bem formadas
Mosto	Teor alcoólico provável	Médio
	Acidez total	Média

Perfil de Microssatélites		VVS2	VVMD5	VVMD7	VVMD27	VrZAG62	VrZAG79
	Alelo 1	145	226	235	181	188	245
	Alelo 2	153	236	235	189	194	245

Aptidões Culturais e Enológicas

Produção média, podendo ser elevada com materiais selecionados e condução adequada. Pouco susceptível ao míldio, oídio e cigarrinha. Medianamente susceptível a carências de magnésio e à podridão cinzenta. Susceptível à escoriose, *stress* hídrico e térmico, perdendo frequentemente folhas nessas condições. Muito susceptível ao desavinho (material tradicional). Casta muito exigente em termos de armação/condução. Prefere porta-enxertos pouco vigorosos. Maturação em época média.

Os vinhos obtidos são encorpados e complexos, por vezes com qualidade muito elevada, onde sobressai o aroma lembrando frutos pretos e flores silvestres. Elevada capacidade para o envelhecimento.

Clones certificados: 17 ISA, 18 ISA, 19 ISA, 20 ISA, 21 ISA, 22 ISA e 23 ISA; 16 JBP, 105 JBP e 112 JBP.

Distribuição