# Effects of time-variable water deficit, defoliation and crop load reduction on the juice and wine composition of Vitis vinifera L. cv. Solaris, analysed by FT-IR Spectroscopy and $^{1}$H-NMR Spectroscopy

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Dissertation to obtain the degree of

**European Master of Science in Viticulture and Enology**

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2019

## Acknowledgements

I would like to thank both of my supervisors, Professor Jorge M. Ricardo-da-Silva for his support and positivity, and Professor Torben Bo Toldam-Andersen for trusting me in the design of my own experiment, supervising my work and providing support, feedback and encouragement. Furthermore, I would like to say thank you to the team in the foodomics laboratory at the University of Copenhagen under Professor Søren B. Engelsen. Finally, special thanks go to Dr. Violetta Aru, who not only is a brilliant scientist but also did never get tired of my questions, was available for support even in her free time and pushed me to do the best work that I could.

## Abstract

Water deficit at different times during the phenological cycle was induced in pot-grown Vitis vinifera L. cv. Solaris while severe defoliation and severe crop load reduction was carried out in field grown Solaris plants. Juice and wine samples were obtained by all treatment groups and measured by Fourier Transform Infrared (FT-IR) Spectroscopy and Proton Nuclear Magnetic Resonance ($^1$H-NMR) Spectroscopy. Chemometric data analysis was performed by PCA on a variety of different data sets. Water stress timing did not alter the composition of juice and wine in Solaris compared to an irrigated control group, but pruning type showed an influence on composition. Single cane pruned vines showed a tendency to higher values in most examined parameters when compared to double cane vines. A clear sample distribution was visible among the field groups. Crop-thinning proved to be a reliable practice to increase sugar content and ripeness parameters while defoliation showed a decreasing effect on sugar accumulation and ripeness and an increasing effect on tartaric acid. $^1$H-NMR was found to be a useful tool to differentiate between the samples of the two experiments in both juice and wine and metabolites responsible for this sample distribution could clearly be identified by PCA. NMR based metabolomics are concluded to be a promising technique for future wine research.

*keywords: grapevine; water stress; defoliation; crop -thinning; $^1$H-NMR*

## Resumo

O déficit hídrico em diferentes tempos durante o ciclo fenológico foi induzido em *Vitis vinifera* L. cv Solaris cultivada em vaso, enquanto que a desfoliação severa e a redução da carga da colheita foram realizadas em plantas Solaris cultivadas no campo. Amostras de mosto ~~suco~~ e vinho foram obtidas para todos os grupos de tratamento e –analisadas por espectroscopia no infravermelho por transformada de Fourier (FT-IR) e espectroscopia de ressonância magnética nuclear de protão ($^1$H-RMN). A análise de dados quimiométricos foi realizada por PCA num conjunto~~s~~ de dados diferentes. O tempo de estresse hídrico não alterou a composição do mosto e do vinho no Solaris em comparação com o grupo controle irrigado, mas o tipo de poda mostrou influência na composição das vinhas podadas, mostrando uma tendência ~~a~~ para valores mais altos na maioria dos parâmetros examinados quando comparada às videiras com poda dupla . Uma distribuição clara das amostras foi visível entre os grupos do campo. A monda de cachos provou ser uma prática confiável para aumentar o teor de açúcar e os parâmetros de maturação, enquanto a desfoliação mostrou um efeito decrescente na acumulação do açúcar e na maturação e um efeito crescente no ácido tartárico. A RMN do protão foi uma ferramenta útil para diferenciar as amostras dos dois ensaios em mosto e vinho e os metabólitos que conduzem à distribuição dessa amostra puderam ser claramente identificados pelo PCA. Conclui-se que a metabolômica baseada em RMN é uma técnica promissora para futuras pesquisas em Enologia.

*keywords: videira; estresse hídrico ; desfolha; redução de carga de colheita; $^1$H-NMR*

## Resumo Alargado

Este estudo investigou a influência do estresse hídrico em *Vitis vinifera* L. cv. Solaris em diferentes períodos durante o ciclo fenológico em Taastrup, Dinamarca. A casta Solaris é uma variedade vigorosa de amadurecimento precoce, com aromas frutados intensos, resistente às doenças fúngicas mais relevantes na viticultura e que tem uma grande importância no relativamente novo país vinícola que é a Dinamarca. As videiras cultivadas em vaso em estufa experimentaram um intenso déficit hídrico durante o final da fase de crescimento dos bagos, pintor e maturação, respectivamente, enquanto um grupo controle foi irrigado o tempo todo. A segunda parte do ensaio foi feita numa vinha, em que foi realizada desfolha severa em dois grupos de tratamento e redução severa da carga de colheita em dois grupos de tratamento, resultando numa modalidade com desfolha, um grupo com monda de cachos e um grupo em que ambos os tratamentos foram aplicados e por fim um grupo de controle não tratado. As práticas vitícolas foram aplicadas antes do pintor. As uvas foram colhidas no início de setembro na estufa e no meio de setembro no campo. As amostras de mosto foram colhidas e analisadas por FT-IR, enquanto um segundo lote de mosto foi congelado para posterior análise por RMN de protão. O mesmo procedimento foi realizado com amostras de vinho. O FT-IR é comumente usado na pesquisa enológica e avalia uma gama de parâmetros importantes no mosto e no vinho de uma maneira facilmente comparável, enquanto a aplicação da metabolômica baseada em RMN na pesquisa do vinho é um campo relativamente novo cuja importância tem aumentado constantemente nos últimos anos . Além disso, e até onde sabemos, esta é a primeira vez que a metabolômica baseada em RMN tem sido usada para investigações sobre o estresse hídrico da videira. Os espectros das amostras de mosto e de vinho obtidos por RMN foram analisados extensivamente em várias combinações, mas devido à natureza complexa e vasta dos dados, foi decidido que uma seleção de metabólitos, ao contrário de todo o espectro ou regiões espectrais, seria a mais adequada para comparação de amostras. Assim, 13 metabólitos no mosto e 9 metabólitos no vinho foram identificados e a linha de base resolvida. A integração de seus sinais permitiu a comparação de concentrações relativas entre tratamentos. Uma combinação do conjunto de dados FT-IR e o conjunto de dados NMR criou uma nova matriz de dados para obter informações adicionais. A análise de dados quimiométricos foi realizada por PCA em amostras de ambos os ensaios. A separação com base no tipo de ensaio foi claramente visível no mosto e no vinho no biplot PCA, tanto na matriz de metabólitos, quanto na matriz de dados combinados de FT-IR e RMN. As amostras de estufa poderiam ser claramente separadas das amostras de campo com base em níveis mais altos de aminoácidos, pH mais alto e níveis mais baixos de ácido málico, ácido cítrico e acidez total. No que diz respeito aos ensaios propriamente ditos, o tempo de estresse hídrico não alterou a composição do mosto e do vinho da casta Solaris em comparação com o grupo controle irrigado. Os resultados do FT-IR revelaram tendências ligeiras, que devem ser interpretadas com cuidado, mas não foi possível separação entre diferentes tempos de estresse no gráfico PCA correspondente. O tipo de poda como fator mostrou influência na composição de videiras com poda simples, apresentando tendência a valores mais altos na maioria dos parâmetros examinados, quando comparado às videiras com poda dupla. Este foi o caso com o mosto e com o vinho.A severidade do estresse hídrico induzida nas videiras pode ser considerada suficiente para revelar possíveis diferenças, de modo que o motivo da falta de diferenças entre os grupos de tratamento possa se basear em outros fatores, como a variedade ou mesmo na falta de resposta da composição

do bago a janelas de oportunidade em matéria de déficit hídrico. No entanto, deve-se considerar que o material vegetal deste estudo foi um tanto diversificado em termos de tipo de poda e histórias de frutificação por causa de ensaios anteriores. Embora o desenho experimental tenha dividido essas diferenças igualmente entre os grupos de tratamento, estudos adicionais com material mais uniforme devem ser usados para testar esses resultados.Em contraste com o ensaio em estufa, uma distribuição clara da amostra foi visível entre os grupos de tratamento de campo. A monda de cachos provou ser uma prática confiável para aumentar o teor de açúcar e os parâmetros de maturação, enquanto a desfolha levou a níveis significativamente mais baixos de açúcares e densidade. O grupo controle mostrou valores dos parâmetros de maturação entre os dois grupos de tratamento, ilustrando bem os efeitos contrários de uma prática de redução de fonte como a desfolha e uma fonte de redução, como seja a redução de carga de colheita. Além disso, observou-se que o grupo em que os dois tratamentos foram aplicados apresentou níveis de açúcar semelhantes aos do grupo mondado, o que implica que o efeito da concentração por meio da redução da força do sink supera a redução da área foliar que assimila o açúcar. O ácido tartárico aumentou significativamente por desfolha, enquanto não foi observada uma redução no ácido málico por desfolha, que é freqüentemente relatada na literatura. O pH foi mais baixo nos tratamentos de desfolha e mais alto nos tratamentos de monda de cachos e entre esses dois para o tratamento misto, sugerindo um efeito crescente de desbaste de culturas, provavelmente por causa de um avanço na maturação em contraste com a desfolha, que geralmente tem um efeito de atraso na maturação. A prolina também demonstrou estar relacionada ao estado de maturação e aumentou a sua concentração com a monda de cachos. Em geral, a razão entre *sink and source* é muito complexa e, embora nem todos os efeitos esperados tenham sido observados (ácido málico), alguns dos resultados estão de acordo com a literatura publicada. É difícil quantificar o resultado da interação entre práticas de redução de *source*, como a desfolha e práticas de redução de *sink*, como a monda de cachos, mas há evidências de que em alguns aspectos, como por exemplo o teor de açúcares, o tratamento de monda de cachos tem muito mais impacto que a desfolha enquanto em outros aspectos, como o pH, a influência pode ser mais igualmente distribuída entre os tratamentos. As diferenças entre os grupos de tratamento são transportadas principalmente para as amostras de vinho, embora a fermentação tenha um leve efeito homogeneizador nos valores. Para o autor, o uso da metabolômica baseada em RMN para responder-a questões de índole vitivinícola é muito promissora para pesquisas futuras. Ele gera acesso a dados que podem não ser facilmente obtidos usando outros métodos. Embora origine trabalho adicional de pré-processamento de dados e ainda enfrente o desafio da interpretação de vários sinais espectrais para os metabólitos do vinho e do mosto, alguns ausentes, a metabolômica baseada em RMN tem o potencial de gerar informações que beneficiarão os domínios da viticultura e da enologia.

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# 1. Introduction

Viticulture, the science of the cultivation of grapevines, is a vast field of research, which spans over multiple scientific disciplines and is trying to answer and investigate highly complex, fundamental questions with big implications for wine and grape producers worldwide. Combining areas of physics, biology, geology, soil ecology and more, viticulture does not suffer from a shortage of hypothetical experimental designs to uncover new insights and add to the already big pool of knowledge. Research departments worldwide are actively pursuing new information in order to increase and ensure grapevine health, to improve wine quality and to minimize, negate or counteract various potential dangers to vines. The evolution of viticulture has produced an arsenal of various practices, targeting different issues in grape growing. Three of the most prominent topics in viticulture; water stress, defoliation and crop level, were included in this study to investigate their influence on the composition of juice and wine, which is the basis for quality and individual wine characteristics. Water stress is considered one of the most fundamental issues in modern viticulture and additional knowledge in that area would potentially improve irrigation schemes and precision water management. Furthermore, knowledge about the connection between water deficit and must or wine composition could enhance strategies to achieve a desired wine style. Defoliation as a technique of grapevine vigour management as well as canopy microclimate manipulation is widely used nowadays but additional insight into its effects on juice and wine composition could also promote a more precise viticultural approach in regard to winemaking. Lastly, crop reduction is a popular tool in viticulture to enhance grape quality, but the concept could also benefit from additional data regarding a variety of enological parameters. The present experiment is an attempt to expand the pool of knowledge on these topics in the context of the cultivar 'Solaris' in the relatively new winemaking country of Denmark. Even though the complex interactions between climate, geographical location, soil characteristics and other factors need to be considered whenever interpreting viticultural research this work aims at adding to existing and encouraging new research. Therefore, aim of the present study is to investigate 3 main hypotheses. First, does the timing of water deficit in regard to the phenological cycle manifest itself in differences in juice and wine composition depending on when the water stress occurred? Secondly, how do the practices of sink reduction through crop-thinning and source reduction through defoliation change juice and wine composition individually? Thirdly, how do these two practices counteract or complement each other concerning juice and wine composition?

# 2. Past research

## *2.1. Phenological Development*

In order to understand the applications of various treatments involved in viticultural research it is important to provide a short overview of the different phenological stages that the grapevine goes through over the span of a season. The major stages are often the ones used for reference when talking about the season and conveying viticultural information. *Bud burst* (sometimes referred to as *bud break*) is defined as the start of new growth by emergence of a new shoot from a formerly dormant winter bud in springtime. *Flowering* (*Bloom*, *Anthesis*) refers to the shedding of the calyptra that are covering the reproductive organs of the grapevine flowers and the consequent fertilization of flowers. *Veraison* (french vérer = to change) represents a change in berry colour, the softening of the berry tissue and the beginning of ripening by sugar accumulation. The time at which the berries are harvested is usually referred to as the stage of full maturity (Keller 2010, p.322). Besides these major stages there are two more stages within the cycle that are often used as reference times in studies in Section 2. *Fruit-set* describes the transition from flowers into the berries, the start of berry development (Centinari 2015).
*Pea-size* refers to a stage after fruit-set and before veraison where berries are approximately the size of peas while still being very hard, green and acidic. A detailed overview of all stages is provided in Fig. 1.

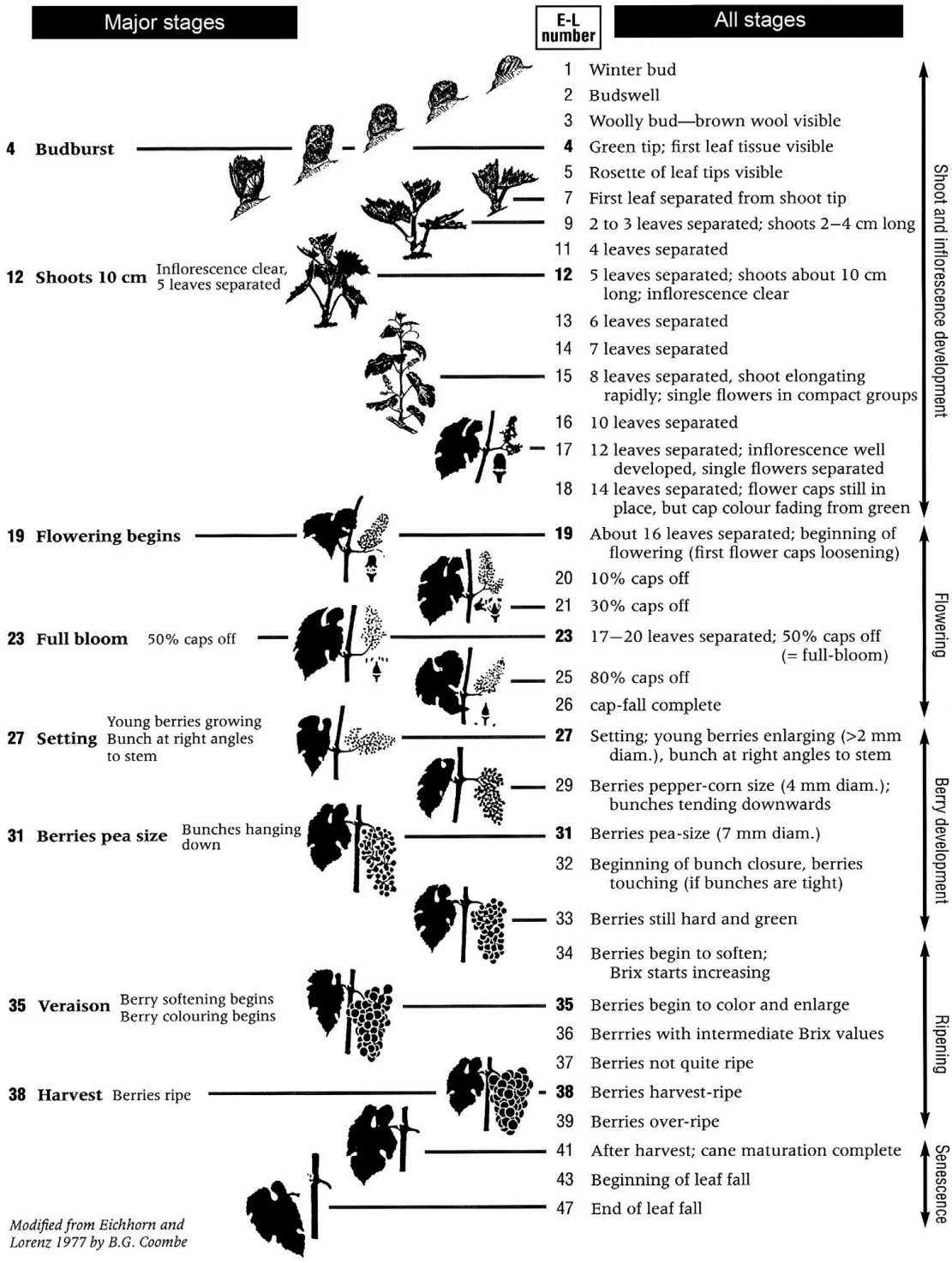


***Figure 1.*** *Overview of the stages during the phenological cycle of the grapevine (Coombe 1995)*

The development of the grape berry itself is also a complex process with a cascade of different metabolic activities within the plant, which change over the course of a season. It is commonly divided into three phases and during development during which they display modifications in size, composition, colour, texture, flavour and in pathogen susceptibility (Conde et al. 2007).

In the initial growth phase (stage 1) growth mainly occurs by cell division and several solutes are accumulated, with the most abundant ones being tartaric and malic acid and reach an apparent maximum after about 50-60 days after flowering. Hydroxycinnamic acids are also accumulated during this period as well as tannins, including monomeric catechin and epicatechin (Kennedy et al. 2001).

Most cultivars experience a lag-phase (stage 2) with a temporary stop in size increase and an accumulation of methoxypyrazines. This stage also corresponds to the end of herbaceous phase of the fruit development. The final stage and second growth phase are characterized by the onset of veraison and with it the beginning of ripening where berries become softer and start to increase in size again, now mostly by cell expansion. The most important metabolic activity in this phase is the import of fructose and glucose into the berry that shift the taste from acidic to sweet. Many aromatic compounds or their precursors are produced in this phase as well. Many of the compounds produced in the first growth phase remain unaltered on a per berry basis but are diluted through water and sugar influx. However, some compounds are degraded because of plant metabolism, such as malic acid, which is metabolized for energy during ripening, or environmental conditions such as pyrazines, which are thought to be degraded upon light exposure (Conde et al. 2007). An overview is provided in Fig.2.

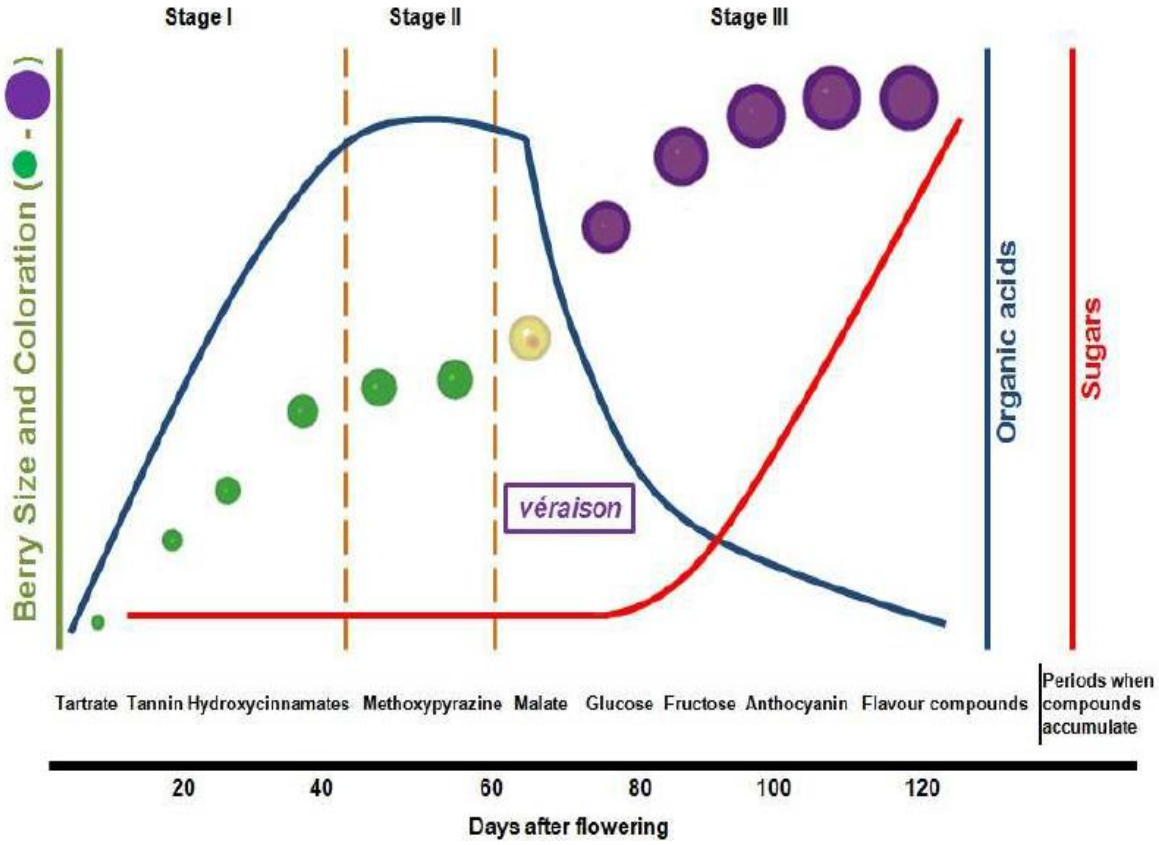


***Figure 2.*** *Berry development regarding size, colour and compounds accumulated as a function of time after flowering (Noronha 2010)*

## *2.2. Water Stress*

Because of its climatic dependence, the future of the grape and wine industry is closely linked to the present situation of climatic factors and their predicted changes in the future (Schultz 2016). The variation in environmental factors and the consequent variations in weather and grape growing conditions can lead to fluctuations in wine prices of up to 2000% from year to year, based on wines produced by the same winemaker on the same plot of land. Additionally, wines from the same year also vary highly in prices depending on the place, and therefore the (micro)climate, where they were grown (Ashenfelter and Storchmann 2016).

According to Williams and Matthews (1990) water shortage probably represents the most dominant environmental constraint within production areas and even in moderate temperate climates grapevines often face some degree of drought during the growing season (Sadras et al. 2012). Indeed, water influences various things like canopy development, vine microclimate, yield and fruit composition, so a lack thereof is considered the major limitation in plant growth and yield formation (Kramer and Boyer 1995). Some literature even considers water scarcity as influential on yield and quality as all other restraining factors combined and can impact all aspects of development, from flower initiation to final fruit size (Zarrouk et al. 2016).

In regard to future projections, it is assumed that the global climatic shift will increase air temperatures and change precipitation patterns but the latter are generally considered uncertain and very difficult to predict even though it is speculated that rainfall will increase in higher latitudes and decrease in lower latitudes. There is, however, evidence that leads to the assumption that potential evapotranspiration (the sum of water evaporating from the soil and water transpiring from plants) will increase as a result of bigger water holding capacity of the atmosphere. It is important to note however that this cannot be understood as a definite fact with definitely predictable consequences on the entire ecosystem since there are countless factors involved and global and regional trends do not necessarily follow the same pattern (European Commission 2009, Intergovernmental Panel on Climate Change [IPCC] 2013, Schultz 2016). Despite this complexity and unpredictability, some research predicts a general increase in water deficiencies in most grape-growing regions (Bates et al. 2008) and an increase in temperatures and severe dryness in specific regions like Portugal (Santos et al. 2019).

Water is seen as one of the most fundamental constituents of a functioning plant metabolism and is, together with carbon dioxide, the basis for every sort of growth and development. Naturally it is supplied by rain or snowfall and the portion that remains in the soil and it is not drained by gravity is temporarily stored for plant use. This available water is highly dependent on the type of soil and its water holding capacity, as well as the climatic conditions on site that determine how fast the water evaporates from the ground surface. The amount of rainfall varies greatly from region to region and from year to year (Keller 2015, p. 272-273). The physiological mechanisms that occur within the plant when experiencing water deficit are highly complex. Some of the characteristics are a decrease in the inner cell pressure (turgor), consequently a decrease in relative water content of the cell (percentage of water when compared to the amount of water at full turgor pressure) and a reduction in cell size. This means an increase of the concentrations of the solutes in the cell and lowering its osmotic potential. Water stress also directly impacts the opening and closing behaviour of the stomata (small openings on the leaf that regulate gas exchange). This is fundamental since closed stomata means a reduction in water loss by

transpiration but also a limited uptake of $CO_2$ as a carbon source for photosynthesis. Therefore, one major consequence of water stress is a reduction in growth, decrease in leaf water content and consequently a more porous canopy that directly influences other parameters like sun radiation impact, wind and temperature. Phytochemically, there are several mechanisms triggered by water stress, one important factor is the hormone abscisic acid which acts as a stress signal and is involved in the closure of stomata, inhibition of cell expansion and limiting shoot growth. But it is just one part of a complex stress response cascade that is based on changes in pressure within different plant compartments, alterations in pH and changes in concentrations and uptake and exchange rates of minerals (Keller 2015, p. 276-279).

Enological and viticultural research has laid a big emphasis on the study of consequences of water deficit on grapevines, bridging the gap between enology and viticulture, ranging from investigations on plant physiology (Bertamini et al. 2006, Castellarin et al., 2007, Costa et al. 2012) to chemical composition of berries, must and wine (Esteban et al. 1999, Roby et al. 2004, Bouzas-Cid et al. 2018) to evaluating water stress from a sensorial perspective (Matthews et al. 1990, Chapman et al. 2005, Bonada et al. 2015***)***. Other water deficit research is aimed to explore the physiological thresholds and optimize viticultural practices such as regulated deficit irrigation (Romero et al. 2010) or partial root-zone drying (Pedreira 2005). Based on the fact that water stress reduces carbon uptake and changes carbon metabolism (Palliotti 2009, McDowell 2011) it is of high interest how this influences compounds relevant for enology and plenty of research has been carried out over the last few years.

Etchebarne et al. (2010) investigated the influence of various levels of water availability and leaf:fruit ratios on berry composition in 'Grenache' at several stages during the growing season. The authors confirmed water availability as a more crucial driver for changes in berry composition as opposed to numbers of leaves per primary shoot (with one cluster per shoot). At harvest, berries from well-irrigated vines showed significantly increased total dry matter and lead to a faster sugar loading rate during ripening. Berries from water-deficit vines showed lower levels of sugars and cations. Furthermore, trends emerged in irrigated vines of increased levels of titratable acidity, tartaric acid and changes in mineral composition although these differences showed no significance. Interestingly, no effect of irrigation on pH level was observed even though it is sometimes found in similar studies, like Intrigliolo and Castel (2010). In this study the authors conducted a 2-year study with 'Tempranillo' where they looked at different water stress treatments and various crop-levels per vine in regard to their impact on berry and wine composition. Two deficit irrigation regimes were carried out: one mild stress treatment over the entire season and one severe stress treatment applied at veraison and compared to a non-irrigated treatment. In one of the two seasons irrigation had a big impact on wine composition where irrigation significantly increased pH and potassium content and decreased total titratable acidity. The increased water availability lead to an increase uptake of potassium and then consequently caused a decrease in tartaric acid through more precipitation as bitartrate potassium salts besides dilution effects through increased berry size, which were more pronounced than in the second year because of lower yield levels. However, there were no differences between the two irrigation regimes themselves. The other season with higher yield and lower dilution effects, showed increased levels of malic acid in wines from irrigated vines, again probably based on the fact that water-deficit and lower mineral influx can lead to reduced vigour and vegetative growth (Keller 2015 p. 276-279)**,** which then means reduced canopy

size, reduced internal shading effects and consequently higher degradation effects of malic acid through higher sun exposure and canopy temperature. The increase in pH by irrigation was consistent across both seasons. The tendency to increased levels of pH by irrigation was also found by Freeman and Kliewer (1983) in a study on 'Carignan' vines in California. They also observed significantly higher levels of arginine and proline. This is in agreement with the hypothesis that higher water availability leads to an increase in the very mobile nutrients like potassium and nitrogen which are then visible as increased pH (by the same mechanisms as described above) and higher levels of amino acids, who have nitrogen as an important component. However, the difference in pH was only observed in one out of 3 seasons and the accompanying decrease in titratable acidity that was seen in the study by Intrigliolo and Castel (2010) above was not observed at all. Concentrations of soluble solids and levels of anthocyanins in berry skins and wine were lowered by irrigation. Irrigated vines experienced a delay in ripening but did not show an effect on titratable acidity. The authors also compared different treatment types (K fertilization, irrigation and crop level) but declared water availability as the most influential factor in the changes observed in the study. In contrast, Esteban et al. (1999) found significantly decreased pH levels in irrigated 'Tempranillo' vines in 2 out of 3 seasons as well as significantly higher levels of titratable acidity in all 3 seasons, mainly caused by higher levels of malic acid. Furthermore, irrigation led to a higher yield and higher berry weight in all three years but also to significantly higher °Brix values in two out of three seasons, implying that dilution effects of increased berry size through irrigation can be counteracted by an elevation of metabolic activity rates. This study was carried out in a warm climate (Spain), with very little precipitation (33 mm – 124 mm of effective rainfall over 3 seasons) so irrigation did not cause a delay in ripening but instead accelerated it in 2 out of 3 seasons even despite an increase in vegetative growth (as indicated by increased pruning weight). This additional vegetative growth increased canopy density (as indicated by higher leaf-area-index) and lowered internal canopy temperature and lowered malic acid degradation. Combined with a shorter ripening period that leaves less time for the degradation of organic acids could explain the higher titratable acidity and lower pH levels in irrigated vines, somewhat countering dilution effects. This study also concluded that differences in various wine parameters were positively correlated to the severity of water stress. Since the results regarding pH are in contradiction to the findings of Intrigliolo and Castel (2010) despite a similar climate and the same variety, the authors argued that environmental factors and soil characteristics could be the reason for differing conclusions. In the study of Intrigliolo and Castel (2010) nitrogen and potassium fertilizers (among others) were applied, which could lead to increased potassium and increase precipitations of tartrates. The study of Esteban et al. (1999) does not mentioned fertilization. It seems clear from the research laid out above that there are processes at play in regard to water deficit that impact various factors like yield, vegetative mass, photosynthesis, rate of ripening, nutrient uptake and many more. Furthermore, climatic conditions and soil characteristics seem to play a role too. This highly complex system apparently can produce results in either direction with higher and lower values for specific parameters like pH or acidity. Even though it is complicated, it is crucial to consider as many factors as possible when attempting an interpretation of the observed results. Nadal and Arola (1995) also observed the aforementioned increase in titratable acidity in wines and musts from irrigated 'Cabernet Sauvignon' plants as well as higher levels of malic acid. Furthermore, irrigation increased potassium in musts and levels of total nitrogen in wines, possibly again by an increased uptake through

additional water in the soil. Values for anthocyanins and tannins were found to be lower in irrigated plants although the authors speculated that this result was mainly caused by one single rain event before harvest and could not be reproduced in subsequent years. The issue of a lack of consistency across seasons when it comes to results is a common thread across many studies of this kind. Another example is a study by Wenter et al. (2018) who also investigated the influence of water stress on berry composition in 'Sauvignon blanc' in a mountain environment. Even though an increase of titratable acidity and °Brix was found in irrigated vines when compared to non-irrigated ones, the results were only significant in one out of two seasons, probably because the climatic conditions and the dynamics along the phenological cycle varied substantially between the two years. One year was much drier and received only half of the cumulative rainfall than the other season and showed higher temperatures. Consequently, the non-irrigated treatment showed stronger signs of water stress in the drier season (lower pre-dawn leaf water potential and lower soil water content) as opposed to the non-irrigated group of the other season. Additionally, a severe drought in the cooler year during flowering heavily reduced berries per cluster in the non-irrigated group. Vegetative parameters like pruning weight and yield were lower and stronger compromised by the dry season, as well as berries per cluster and weight per berry. In the cooler, wetter season the non-irrigated vines accumulated sugar slower but showed significantly higher °Brix at harvest. In the drier season, however, the fully irrigated control group showed the highest level of sugars. This might be explained by leaf transpiration and assimilation rates having been severely suppressed in the non-irrigated vines. The non-irrigated vines in general showed lower levels of titratable acidity. Interestingly, in the cooler season there was a significant difference between the values even though pruning yield per vine (as an indicator for vegetative growth) showed no differences so the hypothesis of slower malate degradation because of lower canopy temperature as a result of stronger vegetative growth is questionable in this context. Similarly, Bouzas-Cid et al. (2018) found big variability over the three consecutive seasons in a study on 'Treixadura' in the warm climate of Spain. Must and wines produced from irrigated plants tended to be higher in acidity than from rain-fed vines. Additionally, irrigation generally seemed to lower pH and in one of three seasons significantly increased proline content and decreased tryptophan content, in another year irrigation significantly decreased cysteine and phenylalanine content. The authors also measured an extensive list of other amino acids that didn’t show any correlations to treatments and an extensive list of volatile compounds. Only two (geraniol and alpha-terpineol) showed significant differences between treatments but only in one season. Almost all of them showed significant differences between seasons, however. In yet another study, when comparing different irrigation timings and regimes across five seasons on 'Tempranillo' in a warm, dry region of Spain, Intrigliolo and Castel (2009) found large differences in the response to water stress but none were consistent across all seasons. They compared pre- and post-veraison irrigation regimes and saw an increase in yield caused by bigger berries through irrigation but no differences among irrigation timings. The ratio of source (total leaf area) and sink (yield) was not influenced by irrigation regimes, probably because higher yield was met with increased vegetative growth. There was however a big difference between seasons and a significant influence of the treatment x year interaction, suggesting that irrigation will have different influence depending on the season with high yielding vintages benefiting more from additional water supply. Once again however, a trend of increased pH levels through irrigation was observed, even though the differences were only significant in one season and again independent

of the timing of irrigation. Furthermore, irrigation seemed to increase malic acid and decreased tartaric acid but with no influence of the application timing. Post-veraison irrigation increased must sugar content, probably because of a higher assimilation rate during the ripening period while pre-veraison irrigation showed a trend of increased phenolic substances. Zarrouk et al. (2016) investigated the influence and interaction between water deficit and elevated temperatures on berry metabolites in 'Tempranillo' in South Portugal. They concluded that water deficit has a bigger overall impact on berry ripening, but temperature has also a fine-tuning effect in interaction with water stress, especially if the effects of deficit irrigation are desired. The authors also found that the observed negative effects of water stress and high temperatures on anthocyanins result from decreased biosynthesis during the onset of ripening and from degradation in later stages.

From the studies mentioned above it is obvious that the variability caused by the factor *season* has a big impact on the overall results and is limiting the ability to draw meaningful conclusions. This is caused by the high complexity of a vineyard, which arises from the countless interactions between soil ecology, climatic conditions and plant physiology. It is hard or near impossible to control for all of these factors, but one approach to reduce the overall complexity is to conduct studies with potted grapevines in a greenhouse environment. There, several factors, such as nutrient availability, irrigation and wind can be controlled, and the complexity of the soil matrix is reduced by a predetermined volume of soil in each pot with a known composition. Sivilotti et al. (2005) investigated the effect of water stress on berry composition in potted 'Merlot' grapevines but in contrast to the research mentioned before found no differences in pH, sugars or titratable acidity between the treatments, once again supporting the hypothesis that field trials can produce radically different results based on factors that can be hard to identify. Especially studies that focus on different timings of water stress during the phenological grapevine cycle, which is the basis for one part of the presented work, greatly benefit from a closed experimental set-up where precipitation will not disturb and blur results. Hardie and Considine (1976) conducted an experiment on container-grown 'Cabernet franc' plants where water stress was induced at different phenological stages. All stress treatments produced lower yield than the irrigated control but for different reasons. Stress nears flowering caused reduced fruit-set and desiccation of clusters or parts of clusters, whereas stress near veraison lead to smaller berry size and consequently lower yield. All stress treatments experienced delayed maturing and fruit from vines that were stressed post-veraison during ripening showed significantly lower sugar levels. The authors argue that this is solely based on reduced photosynthetic activity due to reduced stomatal $CO_2$ uptake during the highest sugar accumulation period. Furthermore, the delay in ripening seems to partially be caused by the diversion of available carbohydrates into the growth of lateral shoots and berries during the lag-phase seem to be most sensitive to delayed ripening induced by stress. Levels of titratable acidity and pH were generally higher in stressed berries, but the author specifically noted that they believed these differences can be explained by differences in maturity alone and that there is no evidence for an additional effect of water stress on acidity or soluble solids content. Another advantage of an experiment in a closed-system (greenhouse) environment is the possibility to determine the severity of water stress regardless of climatic conditions. For this experiment the phases of water stress were quite severe with the hope of it producing clear results.

At the same time studies conducted in vineyards are much more realistic in terms of the conditions of commercial grape growing. In order to reconcile these two fundamental facts, the present work combines a greenhouse trial and a field trial to obtain accurate results with as much factors controlled as possible while simultaneously studying the impacts of other factors under realistic conditions. The manipulations in the field, defoliation and crop thinning, were also applied very extensively, arguably higher than what is seen in most commercial vineyards, in order to visualize effects more easily, if they are present.

## *2.3. Defoliation*

The productivity of a grapevine is ultimately dependent on the photosynthetic capacity of the plant's canopy, which is defined as all aboveground parts of the vine. Because photosynthesis of green tissue other than leaves, namely berries and shoots, is minor and never exceeds respiration, leaves are the main location of effective photosynthesis and their activity and overall state largely determines growth, yield and fruit composition (Keller 2015, p.163). In general, young leaves produce more organic acids, while mature leaves produce more sugars (Hunter et al. 1990), which is important because photosynthesis is thought to be internally partially regulated by the composition of carbohydrates in the mesophyll (Correira et al. 1990, Krapp and Stitt 1995).

Leaves are the source of all organic compounds generated by photosynthesis, so it is of interest how their reduction will manifest in changes of photosynthetic rates, assimilate allocation patterns and chemical composition of grapes. Defoliation can lead to an increase in photosynthetic activity in remaining leaves; the level of increase depends on the severity of defoliation and the location of leaf removal. Furthermore, assimilate allocation can be redirected to the defoliated zone and an increase in assimilate flux has also been observed during midday hours, a time where the normal transport rate in non-defoliated vines usually almost ceases (Chanishvili et al. 2005).

Another aspect that is directly tied to defoliation is the increased exposure to UV-radiation by the sun. Its destructive nature will trigger several different mechanisms in the grape berry, the sunlight induced increase in temperature will influence enzymatic activities and it has been investigated how these alterations will reflect back on must and wine composition (Bergqvist et al. 2001, Spayd et al. 2002, Martinez-Lüscher et al. 2013).

The concept of full or partial defoliation, meaning the removal of leaves in the fruit bearing zone of the canopy, serves a multitude of purposes. Facilitating mechanization, improving micro-climate to regulate disease pressure (Smart 1985, Staff et al. 1997, Pierri and Fermaud 2005) as well as influence yield components, grape composition (Poni et al. 2006, Intrieri et al. 2008) and wine aromatics (Roberts et al. 2007, Vilanova et al. 2012, Hernandez-Orte et al. 2014).

Much research was done on the effects of defoliation all across the viticultural world. Baiano et al. (2015) compared three different types of defoliation with varying orientations with 'Nero di Troia' vines. Removal of 75% of leaves from the east side of the canopy, 75% from the east and the west side and 100% from both sides in 2 sequential steps were compared to a non-defoliated control. The defoliation drastically increased the flux of photosynthetic active radiation available for bunches (PPF, µmol/m$^2$/s) on the respective side of the canopy, besides increased berry temperature. The treatments were carried out at

full veraison and the second step of the 100% treatment 2 weeks before harvest. In must, the vines from the most severe defoliation (100%) showed the highest °Brix value, according to the authors through concentration of the grape juice, possibly by increased water loss. Another explanation might be that remaining leaves after partial defoliation can significantly increase their photosynthetic activity (Hunter and Visser 1988) and all leaves received significantly higher levels of photosynthetic active radiation.
The non-defoliated control and the treatment with 75% removal on both sides showed the highest values for titratable acidity in must. 100% defoliation and east-side defoliation lowered values for titratable acidity, malic and tartaric acid. This is somewhat reasonable, since according to Valdivia (2001) greater exposure of bunches to radiation increases cell respiration and leads to greater consumption of organic acids, and the two groups with the highest acidity received the smallest amount of radiation. The fact that radiation was lower in the east-west defoliation treatment than the east-only defoliation can be speculated to be caused by reflection effects or the fact that morning sun is more intense. Defoliation did not influence anthocyanin synthesis, which is reported to be suppressed between 30-35 °C in some varieties (Kliewer and Torres 1972, Spayd et al. 2004***)*** so it stands to reason that 'Nero di Troia' is more tolerable in that regard since measured berry temperatures surpassed this range. However, defoliation decreased the total phenolic content in the skins. This is surprising, since the authors state that lower-light conditions are known to decrease skin weight and skin to berry ratio and the non-defoliated vines showed the lowest skin dry matter percentages. It could be speculated that the high berry temperature in defoliated vines did somehow negatively influence pathways that produce phenolic compounds. In wines, the alcohol content followed the same trend as sugar levels in must, with the non-defoliated vines showing the lowest levels and it increased with severity of defoliation. Therefore, it could be reasoned that the hypothesis of increased cell respiration through elevated berry temperature induces water loss and leads to a concentration effect that balances the effect of reduced leaf: fruit ratio. Hunter et al. (1991) also compared different levels (33% and 66% of all leaves on all shoots removed) of defoliation, initiated at different times during the phenological cycle on ‘Cabernet Sauvignon’ vines in Stellenbosch, South Africa. Defoliation was done around bud break, berry set, pea-size stage and veraison. Concerning total soluble solids (TSS), selected significances were found, 33% defoliation at berry set and 66% defoliation at bud break and veraison lead to significant higher sugar levels but no overall significant effect of defoliation or timing could be established. Apart from the mechanisms described above, remobilization of stored of carbohydrates and an increased sink strength (Kliewer and Antcliff 1970, Kliewer and Bledsoe 1987) are also listed as possible reasons for increased TSS in some cases of defoliation. Total titratable acidity (TTA) was slightly higher in defoliated vines, but TTA in vines defoliated at veraison was significantly lower. This contrasts with other research suggesting that defoliation should lead to lower TTA and shaded conditions to higher TTA (Kliewer and Lider 1970, Wolf et al. 1986). The authors of this study argue that this might be explained by an increased lateral shoot growth (Hunter and Visser 1989) that produce more young, immature leaves who synthesize more organic acids and if this effect was induced too late in the veraison treatments this could lead to the lower values. But it is also emphasized that the relationship between TTA and the amount of young leaves is still speculative. It is still plausible since, according to the authors, South African vineyards often suffer from an excess in vegetative growth. No difference in pH were found among treatments and also no significant differences of malic or tartaric acid so the significantly lower TTA in vines defoliated at veraison is considered to be

based on other acids not measured in this study. Concerning organic acids, Bubola et al. (2012) investigated the changes in sugars, phenolic and organic acids according to different defoliation regimes in the variety 'Istrian Malvasia' in Istria, Croatia. Defoliation was carried out before flowering, at berry-set and veraison. Similarly to the study above, they found significantly higher levels of TSS in grapes from vines that experienced defoliation at veraison, presumably because of sudden exposure to sunlight and subsequent increased water loss through transpiration. Neither TTA nor pH differed significantly among treatments. However, significantly higher levels of tartaric acid were found in the non-defoliated control and in the group defoliated at veraison. This is in contrast to other research that suggests that early leaf removal increases the levels of tartaric acid (Poni et al. 2006) and is based on the hypothesis that early increase in light and temperature can lead to higher synthesis of tartaric acid (Tardaguila et al. 2010). One explanation for differing results might be a genotype-based difference in canopy porosity, a difference in severity of defoliation between studies as well as a big discrepancy in climate and temperature between the experimental sites of studies. Depending on sun radiation and air temperature defoliation can elevate canopy temperature from a limiting to an optimal or from an optimal to an excessively hot and therefore yet again limiting level. Moreover, in contrast to other studies (Poni et al. 2006, Tardaguila et al. 2008***)*** levels of malic acid were significantly higher in early defoliated vines than in vines defoliated at veraison. This is surprising since malic acid is more prone to degradation by increased temperatures (Ruffner 1982) but the authors argued that early defoliation lead to enhanced lateral growth and consequently re-establishing a similar microclimate in the canopy as the control group. Several hydroxycinnamic acids were analysed and all of them showed significantly lower values in the group with defoliation before flowering while berries from vines defoliated at berry-set showed the highest levels of most phenolic acids. Since sunlight exposure is crucial for synthesis of phenolic compounds (Downey et al. 2006) it can be argued that light exposure in the early phases of berry development can enhance phenolic acid synthesis. The trend of differences in juice values carried over into the wine samples but the significances disappeared, probably due to standardization through controlled winemaking (inoculation with specific yeasts, controlled temperature, etc.).

Uriarte et al. (2012) conducted leaf removal experiments with 'Tempranillo' in East-Spain and West-Spain and observed very different results depending on the specific geographical location. Yield was reduced in one case (East) but not in the other one. They also found contradicting results in relationship with pH values from both experiments, observing increasing and decreasing tendencies in defoliated vines. In East-Spain defoliation reduced total leaf area significantly and reduced fruit-set but because of the even more pronounced reduction in yield led to a higher leaf:fruit ratio. This led to a concentration effect as displayed by generally higher levels of sugars, total polyphenols, anthocyanin and tannins. The differences were mostly only significant in one out of two years and mostly only present in the earlier defoliation timings (pre-veraison). Higher levels of tartaric acid were significant in both seasons and in all defoliation timings, supporting the hypothesis mentioned in the study of Palliotti et al. (2012). Malic acid levels were lowered but only the pre-veraison treatments. In the experiment in West-Spain, yield and fruit-set increased through defoliation which is in contrast to most research presented here. There was only one defoliation timing and it was carried out much less severely than in East-Spain. This also resulted in a similar total leaf area as the non-defoliated control group, possibly by compensation through promoted lateral shoot growth. However, leaf:fruit ratio still increased significantly in defoliated vines.

West-Spain showed contradicting results to East-Spain with slight tendencies of increased levels of malic acid, titratable acidity and decreased tannin concentration. None of these results was significant, however. The differing experimental conditions, however, make it impossible to attribute these differences in results solely to geographical location but rather the big differences in severity of defoliation (37% reduction of TLA in West-Spain, as opposed to 60-96%, dependent on defoliation timing, in East-Spain) could be an important factor for the lack of differences between treatments in West-Spain. However, the increased leaf:fruit ratio in West-Spain would suggest a concentration effect but none was observed. This highlights the difficulty of comparing geographic locations when establishing different experimental conditions.

The influence of defoliation on aroma compounds was also subject to research in the past (Diago et al. 2012, Ponti et al. 2013, Risco et al. 2014). Morano et al. (2016) investigated the influence of early leaf defoliation on the volatile composition of wines. The study was conducted over 3 years in a warm climate on 'Tempranillo' vines. Defoliation was defined as the removal of the first seven basal leaves before flowering and compared to a non-defoliated control. The leaf area removed (LAR) was measured electronically and ranged from 36-50% of the total leaf area (TLA). Furthermore, a clear vintage effect was demonstrated, with the greatest effect of defoliation occurring in the hottest and driest vintage. Defoliation significantly reduced yield in two out of three seasons. In all years, defoliation produced an acceleration in ripening of around 10-13 days. In the hottest and driest year, defoliation increased levels of alcohols, C6 alcohols, acetates, esters and volatile acids but it has to be noted that in that year the control group was affected by noble rot *Botrytis.* In the years where botrytis was absent defoliation significantly increased acetates, carbonyl compounds and lactones in the second year and lactones, volatile acids, acetates, carbonyl compounds and alcohols in the third year. The level of increase of individual aroma compounds was highest in the first year where climatic conditions were marginally drier and warmer and leaf removal was done in a less severe fashion than in successive years. The authors concluded that the vintage effect was more influential on volatile compounds than the treatment effect, even though early defoliation reliably changed the aromatic composition.

This can be related to the findings of Tardaguila et al. (2008) who found significantly higher aroma complexity, mouthfeel and general sensorial preference in 'Grenache' wines from early-defoliated vines compared to the non-defoliated ones and to vines defoliated at veraison. Early defoliation also significantly increased colour intensity, fruitiness, tannin quality and defoliation in general decreased vegetal characteristics. Yield components, including total leaf area and total leaf area: yield ratio, were unaffected by defoliation treatments, according to the authors probably due to the low severity of defoliation or by the yield-reduction effect, which defoliation can sometimes also elicit, as outlined by the studies above. The same study, however, did not find any differences in enological parameters such as alcohol content, pH, titratable acidity or tartaric acid. Only malic acid was significantly reduced by early defoliation. The authors did not provide an explanation for the lack of differences in various yield and enological parameters but it can be speculated that it is based on the complex variety-soil-climate matrix or, as stated above, the low level of defoliation, which would go in accordance with the lack of a difference in total leaf area and related parameters. Palliotti et al. (2012) also investigated the effects of defoliation on aromatics and flavour characteristics, besides a range of enological and viticultural parameters. Defoliation pre-flowering was compared to a non-defoliated control on a high-yielding Italian

cultivar called 'Ciliegiolo'. One of the main goals of the researchers was to limit yield to the legal limit of t/ha through early source limitation. This was clearly achieved with significantly lower levels of yield, less berries per cluster, and lower weight per berry in the defoliated treatment. Must composition did not differ between treatments except for higher levels of total soluble solids (TTS) and consequently higher alcohol levels in wines, besides higher levels of anthocyanins, total phenolic content and colour intensity in defoliated vines. So overall, a concentration effect was observed. However, besides stating that the defoliation was carried out severely, the authors did not provide information on total leaf area or the fruit: leaf ratio of either treatment. Therefore, it is difficult to assess the balance of the counter-acting mechanisms of leaf, and therefore source, limitation and fruit-set, and therefore sink limitation. The concentration effect would point to the reduction in yield as the more dominant factor in this particular case and the authors argue that the post-defoliation balance of source-to-sink came out positively due to enhanced foliage efficiency. Interestingly, the increase in TTS was not paralleled by an increase in must pH and a decrease in total titratable acidity, which is assumed to be due to a higher tartaric acid synthesis through increased sunlight exposure based on research by Kliewer and Schultz (1964). A decrease in rot infections was also observed through better wind canopy penetration and consequently lower levels of internal canopy moisture. Regardless of the mechanisms, wines produced from defoliated vines showed enhanced aromatic and flavour characteristics. Additionally, the wines were more suitable for aging, indicated by higher flavonoid content. On the topic of aromatics, defoliation research also addresses very specific but important questions, for example the sensorial characteristic of certain ‘Sauvignon blanc’ wines. Arnold and Bledsoe (1990) investigated vegetal and fruity aromas in ‘Sauvignon blanc’ wines in relationship with leaf removal treatments. Three different defoliation severities were carried out at three different timings during the season and compared to a non-defoliated control. Descriptive sensorial analysis of two specific vegetal aromas and two fruity aromas was done. Vegetal flavour in the mouth was also evaluated. Defoliation was able to highly and significantly reduce vegetal aromas and vegetal flavour but only if carried out at the most severe of the three levels and only if applied at the two earlier treatment timings. The late treatment did not show the same efficiency in reducing the aromas. Therefore, it stands to reasons that severity and timing both are crucial factors to achieve the desired outcome. Fruity aromas were not significantly changed by defoliation.

Some viticultural questions, more specifically questions of defoliation effect on the storage of carbohydrate reserves in trunk and roots and its implications for the following season, were investigated by Bennet et al. (2005). 'Chardonnay' vines were severely defoliated (all but four basal leaves were removed) in monthly intervals, starting four weeks after flowering and compared to a non-defoliated control group. Defoliation caused a highly significant reduction in carbon reserves stored in the trunk and the roots. This came with big implications for the following season, where the number of inflorescences per shoot and the number of flowers per inflorescences were reduced by up to 50%, compared to the control group. Consequently, yield, shoot growth and total pruning weight all were reduced. The authors tried to highlight the negative implications in terms of fertility and productivity that can arise from severe defoliation practices based on a lower starch reserve accumulation through severe reduction of leaves as carbon assimilation sources. This is especially of importance in cooler wine growing regions with no post-harvest carbon accumulation period, for example in New Zealand, where the study was done.

## *2.4. Crop-Thinning*

Yield is a crucial factor in viticulture, since it directly represents the amount of product a grower obtains from a given agricultural site. However, yield as a total parameter is not a reliable indicator of quality even if there persists a notion of an inverse relationship (Currle et al. 1983). There is research that either found no correlation (Keller et al. 2005, Bowen et al. 2005) or even tendencies of increased quality through increase yield (Chapman et al. 2004). Some research suggests that climatic conditions, vineyard characteristics and other factors are much more important in determining fruit composition and wine quality (Ought and Nagaoka 1984, Bowen et al. 2011)**.** However, the yield per individual plant, often termed crop load, is an important variable since it is a fundamental factor in the vine's sink-to-source ratio, so theoretically a reduced sink strength would lead one to believe that more assimilates are available for the remaining sinks. But this always has to be considered in context and it is important to consider the reason for lower crop load per vine. For example, higher planting distance between vines usually lead to higher yield per plant but alongside that vine vigour and leaf area also increases so the sink: source ratio and fruit composition could potentially remain unaltered (Winkler 1969). Higher planting density is often associated with lower yield per plant and higher titratable acidity and juice pH, but more based on shoot competition for sunlight between vines rather than based on individual crop load (Falcetti and Scienza 1989). As stated in Section 2.2 and Section 2.3 both water deficit and defoliation can lead to lower yield levels but since mineral uptake, which is related to water availability, and sunlight exposure and temperature, which are related to defoliation, all can influence fruit composition. It is important to take all these factors into context when investigating crop load as a basis for differences in various viticultural and enological parameters. In the case of water, it can be argued that is has a far superior effect on fruit quality than crop load itself, where excess amounts of it can decrease quality even in situations where crop thinning was applied to reduce yield while a moderate deficit has been shown to even raise quality in high-yielding plants (Bravdo et al. 1985). Despite other factors, crop reduction can still have impact on a vine's performance. Balanced vigour is a term describing a good relationship between vegetative and reproductive growth, a good balance between source and sink locations where ripening of fruit occurs at a desired pace and time while there is still sufficient development in terms of leaf area and shoot growth. Full ripening of 1kg of fruit is estimated to need from 1 - 1.5 $m^2$ of leaf area but this is also dependent on the trellis system (Keller 2015, p.262). An excessive level of fruit in regards to the leaf area (overcropping) would mean an inability for the source locations to provide sufficient assimilates to all sink locations and this can reflect back in a delay in ripening, besides decreased levels of colour pigments, pH, sugars and amino acids (Miller and Howell 1998; Intrieri et al. 2001). These mechanisms can sometimes be desirable when higher levels of acidity, lower sugar levels or lower pH is preferred, for example with white wine in a warm area or in the production of sparkling wine. More commonly however, crop-thinning is performed in order to obtain the effect of increased levels of sugars and amino acids by lowering the sink:source ratio (Santesteban and Royo 2006, Keller et al. 2008**).** However, if yield per vine is too low (undercropping) then it is also possible that there is an excessive amount of vegetative growth, which can have a detrimental effect on quality through shading and dense canopies. Sometimes overcropping or undercropping can also be results of other factors such as inadequate pruning techniques or pest and diseases that damage leaves and lower photosynthetic activity, consequently reducing source strength. Since overcropped vines

usually show a delay and a slower rate of ripening, crop-thinning is usually carried out to accelerate ripening by reducing crop load, besides potential concentration effects mentioned above. In certain seasonal conditions this is not necessary if a delayed ripening period can be met with a later harvest, but since later harvest dates usually mean higher risk of rain events and disease pressure, delayed ripening is usually undesirable (Keller 2015, p.262-264). Since a vineyard is such a complex ecosystem research does not always find the expected results that would be proposed by these general guidelines. In general, there are two main types of direct crop reduction techniques, which were compared in 'Syrah' by Gil et al. (2013). Cluster-thinning is defined by removing entire clusters from the vine, often times the distal one(s), if there are more than one on a shoot. Berry-thinning consists of removing the tips of clusters, leaving the shoulder part that is nearer to the rachis, because some research suggests that this part ripens earlier and better (Tarter and Keuter 2005, Figueiredo-González et al. 2012). Berry-thinning was applied around when berries were around pea-size and cluster thinning was applied mid-veraison by removing 50% of clusters from each vine. One control group was left untreated. Yield was reduced slightly in the berry thinning treatment (22%) and significantly (43%) in the cluster-thinning treatment. Berry weight and berry volume increased in both treatments, but again, only cluster-thinning produced significant differences. Both treatments advanced maturity and berry-thinning also decreased the heterogeneity of maturity between the individual berries within a cluster. This increased maturity was also observed in a higher value of total soluble solids, higher levels of pH and lower total titratable acidity and slightly elevated grape juice density. The differences carried over into wines produced from the grapes of the three different treatments, although the differences became smaller and some significances disappeared, according to the authors arguably because of the same yeast and fermentation conditions used and because of the very young age of the wines. Cluster-thinning also lead to the highest anthocyanin concentration in wines and berry-thinning showed the highest value for total phenolic index. Additionally, differences were observed in a big spectrum of various kinds of anthocyanins, phenolic acids and non-flavonoid compounds. A trained panel of tasters were able to distinguish the wines from both treatments from the control group more than 50% of the time, but not able distinguish between treatments successfully. In summary, cluster- and berry-thinning can both be useful tools to reduce yield and enhance maturity and quality, with an economic disadvantage towards cluster-thinning because of a greater reduction in yield.

Palliotti and Cartechini (1998) compared three different thinning levels (0%, 20% and 40%) on three red cultivars in Central Italy for three consecutive seasons. Thinning was carried out by removal of distal clusters along the shoot at veraison. Yield was only significantly reduced with the most severe thinning level (40%) in all cultivars in two out three seasons because there was a compensatory effect through significantly increased cluster and berry weight induced by crop reduction, which normally is the desired crop-thinning effect: unchanged yield levels with simultaneously increased berry quality. This indicates that yield regulation by crop-thinning is highly dependent one the season. The two seasons with lower yield were characterized by low amount of degree days and heavy rain in summer, so in the third season, which was warmer, the effects of increased cluster and berry weight even compensated for the most severe thinning treatment. All cultivars showed the same significance and similar patterns in terms of yield. Total soluble solids, anthocyanins and polyphenols all were increased parallel to the intensity of the thinning treatment, but again, only in the two cooler years. Apparently in the warmer third year, this

desired enhancement effect was absent because cluster-thinning provided no advantage in terms of ripening and maturation because of more favourable conditions all season long. Titratable acidity tended to be decreased and must pH to be increased by cluster-thinning but with only very few selected significances. The authors conclude that crop-thinning efficiency highly depends on the year and seems to be more effective in years with unfavourable conditions, as well as in high fertility soils and very vigorous cultivars. Crop-thinning can also be mechanized. Tardaguila et al. (2008) used a machine harvester to remove parts of clusters based on vibration in the canopy caused by adjustable bow rods. The experiment was conducted in two Spanish vineyards on 'Tempranillo' and 'Grenache' and treatment was applied around veraison and lead to reduction in fruit of around 65% in both varieties. Consequently, total yield was reduced significantly in both varieties and in both years of the study. Since the vibration was induced below the fruiting zone the reduction mainly happened by a removal of individual berries from clusters, rather than entire clusters themselves. Therefore, a significant reduction in berries per clusters, cluster weight and cluster compactness were also recorded. When it comes to fruit composition, mechanical thinning significantly increased sugar concentration in both years in 'Tempranillo' and in one out of two years in 'Grenache'. The year without significance in terms of sugar in 'Grenache' could be due to a very unusual late harvest date, according to the authors. pH, tartaric and malic acid were unaffected by the treatments, while 'Tempranillo' showed significantly lower values for titratable acidity in the mechanically thinned vines. A general advance in maturity was associated with mechanical thinning, which leads to believe that damage to the canopy caused by the treatment was minimal and did not interfere with the expected higher values for leaf: fruit ratio. Total phenolics on a per berry basis were significantly increased by thinning in one year in 'Tempranillo', and on a per berry fresh weight basis for 'Grenache' but could not be replicated significantly in the following seasons, even though trends of increased levels were visible. Despite the advantage of very low cost of mechanical crop thinning in comparison to manual labour, it can only be applied in circumstances where the trellis system, trunk height and vineyard layout are compatible with the features of the machine used. The economic advantage of mechanization would only be viable if it can be assumed that mechanical crop-thinning produces the same quality grapes as manual crop-thinning. This question was addressed by Petrie and Clingeleffer (2006) in two vineyards of 'Cabernet Sauvignon' in two different regions of South Australia. They investigated whether mechanical crop-thinning would result in similar values of yield reduction and how big the impact of damaged cluster and leaves was. Therefore, they compared a treatment with mechanical thinning, a manual thinning treatment and a mechanical thinning treatment with subsequent removal of all damaged parts to an untreated control group. The machine targeted the lower third of the canopy and similarly, all clusters were removed from this defined zone for the manual group. Crop thinning was carried out at pea-size stage in both vineyards. In both sites, crop thinning reduced yield significantly, machine and manual treatment showed similar values. Mechanical thinning with additional removal of injured clusters further lowered the yield. There were differences between the two sites in terms of yield reduction with 24% for site 1 and 45% for site 2 respectively. However, the control group showed almost twice as much yield at site 2 as at site 1, which might be a consequence of environmental conditions since the vines were trained in the same way, as consequence of different irrigation techniques (sprinkler at site 2, drip irrigation at site 1) or as a consequence of different planting densities since yield was measured as kg/meter of row and the authors did not provide information about

vine spacing. The average berry number per cluster was unaffected by treatments at both sites while average number of bunches per meter of row significantly decreased by thinning, suggesting that the machine primarily removed entire clusters. Average berry weight was increased by hand thinning at site 1 but unaffected by machine treatments while at the other site all treatments increased average berry weight. Total soluble solids were significantly increased by all treatments at both sites with the combined treatment (machine and manual) not showing a higher value than the other treatments, therefore implying that the increase in sugar does not behave linearly to decreasing crop load and stops after a certain threshold of yield is reached. Compared to the control group, titratable acidity was only significantly reduced at site 2 with no differences between treatments in either site. At site 2 all thinning treatments experienced an acceleration of ripening of at least 9 days (defined as the time to reach a certain sugar level). Both anthocyanins and total phenolics were increased by all treatments at site 2, while at site 1 only the treatments involving mechanical thinning increased these values. Interestingly and despite the differences in final sugar levels between the treatments and the control, the rate of sugar accumulation was consistent among all sites and treatments. The authors speculated that this either means that the changes induced by crop thinning already took place before the first maturity sampling date or that crop thinning removed predominantly clusters within the canopy that were less ripe. In any case, these results imply that crop load and berry size do not necessarily impact rate of sugar accumulation. In summary, the absence of significant differences between hand and mechanical crop thinning suggests that the damage of canopy by the machines was minimal and mechanical crop thinning can produce similar results than manual crop thinning.

Investigations into the influence of crop-thinning on phenolic composition were also conducted by Bubola et al. (2011). 'Merlot' vines were subject to two different severities of crop-thinning treatments and compared to a control group. 30% and 60% of clusters were removed from the upper parts of shoots at veraison. As already seen in research mentioned above, yield was significantly reduced by crop thinning, parallel to its severity. Berry weight and consequently cluster weight were significantly increased in the same fashion. Ripening rate and maturity were heavily enhanced by crop-thinning, which led to a discrepancy in harvest date of 2 weeks between 60% treatment and control and 1 week between 30% treatment and control group respectively. The most severe crop-thinned treatment also showed significantly higher sugar values than both the more moderate treatment and the control group. Titratable acidity was significantly lower in both treatments when compared to the control group, probably caused by enhanced maturation and dilution effects through increased berry size. Levels of total soluble solids increased parallel to leaf area-to-yield ratio, more assimilates could be distributed among fewer sink locations. In wines produced from the trial fruit, the most severe crop-thinning led to the highest levels of alcohol and the highest pH and both treatments kept their significantly lower levels of titratable acidity. In terms of phenolics, the 60% crop-thinning treatment significantly increased the concentration of total phenolics, total anthocyanins and colour intensity, whereas 30% reduction increased total anthocyanins significantly and total phenolics slightly. The severe treatment also saw highest levels of all individual types of anthocyanins. The authors argue that these changes are especially desirable for wines meant to be aged but the loss of yield has to be considered from an economic standpoint. Timing of crop-thinning in relationship to changes in fruit composition was studied by Kok (2011) on 'Sauvignon blanc' vines in Turkey. No irrigation was used but the seasonal climate

provided sufficient rainfall (578,76 mm over the year) and temperatures were relatively mild (13,91°C total annual average). Manual crop-thinning was carried out by reducing the number of clusters from 25 to 15 by removing all but the basal clusters on the shoots. Treatments were carried out on different times during the phenological seasons and differentiated between pre- and post-veraison: 4 weeks, 6 weeks and 8 weeks after flowering were the groups before veraison and 10 and 12 weeks after flowering comprised the treatments after veraison. A control group was not crop-thinned. Must pH, total acidity, total soluble solids, potassium and sodium was analysed as well as levels of free and potentially free volatile terpenes, which are important aroma compounds in this variety. Individual yields for treatments were not assessed but on average crop thinning reduced the crop load by 37,5% per vine. Various berry and cluster attributes (width, length, weight) were measured but did not show differences. Measurements were obtained at three different sampling dates, 3 and 7 days before harvest and at harvest itself. Statistically significant differences were only seen in must pH where all three pre-veraison treatments showed increased levels. This can probably be based on the effect of enhanced ripening described in the studies above. In the control group this effect was not induced and in the post-veraison treatments potentially too late, explaining the similar values for these 3 groups. These results were consistent on all three sampling dates, even though differences were smaller at harvest. Even despite the absence of significance a similar pattern emerged in almost all parameters measured, as well as very similar results on all sampling days. For volatile terpenes the lowest values were always found in the control group and in the group that was crop thinned the latest, while in the groups thinned 6 and 8 weeks after flowering the levels always were the highest. Similarly, total soluble solids were always highest in the middle two treatments (6 and 8 weeks after flowering) while the control was the lowest in two out of three sampling dates. In contrast, total titratable acidity was always the lowest in the 6- and 8-week treatment and always the highest in the control. These findings nicely support the hypothesis of advanced ripening through crop-thinning. This pattern of the 6- and 8-week treatments producing the highest and the control group the lowest values was also observed in both micronutrients, even though the differences were more pronounced in sodium than in potassium. What is remarkable however was the fact that the group that was crop thinned 8 weeks after flowering showed the highest values for all parameters measured on all sampling dates, except for total acidity, where it showed the lowest values on all sample dates. This would lead to the hypothesis that there is somewhat of an ideal timing of crop thinning in regard to the increase of desired compounds, which in this case, was shortly (7 days) before veraison. Post-veraison treatments were apparently crop thinned too late for a comparable effect, sometimes showing the same values as the control group. However, it is still uncertain if this outcome was a result of the crop thinning practice alone and to what extent it was influenced by crop thinning severity and climatic factors like for example water supply and temperature. It would be beneficial to try to reproduce this result in a variety of different regions with different environmental conditions.

As stated in Section 2.2 defoliation can also lower yield in grapevines, therefore it is interesting what the differences are between crop thinning and defoliation as tools for lowering crop load and yield. Tardaguila et al. (2012) compared a mechanized defoliation technique and a mechanized crop thinning technique to a non-defoliated, non-thinned control group in 'Tempranillo' vines. Treatments were applied at two different timings respectively: before flowering and at fruit-set for defoliation and at bunch closure and at veraison for mechanical crop-thinning. Experimental location was a non-irrigated vineyard in

Spain over two seasons. Mechanical defoliation was based on an air pulse directed at the canopy that removed either entire leaves or parts of them while mechanical crop-thinning was based on the same vibration technique as described above. Both defoliation treatments significantly lowered yield per vine compared to the control in both years while crop thinning lowered yield per vine in both years but only oneseason was significant. In terms of timing, in season one yield was significantly lower when defoliation was applied at fruit-set compared to pre-flowering treatment, while in the second year yield per vine in the treatment of defoliation prior to bloom was significantly lower than when applied at fruit-set. Different timings of crop-thinning did not effectively change yield per vine. Number of bunches per vine was unaffected by technique or timing but significantly lower than the control in all cases. Fruit-set was reduced by defoliation in both seasons but the reduction in season 1 was much more pronounced with significantly lower fruit-set when defoliation was applied at fruit-set compared to pre-flowering and no clear differences were observed in season 2. Berry weight was not affected by defoliation in both seasons except at fruit-set in season 1 where defoliation lead to significantly lower berry weight. Crop-thinning, however, led to heavier berries, especially when crop thinning was carried out at bunch closure. All treatments at all timings reduced cluster compactness in season 1 and defoliation also in season 2. Total leaf area at harvest was not affected by the treatments but the ratio of total leaf area:yield was increased by both treatments on average. In terms of juice composition defoliation led to significantly increased levels of total soluble solids (TSS) consistently regardless of timing or year. Thinning only increased TSS significantly in season 1. The same pattern was observed for pH, where the groups were always significantly higher and thinning only in season 1. Furthermore, in season 1 titratable acidity was significantly decreased by all treatments whereas in season 2, despite a trend towards lower levels in treatments, no significance was observed. Apparently, there is a high variability caused by the season, which is even more visible when individual organic acids are measured. In season 1 malic acid content was lowered by all treatments, most of them significant whereas in season 2 not even a trend was emerging in the results. Concerning tartaric acid, season 1 showed clearly decreased levels for defoliation and increased levels for crop-thinning, while there was yet again no difference in season 2. Anthocyanins were increased reliably in all treatments over both seasons with only the later crop thinning treatment not being significant, similarly, total phenols were increased by all treatments in both seasons, most of the time significantly. Comparing the two techniques, crop-thinning seems to be a “safer“ way of reducing final yield because there is no influence on fruit-set like there is in early defoliation, either through direct removal of clusters and flowers by this air-pulse based method, or indirectly by a decreased carbohydrate supply to the clusters because of leaf, and consequently source, limitation (Caspari et al. 1998). The authors conclude that even though both techniques represent an effective way of crop reduction and improvement of fruit composition, early defoliation might be a more consistent tool in regard to seasonal variation in climatic conditions and shows better results in terms of chemical composition of the mature fruit.

It is important to note that the research in this short overview was done by conventional methods of must and wine analysis, which includes practices like titration for determining acids, infrared spectrometry for sugars and standard protocols for determination of ethanol and pH.

# 3. Analytical Methods

## *3.1. Fourier Transform Infrared Spectroscopy and WineScan*

### 3.1.1. Principles

Fourier Transform Infrared Spectroscopy (FT-IR) is an analytical technique based on measurements in the Near-Infrared (NIR) and Mid-Infrared (MIR) range of the electromagnetic spectrum. The principle underlying it is the fact that the bonds of functional groups within a sample will be excited upon exposure to Infrared radiation by absorption of energy, and the specific frequencies of absorption can then give insight into the structure of the substance measured. The absorption intensity can be plotted against wavenumber or wavelength. Modern FT-IR instrumentation produces a complex time-dependent response, the interferogram, which is essentially the variation in absorption intensity as a function of interferometer displacement. The process of mathematically transforming this interferogram from the time domain to the frequency domain gives rise to the conventional Infrared spectrum (Bauer et al. 2008). FT-IR instruments have some advantages over traditional dispersive Infrared spectrometers because of their bigger signal-to-noise ratio, their higher accuracy of wavenumber, their wider scan range, shorter scan time and higher resolution (Birkner and Wang 2019). Qualitative and quantitative IR spectroscopic methods require multivariate calibration algorithms to model the spectral response to chemical or physical properties of the calibration samples. Measured spectra often have to be pre-processed through a series of mathematical procedures, including various forms of scaling and corrections to yield a suitable absorbance spectrum. Statistical models are usually applied to the data, most based on the concept of Principal Component Analysis (PCA) (Bauer et al. 2008). In chemometrics, the chemical discipline that uses mathematical and statistical methods to design or select optimal measurement procedures and experiments and to provide maximum chemical information by analysing chemical data (Otto 2017), the most commonly used technique is called Partial Least Squares Regression (PLSR) (Wold et al. 2001).

Since its development and successful implementation into the scientific world FT-IR has been used in vast range of disciplines, from microbiology (Naumann et al. 1991), material sciences (Tyagi et al. 2006), medicine (Petibois and Déléris 2006) and environmental sciences (Azarraga and Potter 1981). Fig. 3 shows the application of FT-IR spectroscopy in wine research over the last 18 years which has been steadily on the rise even though practical implementation into wineries has been somewhat slow and restricted to large wineries because of high initial acquisition costs (Bauer et al. 2008) but nevertheless interest is growing because it has been a reliable and fast way of simultaneous estimation of various parameters in wine and grape juice (Friedel et al. 2013). *WineScan* is the brand name of a wine analysis device from the company FOSS ANALYTICA A/S and is based on FT-IR. Specifically the model *WineScan FT120* has gathered attention because of its impressive results in terms of speed and accuracy (Nieuwoudt et al. 2004, Patz et al. 2004). After measuring IR spectra of the respective samples, it estimates the absolute concentration of various wine parameters based on regression models that are developed through conventional analysis of wine and grape juice compounds. Accuracy can further be increased by slope-intercept adaptation of the models based on wines from the same region as the samples intended for measurement by *WineScan* (Aru et al. 2018). Infrared spectroscopy

in general offers a rapid and environmental friendly way of measuring a variety of values in wine samples without costly and extensive sample preparation (Kessler 2007, Lachenmeier 2007) and especially modern FT-IR instruments like *WineScan*, who were solely developed for the wine industry, can assess a multitude of enological parameters in a matter of minutes (Patz et al. 2004). These parameters include but are not limited to: organic acids (tartaric, malic, citric, lactic), volatile acidity, sucrose, glucose, fructose, pH, alcohol, relative density, total phenols, total $SO_2$ and sugar-free extract.

### 3.1.2. Applications in Wine Research

FT-IR techniques have been applied over a variety of wine related topics, from discrimination between cultivars (Edelmann et al. 2001), investigating specific compounds (Versari et al. 2004, Boulet et al. 2007), yeast metabolism (Burattini et al. 2008), assessing anti-oxidant capacity (Versari et al. 2010), studying the chemical basis for the sensation of astringency (Edelmann and Lendl 2002) to determining overall wine quality (Kupina and Shrikhande 2003, Edelmann et al. 2003, Agatonovic-Kustrin et al. 2013). FT-IR can also be used for very fundamental wine research as demonstrated by Ricci et al. (2015) who created a database for different tannin molecules in order to systemically categorized their vibrational frequencies and molecular fingerprints. This will help future researchers in this field to easily, quickly and reliably confirm the quality and authenticity of these phenolic compounds and potentially enable the development of wine analysis tools that can automatically discriminate between individual tannin forms. Investigations concerning yeasts important for winemaking is another interesting research field. Adt et al. (2010) showed that FT-IR is capable of discriminating between yeasts at the individual strain level, reliably identifying s*accharomyces cerevisiae* and s*accharomyces bayanus*. Another unconventional, yet very practical, use of Fourier Transform Infrared Spectroscopy in wine research was published by Fu et al. (2009) where white wine in an alternative storage vessel (bag-in-box instead of glass bottles) was subject to different storage times and storage temperatures. Several enological parameters were monitored and correlated to oxygen transmission rate (OTR) as well as to spectral data obtained through FT-IR. The models resulting from Partial Least Squares (PLS) analysis of the FT-IR spectra were able to predict total $SO_2$, free $SO_2$, total phenols, total aldehydes and storage time.
Another application of FT-IR can be seen in a study by Agatonovic-Kustrin et al. (2013) where spectral data obtained from various wines in Australia were correlated with Artificial Neural Networks (ANN) and values of various parameters predicted by the validated ANN model showed excellent correlation with experimentally measured values. Roussel et al. (2003) compared three different techniques to categorize more than 100 white must samples by grape variety. Aroma sensors (“electronic noses “) were compared to Fourier Transform Infrared spectrometry and to Ultraviolet spectrometry. FT-IR showed the most satisfactory results with a low (9,6%) classification error level. The question of wine authenticity is also addressed by FT-IR techniques. Bevin et al. (2006) successfully developed a monitoring system based on *WineScan* measurements of red wine samples in Australia. These spectra then were used to create an index for the samples that then could be used to authenticate samples during transport or processing. As outlined above, Fourier Transform Infrared Spectroscopy is applied in various fields across enology and can be expected to remain useful in future research.

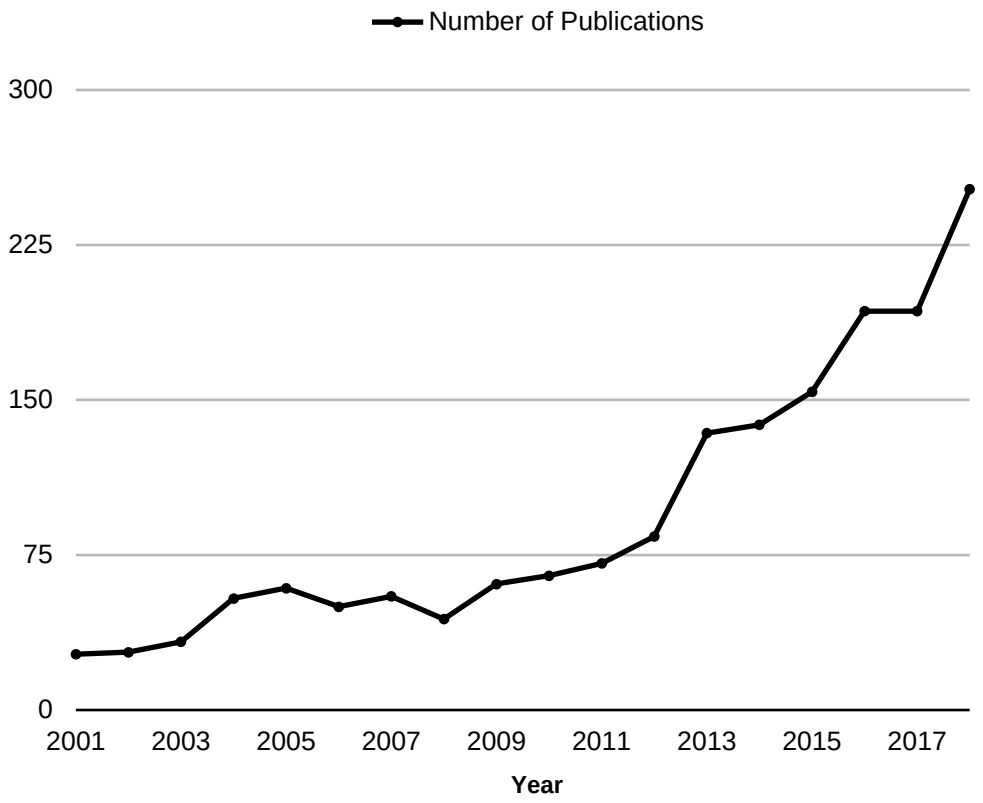


***Figure 3.*** *Wine FT-IR publications per year (2001-2018). Sciencedirect.com was used to search for publications using the terms "FT-IR Wine" in "all fields". All document files were considered.*

## *3.2. Nuclear Magnetic Resonance (NMR) Spectroscopy-based Metabolomics*

### 3.2.1. Principles of NMR Spectroscopy

NMR spectroscopy is a powerful analytical technique widely applied in the chemical and biological fields for the qualitative and quantitative analysis of complex mixtures, kinetic studies and structure elucidation (Ramos 2002).The NMR phenomenon is based on the absorption of energy, in the form of radio frequency (RF), by the NMR active nuclei of an atom (i.e. $^{1}H$, $^{13}C$, $^{31}P$ and $^{15}N$). When placed in a strong static magnetic field (B0), the nuclei can absorb energy from RF radiation from specific frequencies depending on the type of NMR active nucleus, the chemical environment of the nucleus and the location within the magnetic field (if the field is not uniform) (James 1998). When resonance occurs, RF is emitted and will give the interferogram of the observed nucleus. Fourier transformation is then applied to convert the data from the time (t) domain to the frequency domain (Hz).

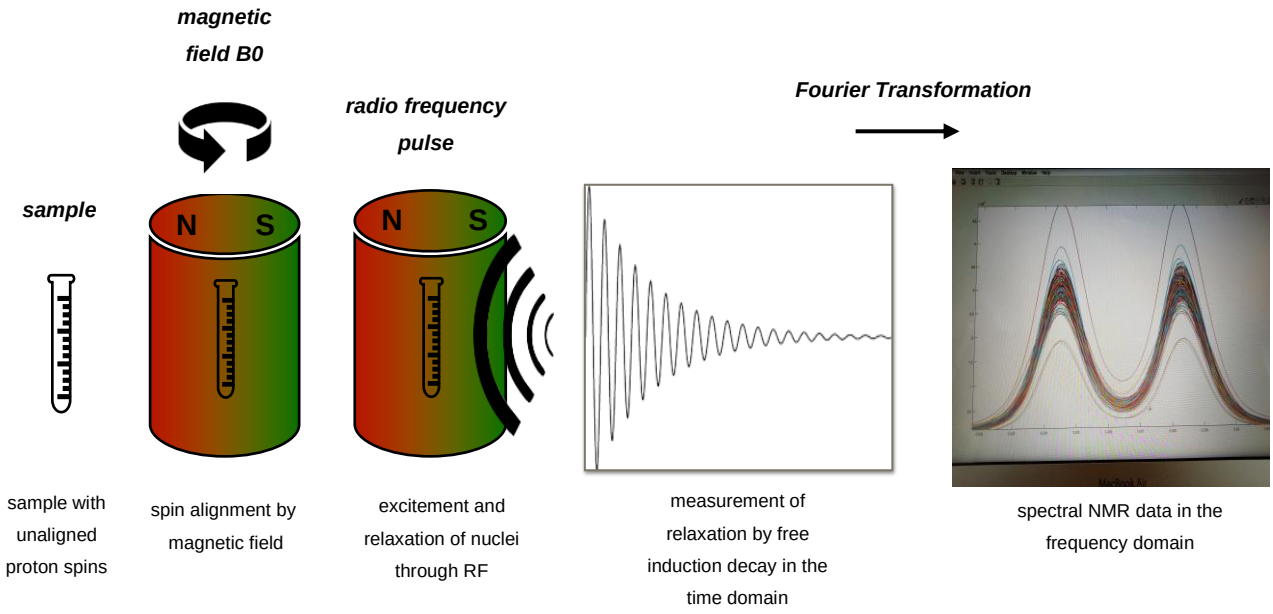


***Figure 4.*** *Simplified scheme of the fundamental NMR principle*

### 3.2.2. NMR-based Metabolomics

Metabolomics is a dynamic research filed, which is focused on the qualitative and quantitative study of the endogenous and exogenous low molecular weight metabolites (<1.5 KDa), known as metabolome, in plant and animals (Oliver et al. 1998, Nicholson et al. 1999). The metabolome viewed as the consequential response to environmental conditions and influences as well as to disease and disruption combined with the fact of it being the product of gene expression makes it a reliable snapshot of the molecular phenotype of the respective sample investigated (Fiehn 2002).

Several scientific fields have already implemented metabolomics as a research tool from clinical medicine to food and environmental sciences (Samuelsson et al. 2006, Oms-Oliu et al. 2013, Viant et al. 2013, Nemkov et al. 2015, Wishart 2016). Particularly analysis of food samples and food matrices by this approach, commonly referred to as foodomics, have successfully proven to be able to produce insights into important questions regarding food quality and food authenticity (Ibáñez et al. 2012, Capozzi and Bordoni 2012). Moreover, foodomics has great potential to enhance and further scientific knowledge in regard to food safety, food traceability and overall food quality. Its anticipated new results in the future are expected to also have big impacts on analysis of human dietary patterns since diet is a factor connected to health that is highly modifiable. Therefore, foodomics increasingly receives attention from laboratories, research institutions and governmental regulatory bodies (Leo et al. 2018). The connection between food characteristics and human health has always been a scientific area of interest and has been approached by foodomics in recent years even though it is highly complex and requires the combination of a multitude of *omic*s platforms and bioinformatical databases (Braconi et al. 2018). Research in food metabolomics can take various approaches with some work focusing on practical aspects such as the degradation rate of meat as a function of storage time and storage temperature (Eraldo do Nascimento Fontes et al. 2019) or comparing the lipid fraction between conventional and organic milk as a quality identification parameter (Tsiafoulis et al. 2019). NMR-based foodomics are also

used to discriminate between types of fruit, either on a product level such as fruit juice (Cuny et al. 2007), on a cultivar level (Koda et al. 2012) and even on a clonal level (Clausen et al. 2012). In regard to food authentication, some works have tied NMR-based metabolomic fingerprints to geographical origin of products such as beef (Jung et al. 2010), salmon (Masoum et al. 2007) or the botanical origin of honey (Schievano et al. 2010). Other foodomics research tries to gain insight into food processing by the human gastro internal tract, the release of food-derived compounds during digestion, the metabolites in present in the body after absorption and the bioactivity of various foods and its consequences on health (Pimentel et al. 2018). Advances and refinement of analytical platforms including chromatographic and spectroscopic methods has promoted a rapid growth in metabolomics applications (Zhang et al. 2012). Especially profiling based on metabolomic fingerprints obtained through NMR represents huge potential in research since the characteristics of numerous molecular constituents of a sample can be identified with a single experiment. Thousands upon thousands of metabolic profiles have been collected over the years, from humans to plants or single-cellular organisms which are stored in online databanks like the human metabolome database (*www.hmdb.ca*) or, with regards to microbiology, the yeast metabolome database (*www.ymdb.ca*).

Nuclear Magnetic Resonance spectroscopy is one of the most applied analytical methods in high-throughput metabolomics. Because of the rich abundance of hydrogen atoms in all kinds of substances NMR spectroscopy that is based on the interactions with these proton nuclei, (1H)-NMR spectroscopy, is the most frequently applied tool in metabolomics and foodomics research. Acquisition time of unbiased data is only a few minutes. NMR spectroscopy is non-destructive and therefore ideal for intact food matrices while its non-selective nature makes it a superb tool for untargeted analysis (which describes the analysis of the entire NMR spectrum as opposed to the targeted approach where only preselected metabolites are analyzed and quantified) (Trimigno et al. 2015). However, NMR spectra are very complex in nature and often contain many signals not-yet-assigned to metabolites and therefore the use of advanced data mining tools is essential to transform the NMR signals into useful data (Khakimov et al. 2015, Bevilacqua et al. 2017). Especially unsupervised Principal Component Analysis (PCA) is a crucial and frequently applied method for the analysis of multivariate data sets (NMR spectra), where K number of variables (data points) are measured for N number of samples. The high dimensionality of a given dataset is lowered, while retaining the variation present in the data set as much as possible (Wold 1987). It accomplishes this reduction of dimensionality by identifying directions, called principal components (PCs), along which the variation in the data is maximal. The PCs are uncorrelated and sorted in descending order of explained variance. By using a few components, each sample can be represented by relatively few numbers instead of by values for thousands of variables. Samples can then be plotted, making it possible to visually assess similarities and differences between samples and determine whether samples can be grouped (Ringnér 2008).

The decomposition of the original X matrix by PCA results in two matrices is known as scores and loadings. The scores (T) correspond to the coordinates of the projection of the samples onto each individual Principal Component, while the loadings (P) describe the linear combinations of the original variables to new variables which then determines the direction of the PCs.

$$X = T * P^T + E$$

*data matrix* *scores matrix* *loading matrix* *residuals matrix*

***Equation 1.*** *Typical structure of a PCA model*

Eq.1 displays the typical structure of a PCA model that aims for the decomposition of a matrix X. T represents the matrix of the scores, P the matrix of the loadings and E the matrix of the residuals. Plotting of the scores (scores plot) represents an informative way of visually assessing the distribution of samples where potential clustering, outliers and general trends can be identified and interpreted. The loadings plot gives insight into how much influence the measured variables have in the model plane. These two plots can be visualized separately or combined and displayed as a so-called biplot, where samples (scores) and variables (loadings) are laid on top of each other to unveil fundamental clustering patterns and correlations. A typical biplot is reported in Fig.5.

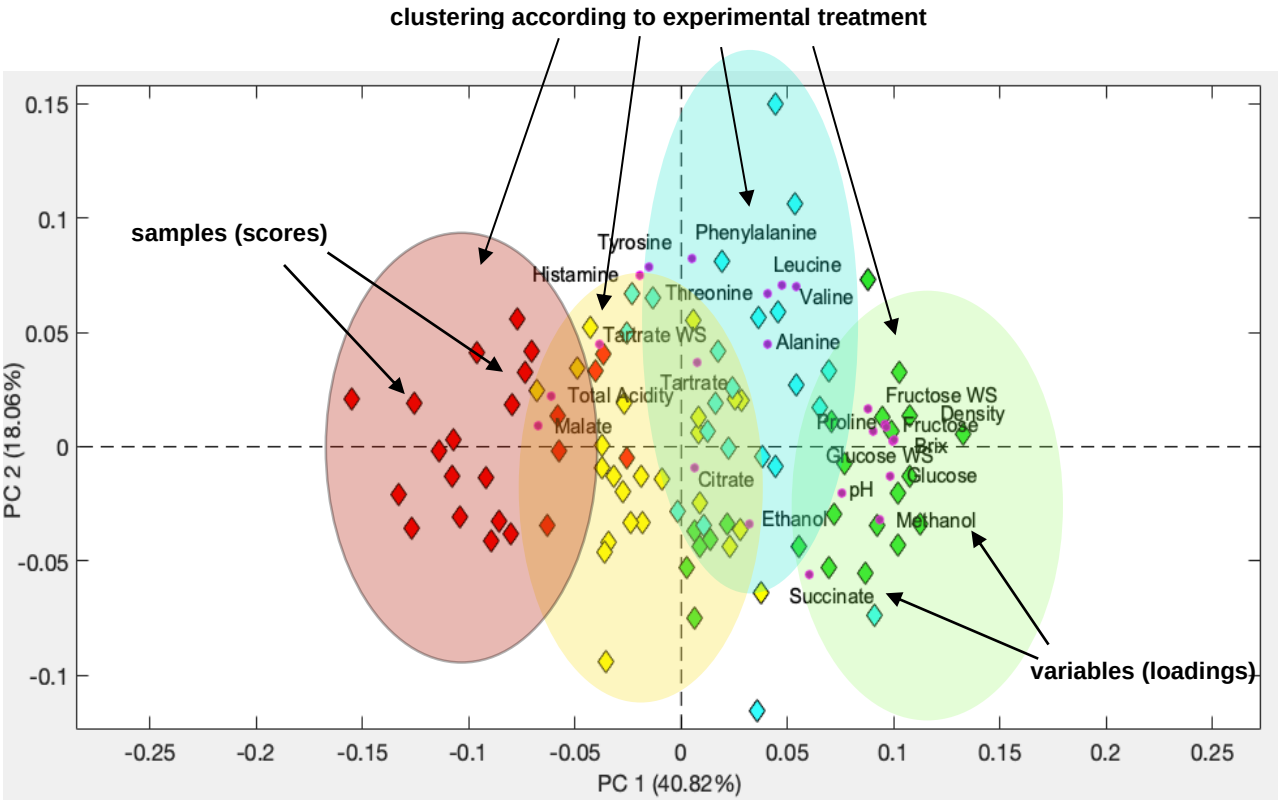


***Figure 5.*** *Representative biplot of the first two components of a PCA model from the presented study; different coloured diamonds (scores) represent samples from different treatments of the experiment; purple dots represent the variables (loadings)*

### 3.2.3. Applications in Wine Research

Analysis of must and wine based on NMR spectroscopy has been steadily on the rise (Fig.6) over the past 18 years. It can be used in a multitude of ways and has seen application all over the wine world. Ali et al. (2011) successfully related the relative concentrations of various metabolites to sensory attributes in 'Riesling' and 'Müller-Thurgau'. Furthermore, they managed to discriminate between two vintages based on the respective NMR profiles. This distinction between vintages based on metabolomic profiles was also observed by Lee et al. (2009) with identical vinification of 'Merou' wines in two consecutive years. Not only did they find a clear separation between the vintages but the results were also in agreement with meteorological data based on sunshine hours and rainfall. Again, the variance in metabolite profile based on different vintages was also found by Pereira et al. (2005) who used 1H-NMR data derived from grape berry skins of various varieties to investigate differences in 3 consecutive grape growing years. However, when comparing different soil types of 5 geographic locations the authors did not find significant variations of the metabolomic profile, therefore emphasizing the well accepted notion that climatic conditions, for example the *vintage effect*, provides greater variance in grape composition than the soil effect. However, Mazzei et al. (2010) managed to differentiate the metabolomic profile of three different wines based on variance in pedological and microclimatic properties, as well as statistically significantly distinguish them from commercial wines of the same variety, underlining the importance of the *terroir* effect. Another interesting application of 1H-NMR in wine research was conducted in Brazil by Pereira et al. (2014). The authors used NMR spectroscopy to analyse commercial and homemade grape juices for the efficiency of sterilization processes based on the presence or absence of ethanol. Furthermore, even winemaking techniques such as malolactic fermentation have been monitored and studied by the means of Nuclear Magnetic Resonance. Lopez-Rituerto et al. (2012) described the progression of malolactic fermentation by monitoring malic and lactic acid at different points in time. Remarkably and in connection to the vintage investigations mentioned above, this study also succeeded in distinguishing between individual wineries close in proximity based on NMR metabolomics. Winemaking techniques were also compared, as done by Baiano et al. (2012). Significant differences, for example in tartaric acid content, were observed in the metabolomic profile when comparing four different winemaking techniques. Also, fermentation was a field investigated by NMR based metabolomics. Son et al. (2009) monitored the metabolic changes during fermentation and also managed to assign individual fermentative characteristics to three strains of saccharomyces cerevisiae, the most common yeast used in winemaking. NMR spectroscopy can also be applied to help answer very specific questions as demonstrated by Pinu et al. (2013). The authors investigated the biogenetic origin of various thiols typically found in ‘Sauvignon blanc’ wines. The precursors of these thiols are not well understood but of high importance since they contribute extensively to the 'Sauvignon blanc' character and therefore represent an economical factor. By combining metabolomic profiles derived from NMR and thiol concentrations derived from gas chromatography–mass spectrometry the authors found various correlations between metabolites and thiols. One rather unconventional application of nuclear magnetic resonance was done by Weekly et al. (2002). The authors used a variation of the traditional NMR spectroscopy to measure contents of various metabolites in unopened, intact wine bottles. The field of wine sensory has also been touched by NMR metabolomics. Rochfort et al. (2010) tried to find alternatives to time consuming and expensive sensory

panels and successfully found metabolomic profiles in ‘Shiraz’ and 'Cabernet Sauvignon' that correlated to sensory attributes. Furthermore, different levels of sun light exposure were investigated, and the shaded treatment was clearly separated in the Principal Component Analysis plots, based on higher levels of malic acid and glycerol. An experienced panel of tasters was able to confirm these results by successfully differentiating between the shaded group and the other treatments. Even the heavily discussed and scientifically difficult to access field of organic and biodynamic viticulture was investigated by NMR based metabolomics. Picone et al. (2016) found that organically and biodynamically grown grape berries show differences in their metabolomic profile, above all through concentrations of amino acids. Very recently a study was conducted at the same university that the experiment of this thesis was done with similar instrumentation used. Aru et al. (2018) used $^{1}$H-NMR spectroscopy to investigate the metabolic profile of wines from 22 grape varieties. By comparing two different sample preparation methods (*wet* and *dry*) and identifying a total of 27 metabolites present in the wines the authors were able to assign clear metabolic fingerprints to 13 of the varieties through Principal Component Analysis. Furthermore, they also found 6 metabolites that were the main drivers of the sample groupings. Results were compared to total concentrations measured by FT-IR and high correlation was observed. The experiment in this study uses a similar approach in terms of analysis tools but with viticultural practices and environmental factors as variables instead of different cultivars. To the best of the author’s knowledge and also in accordance with a review on wine-related NMR metabolomics by Amargianitoki and Spyros (2017) the field of viticulture, viticultural practices and specific environmental conditions (as opposed to a general *terroir* effect) have barely been touched by this method of analysis. The presented study is the first attempt to approach water deficit timings, defoliation and crop reduction treatments through the angle of Nuclear Magnetic Resonance metabolomics and it is based on combining forces between Fourier Transform Infrared Spectrometry and $^{1}$H-NMR based metabolomics to gain a big amount of data in search for potential correlations between treatments and results.

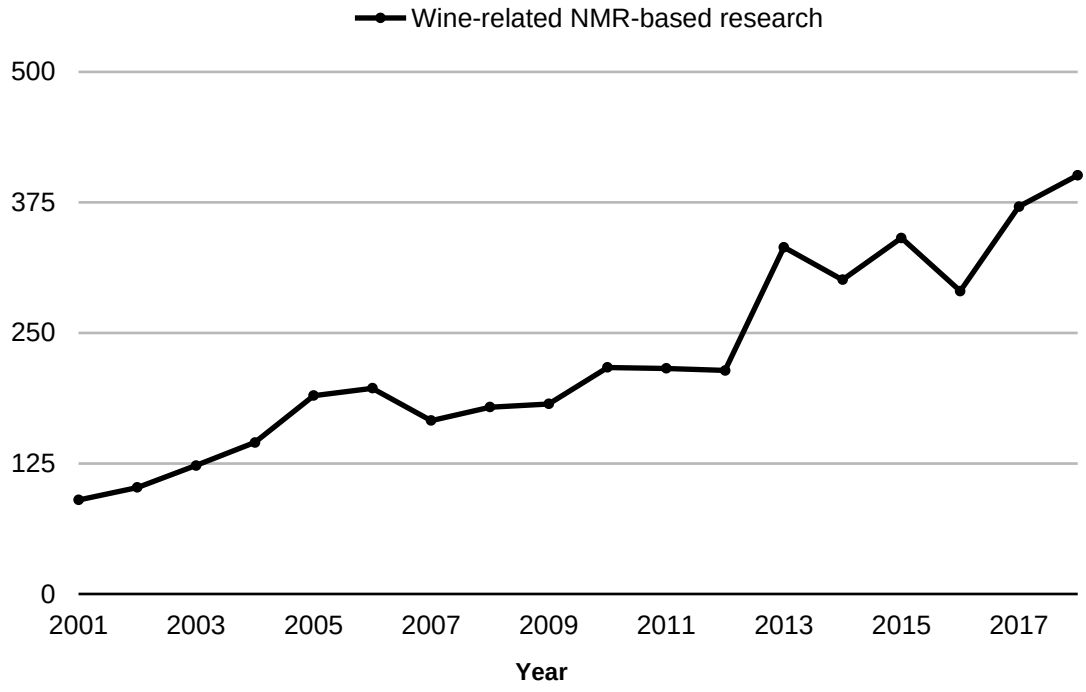


***Figure 6.*** *Wine metabolomics publications per year 2001-2018. Sciencedirect.com was used to search for publications using the terms “NMR Wine” in “all fields”. All document file types were considered.*

# 4. Materials and Methods

## *4.1. Experimental Design*

The experiment consisted of two independent trials, one performed in a greenhouse and the other in a conventional vineyard. The greenhouse trial was based on water deficit studies while the field study looked at the effects of viticultural practices, namely defoliation and crop reduction. Juice and wine samples (8 per treatment from the vineyard and 9 per treatment from the greenhouse) were extracted from both experiments and then analysed by $^{1}$H-NMR Spectroscopy and Fourier Transform Infrared Spectroscopy. A summary can be seen in Fig.7.

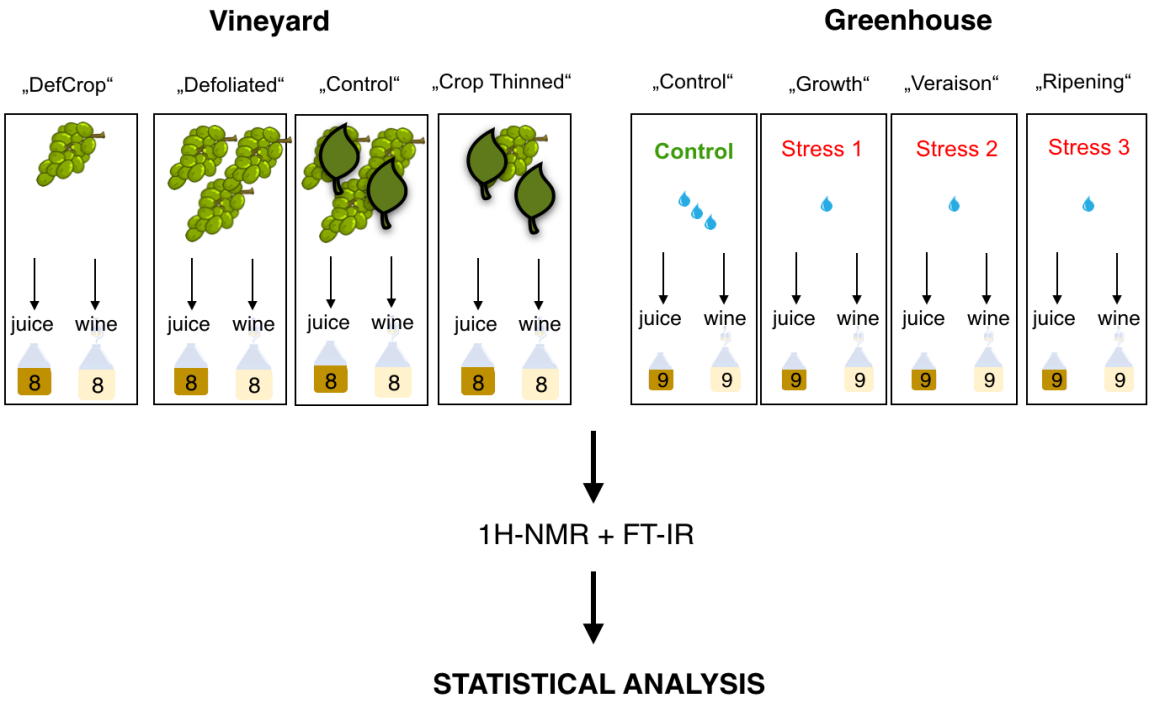


***Figure 7**. Overview of the experimental design*

### 4.1.1. Greenhouse

36 plants of Vitis vinifera cv. 'Solaris' were used for this experiment, grown in 30L plastic pots in a greenhouse at the University of Copenhagen's agricultural site *Pometet*, located in Taastrup, Denmark. The setup consisted of 3 rows of 14 plants each, the first and last one was excluded in order to ensure similar conditions for each plant. The vines used for the trial were historically in the last year of an experiment looking into the effect of early cropping on plant development. They were all of the same age (4 years) but diverse in terms of pruning type, 24 are pruned as a double cane with 1,5m spacing between them, while 12 are pruned as single cane with 1m spacing respectively. Additionally, the plants have different fruiting histories, due to the experimental background, with 8 plants bearing fruit for the first time in 2018, 20 for the second time and another 8 for the third time.

In order to minimize the influence of these factors as well as things like row orientation and proximity to doors and windows 4 trial groups were established with the factors mentioned above distributed equally as can be seen in Table 1.

| | GROUP 1 | GROUP 2 | GROUP 3 | GROUP 4 |
|---|---|---|---|---|
| **Single cane, first fruit** | 2 | 2 | 2 | 2 |
| **Single cane, second fruit** | 3 | 3 | 3 | 3 |
| **Single cane, third fruit** | 2 | 2 | 2 | 2 |
| **Double cane, second fruit** | 2 | 2 | 2 | 2 |
| ***Plants per group*** | *9* | *9* | *9* | *9* |
| ***Plants per row*** | *3* | *3* | *3* | *3* |

***Table 1.*** *Distribution of pruning types and fruiting histories among groups*

#### 4.1.1.1. Technology

The Greenhouse is connected to an automated drip irrigation system and has a rooftop which closes in case of rain or strong wind but normally is kept fully open to allow an environment as natural as possible. Furthermore 2 sensors, placed in the first and third row collect data on relative humidity and temperature every 12 minutes for the whole year.

#### 4.1.1.2. Plant Material

The cultivar 'Solaris' is one of Denmark's most important white varieties, above all because of its adaptation to a short season (early ripening), intense flavours and its resistance to downy and powdery mildew which continue to be an issue in northern winemaking countries. The vines of the experiment are grown in 30L plastic pots and stabilized by wooden poles that are connected by a wire system, the setup strongly resembles the one found in a conventional vineyard (Fig.8 and Fig.9). The vines are grown on SO4 rootstocks in a coarse peat soil.

#### 4.1.1.3. Irrigation

The objective of the experiment was to investigate if water stress, induced at 3 different phenological stages, would change the grape and wine composition. Irrigation was carried out by an automated, centralized drip irrigation system with long tubes running through each of the 3 rows, where individual drippers can easily be added or blinded for every plant according with the desired irrigation level. The flow rate and the irrigation timing and duration can be modified by a central console electronically. For this experiment the irrigation was set to 3 times a day, namely at 9:30 a.m., 03:00 p.m. and 08:00 p.m. with a total flow rate of 2L/hour/dripper. The time per irrigation was set to 15 minutes at the start of the experiment but was increased to 20 minutes due to warm temperatures and subsequent water demand. The water stress groups were irrigated with 2 drippers per plant, while the fully irrigated groups received water from 6 drippers at the same flow rate. From the day of the change to 20 minutes onwards, this results in a total irrigation of 12L per day for the irrigated groups, and 4L per day for the groups experiencing water deficit.

***Figure 8.*** *Greenhouse plants at the start of the season*

***Figure 9.*** *Greenhouse plants at harvest time*

4.1.1.4. Stress Tracking

*Time Domaine Reflectometry*

Time domain reflectometry (TDR) was used to determine the soil moisture content in each pot. The measurements were done at 9:15 a.m. and 14:45 a.m. each day, shortly before irrigation. 25cm metal rods were used in the pots as electric conductors for the measurement. For this soil type a soil moisture content of around 35% is considered full field capacity. This value was established by irrigating a pot until run-off occurred and measuring the corresponding soil moisture content.

*Leaf Water Potential*

Additionally to the TDR measurements the Leaf Water Potential (ψ) was measured twice a week over the course of each stress period except the first one because of unavailability of the equipment. The measurement was carried out in a conventional pressure bomb on a fully developed leaf from the shaded side of the canopy. Typical time of the measurements was around 11:00 a.m. Reduction in stomatal conductance (the gas exchange rate through stomata in leaves and indicator for uptake of $CO_2$ for carbonic compound assimilation as well as indicator of $O_2$ release and location of water loss through transpiration) as well as leaf rolling have found to be good indicators for water stress situations (Kadioglu and Terzi 2007) and are correlated to Leaf Water Potential ψ (Bittman and Simpson 1989).

*Calculation of Water Demand by Crop-Evapotranspiration*

The evapotranspiration $ET_o$ as a parameter for water demand from soil evaporation and plant transpiration was calculated using the Penman-Monteith-Equation (Eq.2) as provided by the Food and Agricultural Organization of the United Nations (*fao.org*). Even though most systems use one value for the crop efficient ($K_c$) that corrects the $ET_o$ for the respective type of crop, in reality this value changes along the season as the plants develop. However, for facilitating calculations and since the experiment started when the canopy was already mostly fully developed a $K_c$ of 0,8 was used for determining $ET_c$ because it is representative for a fully developed grapevine canopy mid-season according to Washington State University (*wine.wsu.edu*). All necessary climatic data was provided by the

meteorological station onsite in Taastrup and was corrected for the temperature measured in the greenhouse.

$$ET_o = \frac{0.408\Delta\left(R_n - G\right) + \gamma\left(\frac{C_n}{\left(T + 273.16\right)}\right)u_2\left(e_s - e_a\right)}{\Delta + \gamma\left(1 + C_d u_2\right)}$$

***Equation 2.*** *Penman-Monteith-Equation with*

**$ET_o$**… reference evapotranspiration [mm $day^{-1}$],

**$R_n$**… net radiation at the crop surface [MJ $m^{-2}$ $day^{-1}$],

**G**… soil heat flux density [MJ $m^{-2}$ $day^{-1}$],

**T**… mean daily air temperature at 2 m height [°C],

**$u_2$**… wind speed at 2 m height [m $s^{-1}$],

**$e_s$**… saturation vapour pressure [kPa],

**$e_a$**… actual vapour pressure [kPa],

**$e_s - e_a$**… saturation vapour pressure deficit [kPa],

**Δ**… slope vapour pressure curve [kPa $°C^{-1}$],

**γ**… psychrometric constant [kPa $°C^{-1}$].

**$C_n$**… constant depending on time frame and ETo model (= 900 in this case)

**$C_d$**… constant depending on time frame and ETo mode (= 0,34 in this case)

#### 4.1.1.5. Stress Periods

The 3 stress periods were loosely based on the general understanding of the 3 main phases of berry development, where different synthesis processes take place at different times, often described by their temporal distance from flowering. Flowering in the greenhouse occurred at the end of May, which puts stress period 1 ( 2.7. - 23.7.) in the berry growth phase (stage 1) overlapping into the lag-phase (stage 2), according to Fig.2. Stress period 2 (23.7. - 13.8.) can be considered occurring around veraison with, again slightly overlapping into the start of ripening (stage 3). The final stress period 3 (13.8. - 3.9.) took place during the final stages of ripening.

*Period 1 - Pre-Veraison*

The first stress period was set from 3.7. until 23.7.2018. Soil moisture content was determined via time domain reflectometry twice every second day, at 9:15 a.m. and 2:45 pm, shortly before irrigation. Due to issues with the equipment, only the stressed and the control group could be measured but it is important to note that the other groups received the same irrigation as the control group. The stressed group showed typical signs of water deficit such as yellowing of leaves and visibly decreased turgor pressure. Due to a failure of the irrigation system on the weekend before the start of the experiment the control group shows a reduction in soil moisture content at the start but quickly recovered after a couple

of days. During the first period one of the control plants was measured on an hourly basis, to follow the evolution of the soil water content over time period from before the first irrigation to shortly before the second irrigation. Depending on the weather conditions on the particular day, sometimes the soil water content changed very little (for example 0,4% difference between measurements before the first and second irrigation with very little variation along the hours as observed on a cloudy day). This implies that on such an occasion the water supply was pretty much in equilibrium with the water demand. In other instances, rainy days could even increase the soil water content between the first and second irrigation, implying that demand was lower than supply, possibly because of increased air humidity and subsequent decrease in evapotranspiration. It is important to note that precipitation did not directly influence water availability in the plants since the greenhouse has an automated roof-closing-system in case of rain. On sunny and hot days, a decrease of between 1-4% of soil water content were usually observed between the two irrigation times. This pattern was also observed in a plant from the stressed group that was measured every hour. The variation between plants of the same group were quite substantial, with some plants quickly rising in soil moisture content and consistently staying above 30% mark, whereas others never reached that level and stayed in mid- or low twenty percent range. Similarly, some vines of the stressed group quickly experienced water stress while others slowly dropped in soil moisture content and just rarely went below 10%. Average values of soil moisture content during stress period 1 were 23,4 % for the control group and 12 % for the stress group respectively.

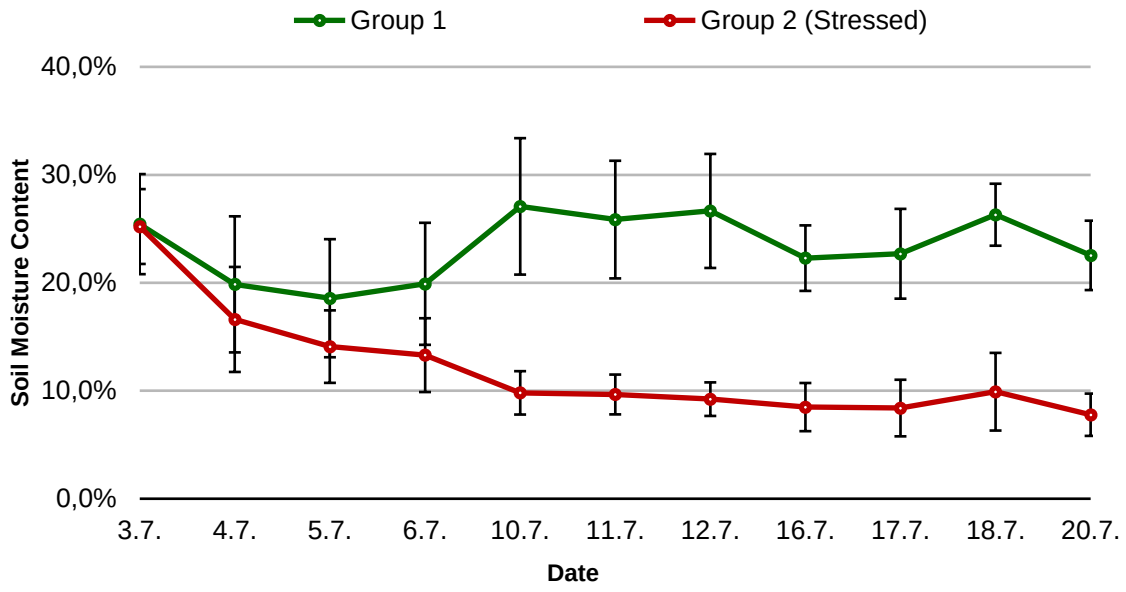


***Figure 10.*** *Tracking of soil moisture content over the first stress period; ; values are group averages of all 9 vines and averages between the 9:15 a.m. and 14:45 p.m. measurements; bars indicate standard deviation per day for each group*

*Period 2 - Veraison*

The second stress phase lasted from 23.7.2018 to 13.8.2018 and included monitoring of all 4 groups. Additionally, leaf water potential was measured twice a week with a pressure chamber at 11:00 a.m. Due to warm conditions the irrigation for all plants had to be increased in order to achieve the targeted soil moisture. The stressed group showed typical signs of water deficit such as yellowing of leaves and visibly decreased turgor pressure. The measurements on an hourly basis of a control and a stressed plant were discontinued, due to little variation over the course of a day. In the first few days the stressed group experienced a quick decrease in soil moisture and then stay around the 8-9% range with a short slight elevated phase in week 2. This is probably due to slightly lower average and maximum temperatures and less wind, as well as much more cloud cover in the first days of August. This can also be seen in the average values for leaf water potential. The average values of soil moisture content during stress period 2 were 22,7% (Group 1), 17,8% (Group 2), 8,3 % (Group 3) and 20% (Group 4). The average values for Leaf Water Potential during period 2 were -0,65 MPa (Group 1), -0,67 MPa (Group 2), -1,04 MPa (Group 3) and -0,70 MPa (Group 4).

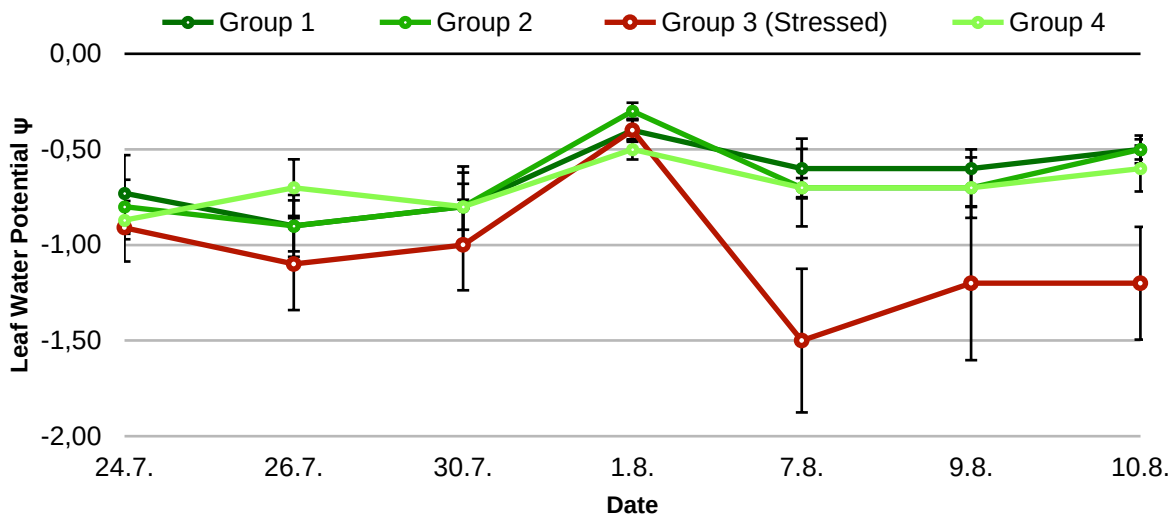


***Figure 11.*** *Tracking of leaf water potential ψ over the course of the second stress period measured at 11:00 a.m. from a fully developed leaf from the shaded side of the canopy; values are groups averages of 9 vines; values in MegaPascal (MPa); bars indicate standard deviation for each day per group*

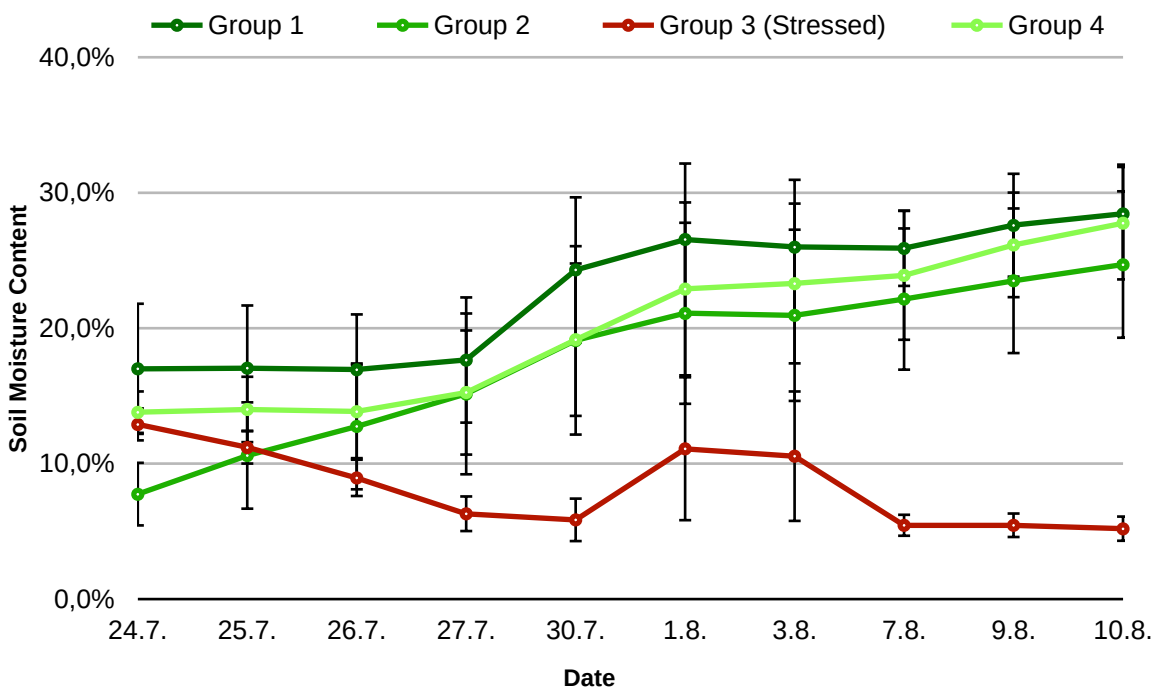


***Figure 12.*** *Tracking of soil moisture content over the second stress period; values are group averages of all 9 vines and averages between the 9:15 a.m. and 14:45 p.m. measurements; bars indicate standard deviation for each day per group*

*Period 3 - Ripening*

The third stress period lasted from 13.8.2018 to 3.9.2018 and resulted in harvest of all the vines on the following day, on the 4.9.2018. Again, leaf potential was measured twice a week during that time, as well as soil moisture content. The stressed group showed typical signs of water deficit such as yellowing of leaves and visibly decreased turgor pressure. Over the first few days group 3 recovered from the previous stress phase and reached similar values than the irrigated groups after approximately one week. Parallel to group 3, group 4 declined in soil moisture content over the first week and then settled in at around 10%, with two consequent days of slightly higher values in the second week. At one date (20.8.) the stressed group showed similar values in Leaf Water Potential as the other groups, and even though there was less wind on that date, overall the weather was similar in comparison to other days in that time frame. Therefore, it can be assumed that this result is based on measurement errors or irrigation issues on that date. When excluding this outlier, the average values for leaf water potential were -0,56 (Group 1), -0,59 (Group 2), -0,66 (Group 3) and -0,93 (Group 4). The average values of soil moisture content for the groups were 30,2% (Group 1), 26,0% (Group 2), 19,8 % (Group 3) and 11,3% (Group 4).

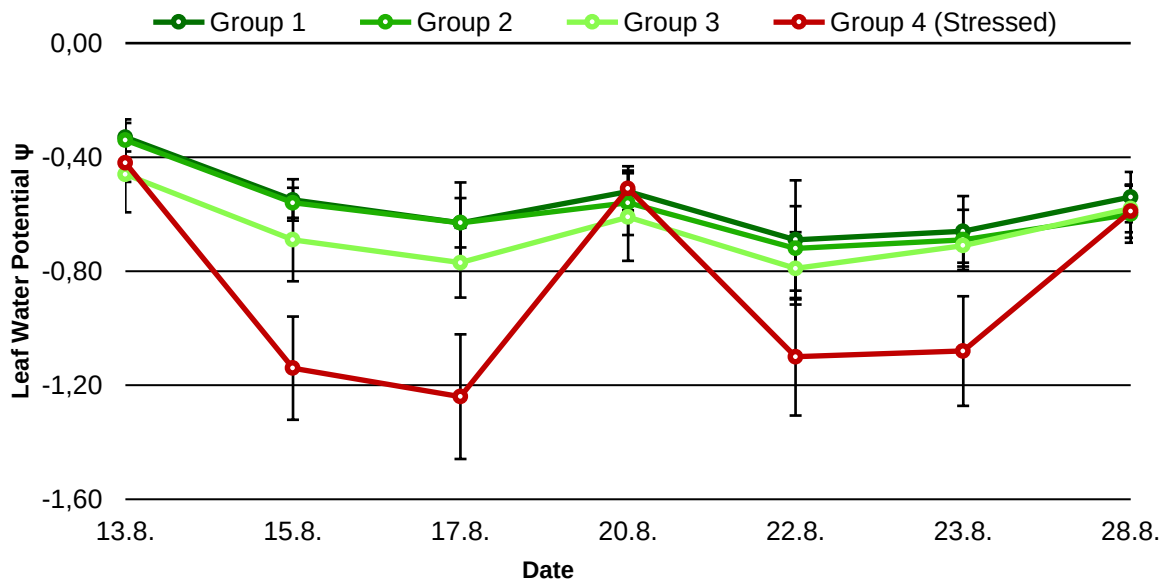


***Figure 13.*** *Tracking of leaf water potential ψ over the course of the third stress period measured at 11:00 a.m. from a fully developed leaf from the shaded side of the canopy; values are groups averages; values in MegaPascal (MPa); bars indicate standard deviation per day for every group*

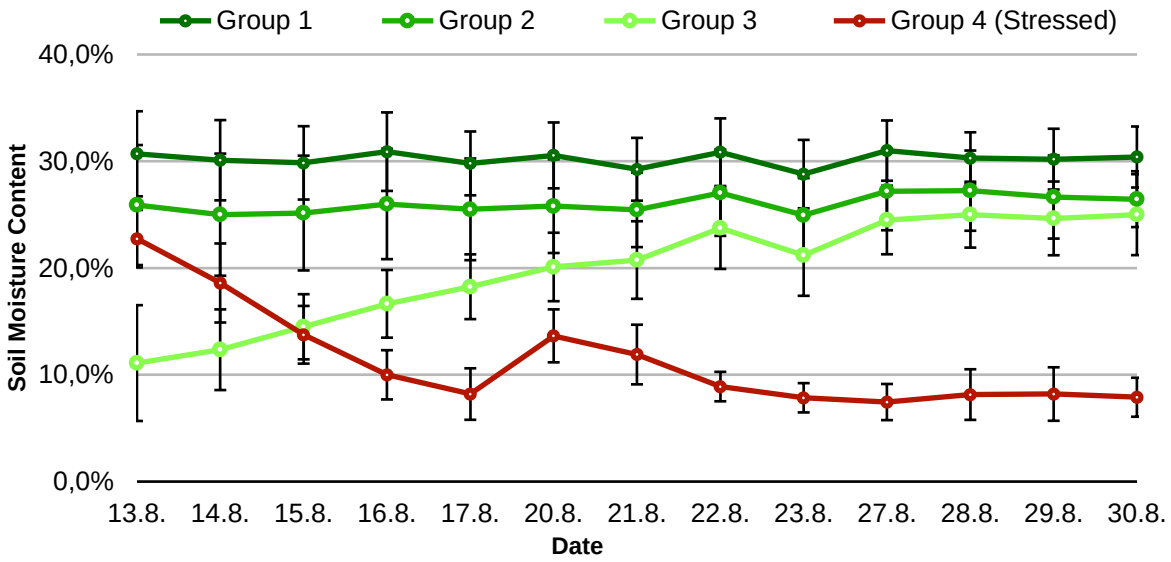


***Figure 14.*** *Tracking of soil moisture content over the third stress period; values are group averages of all 9 vines and averages between the 9:15 a.m. and 14:45 p.m. measurements; bars indicate standard deviation per day for every group*

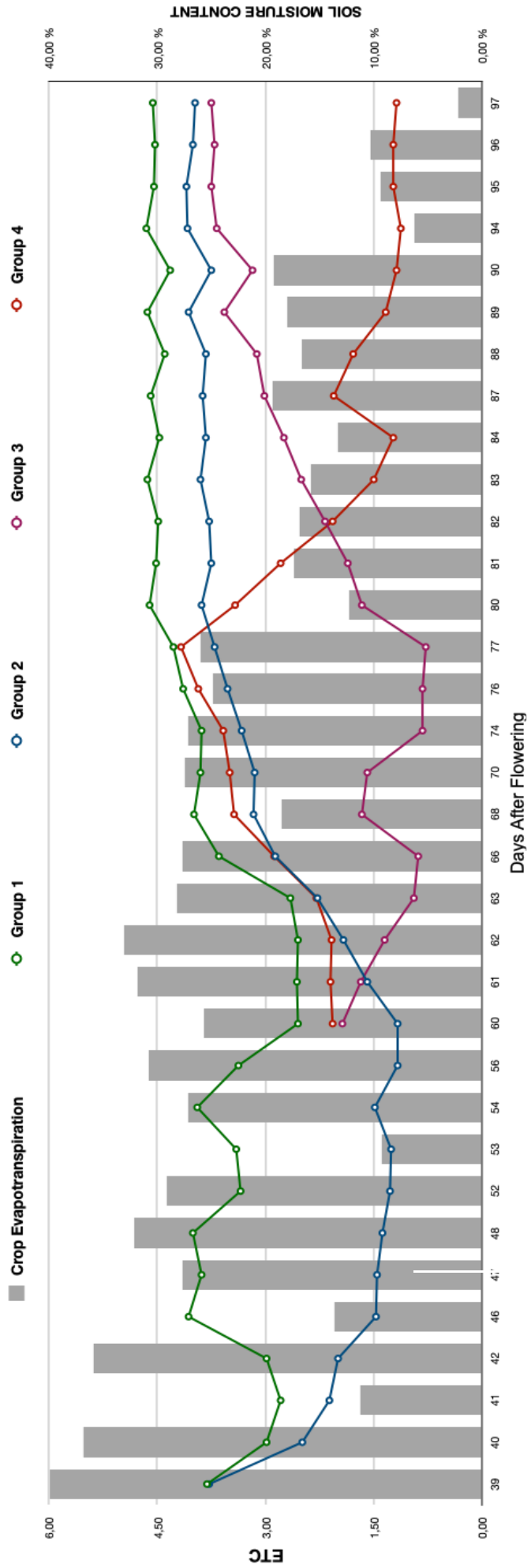


***Figure 15.*** *Overview of the soil moisture content of all groups over the course of the experiment; Group 1 (green) was irrigated at all times, Group 2 (blue) was stressed during the end of berry growth phase and beginning of lag-phase, Group 3 (purple) was stressed around veraison into the beginning of ripening and Group 4 (red) was stressed during ripening; grey bars represent crop-corrected evapotranspiration ($ET_c$), left y-axis shows $ET_c$ in L/m2/day; right y-axis shows soil moisture content in %*

### 4.1.2. Vineyard

***Figure 16.*** *Field trial vineyard in Pometet*

#### 4.1.2.1. Treatment Groups

The second experimental site was a North-South oriented vineyard in *Pometet* with a slight slope to the north-side, where 32 field-grown Solaris plants were chosen, divided in 4 groups of different defoliation and crop thinning treatments. The vines are grown on SO4 rootstocks and planted in 2005. As opposed to water stress, which is difficult to control in a field environment, the variables here were different crop-levels and the absence or presence of a full canopy in the fruiting zone. 8 vines next to each other were subject to each treatment. For the rest of this thesis the treatment groups are referred to as indicated by the names in parentheses.

*Defoliation (Def)*

Defoliation was done during the berry growth phase in the end of June and consisted of removing all leaves manually from the fruiting zone on the east-facing side of the row in order to gently adapt the clusters to moderate morning sun and still shielding them from intense afternoon sun. In the third week of July the leaves on the western side were also removed, exposing the bunches to full sun radiation for the rest of the season. In regards to the phenological cycle this timeframe can be considered as the end of the berry growth phase since the greenhouse at that time was already in the lag phase as stated in Section 4.1.1.5, and the open vineyard is slower in terms of development because of the elevated

temperatures in the greenhouse. So, it is reasonable to assume that both defoliation treatments were carried out before the lag phase and consequently before veraison.

*Cluster Thinning (Crop)*

The manipulation of crop-levels on the vines was carried out manually by removing all existing clusters except 8, all located on operate shoots, preferably equally distributed around the centre of the head of the vine. Conventional vineyard scissors were used. Removed crop load was measured on a per vine basis. The treatment was carried out in the beginning of August. The reduction in crop load was calculated on a percentage basis by adding weight of clusters removed and final harvest weight to obtain total yield per vine and then dividing by weight of clusters removed. Average percentage of crop removal was 67,1 % for the group that was defoliated and crop-thinned (*DefCrop*) and 69 % for the group that was only crop-thinned (*Crop*).

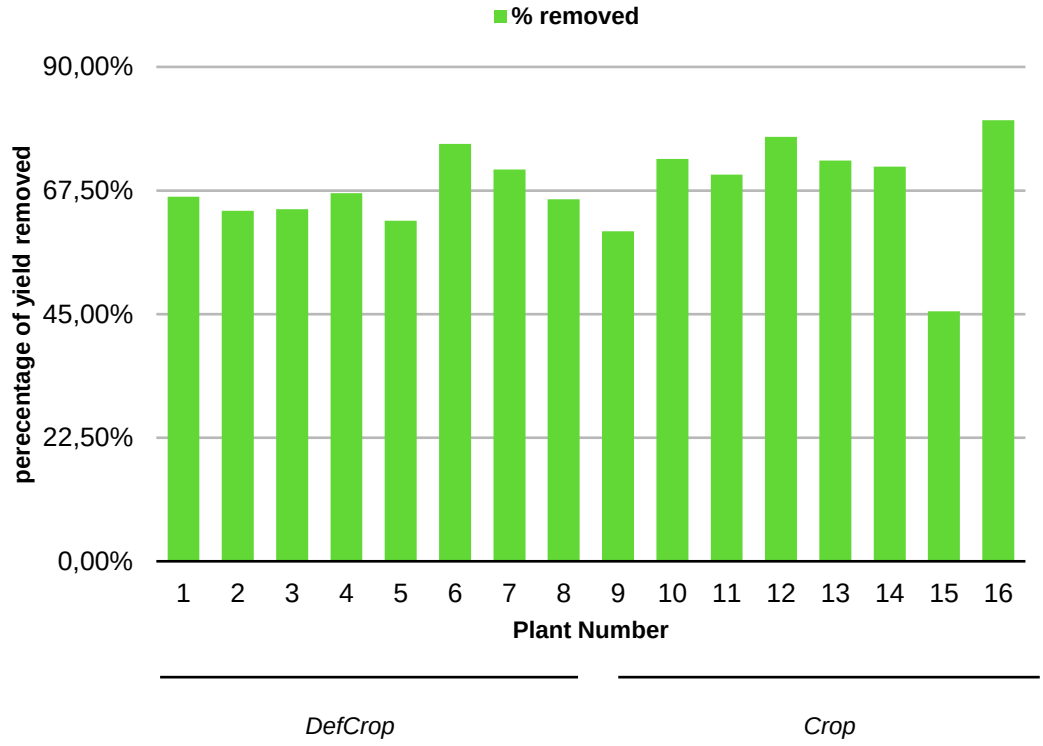


***Figure 17.*** *Overview of crop reduction in %; calculated by adding weight of clusters removed and harvest weight of remaining 8 clusters and dividing by weight of clusters removed; expressed per individual plant*

*Defoliation and Cluster Thinning (DefCrop)*

In order to differentiate influence and investigate interactions between treatments a third group was established, where both treatments were carried out in the same manner and at the same time as mentioned above.

*Control (Control)*

One control group was established were no treatments were carried out but the group experienced all other vineyard practices.

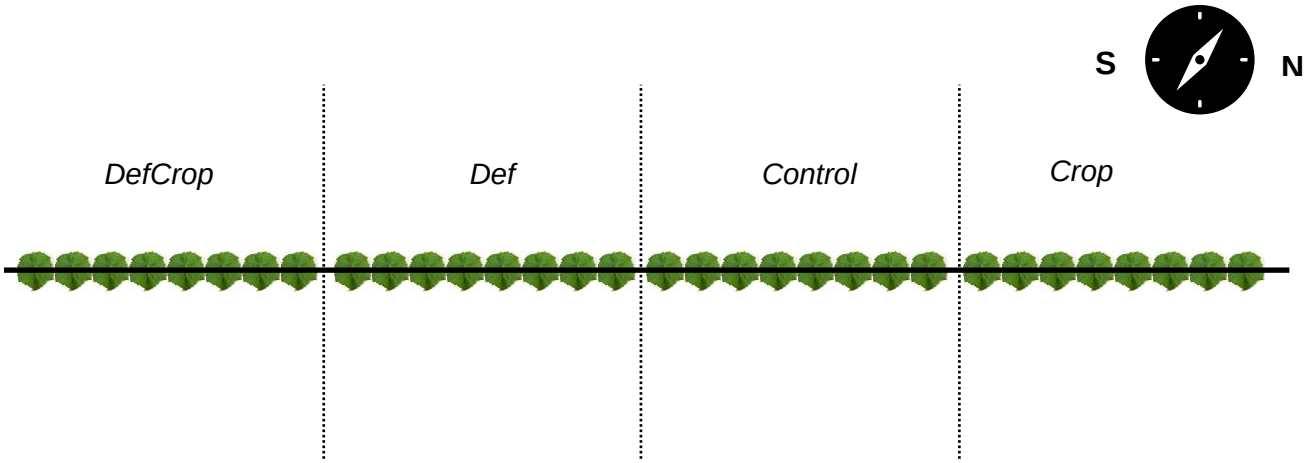


***Figure 18.*** *Scheme of the vineyard trial set-up;*
*each leaf represents an individual Solaris vine;*
*groups were situated along the same south-north oriented row; 8 vines per group.*

4.1.2.2. Vineyard Practices

Besides the experimental treatments regular practices were applied throughout the season. These included hedging of the canopy, removal of weeds growing in between vines and spraying sulphur against fungal disease. All regulatory time-windows in regard to harvest were considered correctly.

## *4.2. Processing*

### 4.2.1. Harvest

On September 4th the fruit in the greenhouse and on September 13th the fruit from the vineyard was manually harvested in the morning, destemmed, crushed and set to macerate for 16 hours at 3°C in small 10L buckets. Every vine was processed separately. After maceration, the individual yields were pressed for 10 minutes with a maximum pressure of 3 bars using a pneumatic 20 L hydropress (Speidel, Germany). Two samples were taken after pressing, one stored at 3°C for 2 days and then analysed by WineScan and the second one frozen at -19°C for future NMR analysis.

### 4.2.2. Fermentation

After pressing the juice was racked into 2L glass jars and inoculated with commercial yeast *Saccharomyces cerevisiae bayanus* (Lalvin DV10TM, Lallemand, Denmark) and moved to the fermentation room, set to 17°C. Fermentation was monitored via density and temperature control.
Fermentation was rapid and uninterrupted and similar for all 4 groups (Fig.19). After fermentation 75mg/L of sulphite was added to the wines (1,5 ml/L of a 5% sulphite solution made from adding sulfuric gas to water and controlling the concentration of the solution with a densitometer) and they were moved to a room set to 3°C for 1 week of cold settling. Wine samples were taken and frozen again at -19°C.

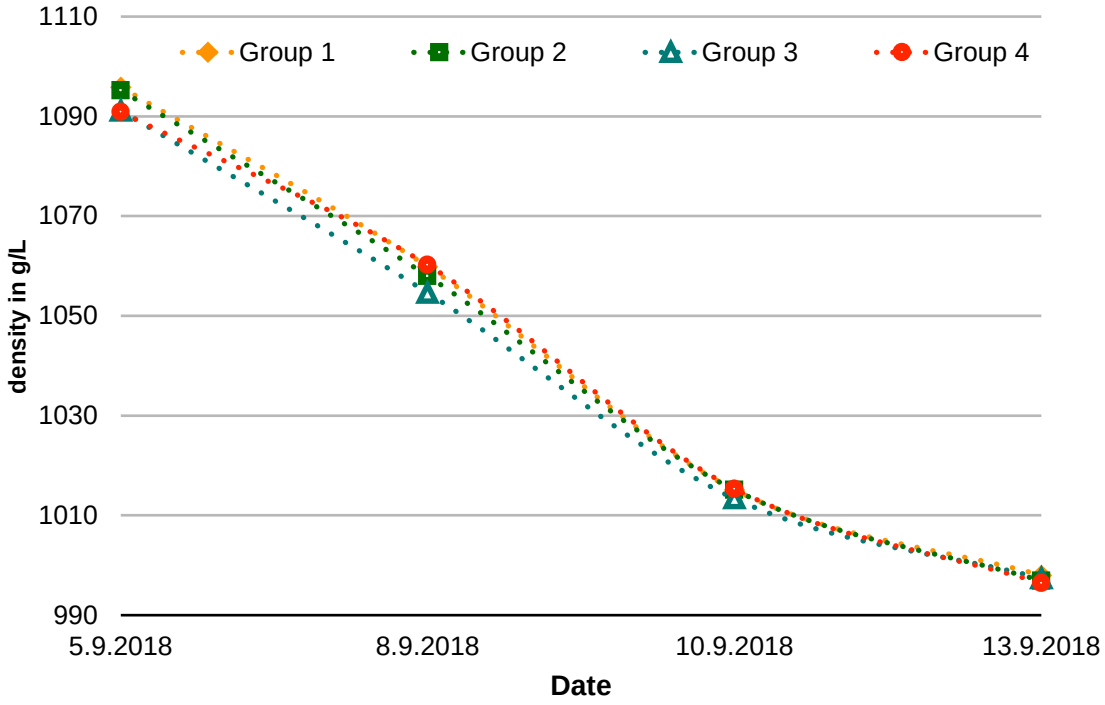


***Figure 19**. Evolution in density during alcoholic fermentation of the juices from the greenhouse trial; values in g/L*[1]

## *4.3. Analysis*

### 4.3.1. WineScan

Samples were analysed by FT-IR spectroscopy using *WineScan* (WineScan FT 120, FOSS A/S, Hillerød, Denmark). Centrifugation of each sample at 4000rpm for 5 minutes was carried out before measurement. The standard protocol of WineScan is based on two separate measurements of a sample, followed by the calculation of a mean of the two, which then represents the result. Juice Samples were analysed after pressing, while wine samples were analysed after fermentation. For the water stress experiment the analysis of the final wines was performed in July 2019 after a prolonged storage in 2 L bottles with a fine lie of yeast. Two samples (1 from the control group and 1 from the second stress period) had to be excluded due to indistinguishable labels.

### 4.3.2. NMR

#### 4.3.2.1. NMR Sample Preparation

Complementary to the FT-IR measurements each individual sample by Proton Nuclear Magnetic Resonance ($^1$H-NMR) spectroscopy. The samples, juice as well as wine, were stored at -89°C prior to measurement. The juice samples were stored in 50 mL falcon tubes while the wine samples were stored in 100 mL plastic bottles. After thawing, 3 mL sample was centrifuged at 2000 rpm at 4°C for 30 minutes to precipitate residual solids. Out of each sample, three replicates were produced in Eppendorf-tubes, each one containing 700 µl sample and 300 µl of 1M $KH_2PO_4$ buffer in $D_2O$ to minimize chemical shift fluctuations due to different pH values. 600 µl of this sample-buffer solution was then transferred into 5

mm NMR glass tubes and placed into a rack in a randomized order. Additionally, 18 pooled samples were produced in the same way, consisting of the cumulation of all samples and representing the chemical average. The pooled samples were placed at the end of each row of the NMR racks. Three juice samples as well as three wine samples were measured 2 weeks before the rest because of a trial measurement related to a different topic. Because of a mistake one juice sample only had 2 replicates, so the second replicate was measured twice.

#### 4.3.2.2. NMR Measurements

$^{1}$H-NMR spectra of the wine and juice samples were recorded on a Bruker Avance III 600 operating at a proton Larmor's frequency of 600.13MHz and equipped with a 5-mm broadband inverse (BBI) probe. Data acquisition and processing were carried out with the TOPSPIN software (version 3.5). A 5-min waiting period was applied for temperature equilibration prior to $^{1}$H-NMR measurements. Spectra were acquired at 298 Kelvin, using the standard pulse sequence for a pre-saturation of the water signal (zgcppr pulse program), a sweep width of 12,626 Hz, a 90° pulse and an acquisition time of 3 s. Data was collected after 128 scans. The relaxation delay (d1) was set to 4 s. The receiver gain (RG) was fixed for all the experiments to an adequate value estimated through several tests. The spectra were acquired in automation using the sample jet system (Bruker BioSpin, Ettlingen, Germany). Spectral chemical shift referencing on the TSP $CH_3$ signal at 0.00 ppm was performed on all spectra. Assignment of metabolites was performed by comparison with the yeast metabolome database *(*http://www.ymdb.ca*/),* with literature data and the profiler function of *Chenomix NMR Suite* (8.4)

### 4.3.3. Multivariate Analysis

#### 4.3.3.1. Pre-processing of the $^{1}$H-NMR Spectra

The $^{1}$H-NMR spectra were imported into the MATLAB software (version 2018b, Mathworks Inc., Natick, MA, USA) and a juice data matrix sized 220 × 30856 (samples x variables) and a wine data matrix sized 213 x 30181 (samples x variables) were built. Regions containing residual water signals as well as noisy regions above and below the relevant ppm range were excluded from analysis. The spectra were aligned using the *icoshift* algorithm. The datasets were imported into the PLS_toolbox (version 7.5.1, Eigenvector Research, Mahson, WA, USA) running in MATLAB. No other pre-processing method other than auto-scale and mean-centring was performed prior to multivariate analysis.

#### 4.3.3.2. Table of Metabolites

For additional metabolomic investigations two new matrices were created, only containing the signals of selected, baseline resolved metabolites. The matrices were sized 213 x 9 (samples x variables) for wine and 220 x 13 (samples x variables) for juice respectively. The integration of these signals enabled comparison of relative concentrations of said metabolites between treatments. The datasets were imported into the PLS_toolbox (version 7.5.1, Eigenvector Research, Mahson, WA, USA) running in MATLAB (version 2018b, Mathworks Inc., Natick, MA, USA). *Autoscale* was used prior to multivariate analysis.

Selected metabolites for juice were alanine, citric acid, ethanol, fructose, glucose, leucine, methanol, phenylalanine, proline, succinic acid, tartaric acid, threonine and valine

Selected metabolites for wine were acetic acid, citric acid, ethanol, glycerol, methanol, succinic acid, tartaric acid, phenylalanine and proline.

#### 4.3.3.3. Combination of Matrices

Relative concentrations of the two metabolite tables were combined with the total concentrations obtained by WineScan analysis to obtain an additional data set with a broader range of compounds for analysis.

#### 4.3.3.4. Principal Component Analysis (PCA)

Principal Component Analysis was used in this study to look for potential correlation between sample groupings and chemical information based on different treatments. Three separate spectral regions were defined as aliphatic (0.00-3.00 ppm), carbohydrate (3.01-5.50) and aromatic (5.51-9.50) and analysed individually. PCA on the full, global NMR spectrum was performed as well. PCA was also performed on the matrices containing the integrated, baseline-resolved signals of pre-selected metabolites as well as on the combined datasets containing both NMR and FT-IR data.

#### 4.3.3.5. Analysis of Variance (ANOVA)

Simple one-way ANOVA was used in R (ver. 3.3.2, 2016) to determine the significance of differences between the values obtained by WineScan.

# 5. Results

## *5.1. Vegetative Parameters*

Primary shoots tended to be shorter, but more numerous in stressed plants, which led to an overall reduction in total fresh weight from primary shoots. The higher number of primary shoots also possibly led to a higher leaf number per plant, as well as the shorter internode length. However, a similar total fresh weight of primary leaves was observed. It can be stated that stress did not influence fresh weight of primary leaves. The total leaf area of primary leaves per plant was higher in stressed plants, but lower on a per-primary shoot basis, due to the higher number of primary shoots.

In double cane plants, primary shoot length was smaller in stressed plants with higher shoot numbers. Therefore, shoots were lower in fresh weight but higher in number of leaves, which, again, led to a similar total leaf area and a lower leaf area per primary shoot in stressed plants. Overall, very similar behaviour in single-and double-cane vines, with differences being the clearly shorter primary shoots in double cane vines and the higher number of shoots. Based on higher number of shoots, total leaf number, total leaf fresh weight and total leaf area was higher in plants with two fruiting canes. Leaf area per shoot is however much lower in double-cane vines because the higher total leaf area is counterbalanced by a much higher number of shoots. Lateral shoot fresh weight was much smaller in stressed single cane plants and slightly smaller in double cane plants, as compared to their respective controls. Both secondary and total leaf area of all shoots was reduced by water deficit, more severely in single cane plants with double cane plants showing lower values for secondary leaf area but are higher in total leaf area because of higher primary leaf area. Ratios of leaf: fruit were highest in the single cane control group with 13cm$^2$ per 1 g fruit and lowest in stressed double cane plants with 10cm$^2$/g. Yield per plant was higher in the double cane vines, especially in the stressed ones.

**Vegetative Parameters**

| | **primary shoot length** | **shoot number** | **shoot fresh weight** | **primary leaves** | **primary leaves fresh weight** | **primary leaf area** | **primary leaf area per shoot** | **yield per plant** |
|---|---|---|---|---|---|---|---|---|
| *unit* | *cm/shoot* | #/plant | *gram/plant* | *leaves/plant* | *gram/plant* | *cm$^2$/plant* | *cm$^2$/shoot* | *kg* |
| **single cane control** | 137 | 15 | 1035 | 215 | 1459 | 52002 | 3461 | 6,72 |
| **single cane stress** | 127 | 17 | 868 | 234 | 1455 | 53313 | 3221 | 6,72 |
| **double cane control** | 114 | 23 | 945 | 323 | 1667 | 62283 | 2798 | 7,25 |
| **double cane stress** | 101 | 25 | 821 | 337 | 1657 | 62551 | 2597 | 8,41 |

***Table 2.*** *Values for various primary vegetative parameters divided by pruning type and absence or presence of water stress; values represent group averages*

**Vegetative Parameters**

| | lateral shoot fresh weight | lateral leaves fresh weight | total lateral leaf area | total leaf area | area per leaf | leaf area per g fruit |
|---|---|---|---|---|---|---|
| *unit* | *gram* | *gram* | *$cm^2$* | *$cm^2$* | *$cm^2$* | *$cm^2$/gram* |
| **single cane control** | 147 | 506 | 22381 | 74383 | 241 | 13 |
| **single cane stress** | 63 | 238 | 12158 | 65471 | 232 | 11 |
| **double cane control** | 69 | 424 | 18999 | 81282 | 193 | 11 |
| **double cane stress** | 55 | 323 | 15148 | 77699 | 187 | 10 |

***Table 3.*** *Values for various secondary vegetative parameters divided by pruning type and absence or presence of water stress; values represent group averages*

## *5.2. Yield*

### 5.2.1. Greenhouse

In terms of yield per plant in relationship to the stress phases, the control group showed the lowest yield levels, followed by the early stress phase and the mid stress phase. The highest yield was observed in the late stress phase which is not statistically significant but was more than 1 kg higher than the control group. The lowest value was found in one of the plants from the mid stress phase (4,56 kg) and the highest value was seen in one of the early stressed plants (10,42 kg). Average cluster weight also did not significantly differ among the groups even though the control and late stress showed higher values and mid stress showed the lowest values. The highest value was found in the late stress group (268 g), while the lowest was found in the mid stress group (168 g).

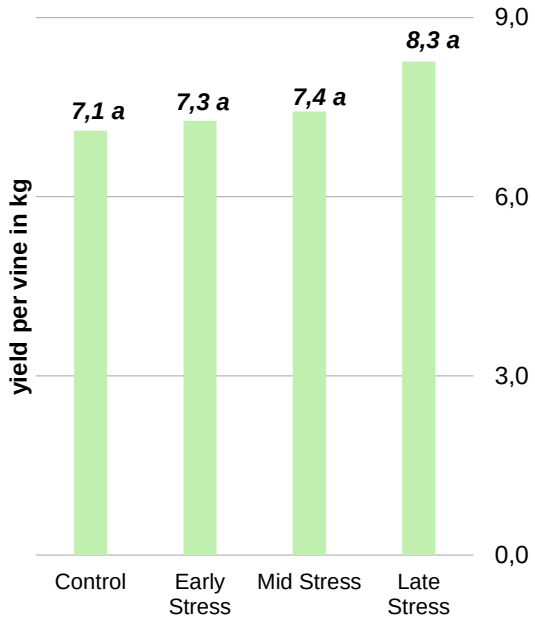


***Figure 20.*** *Yield per plant of the treatment groups; values represent group averages in kg; different letters represent significant differences based on a significance level of p<0,05*

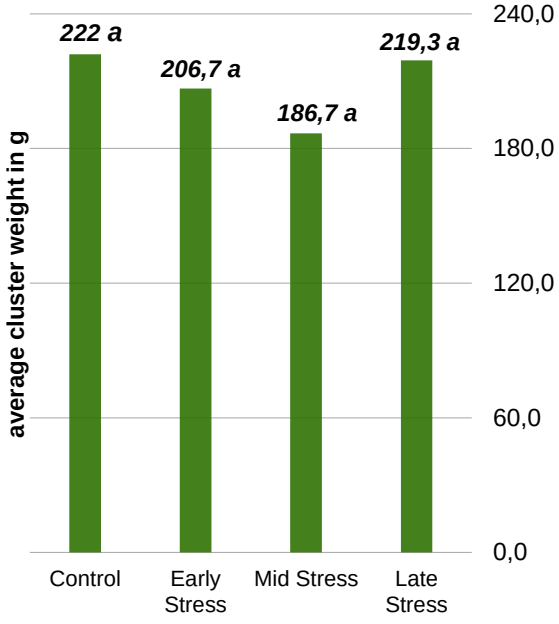


***Figure 21****. Average cluster weight of the treatment groups; values represent group averages in g based on measurement of 10 clusters per vine; different letters represent significant differences based on a significance level of p<0,05*

### 5.2.2. Field Trial

Logically, yield per vine was significantly lowered by crop reduction, while defoliation did not provide clear additional effect. In the vines what were not crop-thinned defoliation showed slightly lower yield values, so the highest yield per vine was seen in the control group (7,02 kg per plant). Average cluster weight however was increased significantly in both crop-thinning treatments and defoliation seemed to furthermore increased average cluster weight. The highest average cluster weight was seen in the group that was defoliated and crop-thinned (211,88 g per cluster) and the lowest value was found in the control group (163,5 g per cluster). Crop-thinning raised the cluster weight by 38.97 grams on average while defoliation raised it by 9,41 grams on average. Yield per vine as well as average bunch weight were both lower in the field trial than in the greenhouse.

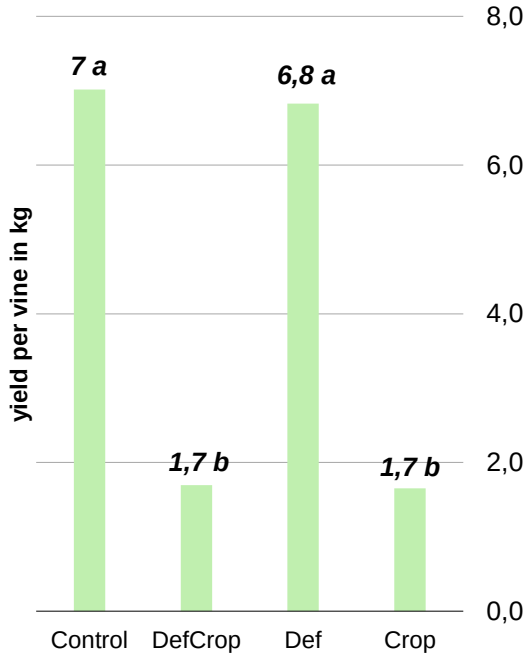


***Figure 22.*** *Yield per plant of the treatment groups; values represent group averages in kg; different letters represent significant differences based on a significance level of p<0,05*

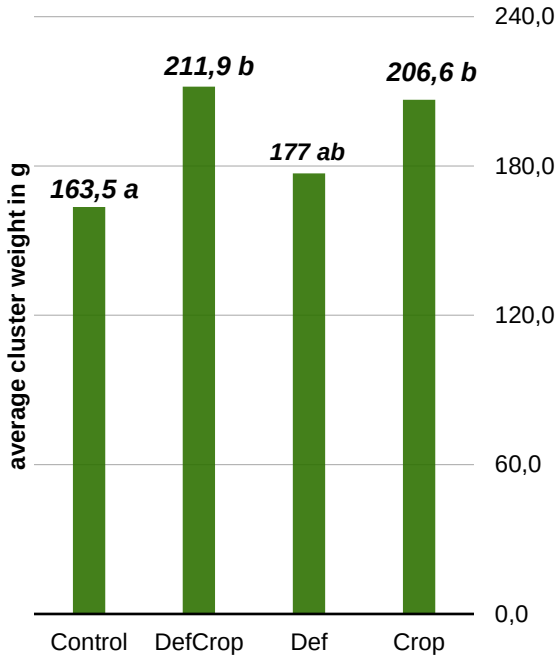


***Figure 23.*** *Average cluster weight of the treatment groups; values represent group averages in g based on measurement of 10 clusters per vine; different letters represent significant differences based on a significance level of p<0,05*

## *5.3. WineScan*

### 5.3.1. Juice

#### 5.3.1.1. Greenhouse

Table 4 shows values for 9 parameters, analysed by WineScan, differentiated by treatment groups. None of the parameters were influenced at a significant level, due to rather big plant to plant variability but some tendencies may be identified. Water deficit tended to lower malic acid content in all treatments to a similar extent, regardless of timing. Total acidity also tended to be highest in the control group, with similar levels in the water stressed groups. However, tartaric acid did not seem to respond to water stress in the same way as malic acid, with the values being comparable among all four treatments. Parameters for sugar content and ripeness, expressed as °Brix, glucose and fructose content and density all showed similar patterns, with the control group and the early stress group showing a tendency to higher values than the later stress treatments. Ammonia and alpha amino nitrogen were found to be very similar in all groups. Only the early stress group tended to have slightly enhanced levels of alpha amino nitrogen. Potassium values were also homogeneous among the groups with slightly elevated levels in the mid stress group. Grape juice pH was not influenced by water deficit. The biggest range in individual values was observed in the mid stress group, showing the overall highest and lowest individual values for glucose + fructose content, °Brix, and tartaric acid, as well as the lowest individual value for malic acid and total acidity. The smallest variation within a treatment group was observed in the early stress treatment.

| | **Control** | **Early Stress** | **Mid Stress** | **Late Stress** | ***significance*** |
|---|---|---|---|---|---|
| **Glucose + Fructose** | 227 | 226 | 216 | 213 | ns |
| **°Brix** | 21,9 | 21,8 | 21,0 | 20,8 | ns |
| **Density** | 1096 | 1096 | 1092 | 1091 | ns |
| **Total acidity** | 7,17 | 6,85 | 6,70 | 6,68 | ns |
| **pH** | 3,19 | 3,19 | 3,20 | 3,20 | ns |
| **Tartaric acid** | 6,2 | 6,2 | 6,1 | 6,1 | ns |
| **Malic acid** | 1,6 | 1,3 | 1,3 | 1,3 | ns |
| **Ammonia** | 112 | 117 | 114 | 114 | ns |
| **Alpha amino nitrogen** | 292 | 307 | 295 | 289 | ns |
| **Potassium** | 1135 | 1114 | 1175 | 1110 | ns |

***Table 4.*** *Results from WineScan analysis of the juice samples from the greenhouse trial, differentiated by water stress timing; test for significance was carried out by ANOVA with a significance level of p<0,05: ns = not significant*

#### 5.3.1.2. Field Trial

*Due to centrifugation issues while preparing the field juice samples for measurement, several samples are missing selected parameter values because some values had to be dismissed because of nonsensical levels. This mainly concerns the first two groups, the DefCrop group and the Def group. Only the parameters pH, tartaric, malic acid and alpha amino nitrogen are concerned. The DefCrop group only produced 4 values for pH and malic acid (instead of the full range of 8) and is missing one value for tartaric acid. The Def group is missing 5 values for pH, malic, tartaric and alpha amino nitrogen. This needs to be taken into consideration when interpreting results derived from analysis. Group 3, the control group is only missing two values for malic and tartaric acid respectively and the Crop group is missing 1 value for alpha amino nitrogen. It is important to note that these measurement issues did not affect the other parameters. Interpretations considering acids and pH should be better based on the wine results, since they did not experience these kinds of issues.*

WineScan analysis of the field juice samples showed several significant differences. Parameters for sugar and ripeness were significantly impacted by the treatments. Crop-thinning raised levels of the sum of glucose and fructose while defoliation lowered it significantly in regards to the control group and also slightly within the two crop-thinning treatments. The same pattern is seen in °Brix and density where defoliation alone led to the lowest, the crop-thinning treatment to the highest and the control group to values in between the former two. Again, defoliation had a slight decreasing effect in the vines that were crop-thinned. Total acidity was significantly lower in the crop-thinning only treatment in regards to the other three groups. pH was lowered by defoliation and raised by crop-thinning, while tartaric acid was lowered by crop-thinning and showed elevated levels through defoliation. Malic acid tended to be lower in crop-thinned vines, but defoliation alone did not clearly impact values. Alpha amino nitrogen did not differ among the groups. The values for pH, malic and tartaric need to be considered as somewhat unreliable because of measurement issues.

| | **Control** | **DefCrop** | **Def** | **Crop** | ***significance*** |
|---|---|---|---|---|---|
| **Glucose + Fructose** | 219 b | 232 c | 202 a | 239 c | * |
| **°Brix** | 21,5 b | 22,6c | 20 a | 23,2 c | * |
| **Density** | 1093 b | 1097 b | 1084a | 1100b | * |
| **Total acidity** | 9,42 b | 9,55 b | 9,77 b | 8,65 a | * |
| **pH** | 3,09 ab | 3,08 ab | 3,00 a | 3,14 b | * |
| **Tartaric acid** | 5,9 ab | 6,3 b | 6,3 b | 5,5 a | * |
| **Malic acid** | 3,6 b | 3,3 ab | 3,6 ab | 3,2 a | * |
| **Alpha amino nitrogen** | 156 | 154 | 154 | 148 | ns |

***Table 5.*** *Results from WineScan analysis of the juice samples from the field trial, Control = no treatment, DefCrop = defoliation and crop-thinning, Def = defoliation only, Crop = crop-thinning only; different letters represent statistically significant differences based on ANOVA with * = p<0.05, ns = not significant*

## 5.3.2. Wine

### 5.3.2.1. Greenhouse

Table 6 shows 9 parameters analysed by WineScan in the wines made from the greenhouse trial. Ethanol was highest in the control group that did not experience water stress and tended to decrease with later and later stress timing. The differences were not significant however. Significant differences were only observed in malic acid content where the control group showed the highest levels, both early and mid stress treatment had significantly lower values and late stress had lower values although not significant. No differences in pH were observed as well as no differences in total acidity. Tartaric acid tended to increase with water deficit especially with mid and late stress but only to a small extend and not significantly. Glycerol slightly decreased in all water stress treatments compared to the control. Volatile acidity was unaltered by water deficit, regardless of timing. Fructose remaining after fermentation was highest in the unstressed control group and similarly lower in the stress groups. Reducing sugar was similar in all groups except in the early stress treatment where it was lower. Only malic acid showed significance in regard to differences among groups.

| | **Control** | **Early Stress** | **Mid Stress** | **Late Stress** | ***significance*** |
|---|---|---|---|---|---|
| **Ethanol** | 13,42 | 13,34 | 13,03 | 12,92 | ns |
| **Malic acid** | 2,01b | 1,77a | 1,77a | 1,82ab | * |
| **pH** | 3,15 | 3,16 | 3,13 | 3,13 | ns |
| **Tartaric acid** | 3,19 | 3,21 | 3,37 | 3,41 | ns |
| **Total acidity** | 7,85 | 7,73 | 7,79 | 7,82 | ns |
| **Glycerol** | 7,31 | 7,21 | 7,08 | 6,97 | ns |
| **Volatile acidity** | 0,13 | 0,16 | 0,14 | 0,12 | ns |
| **Fructose** | 1,23 | 1,00 | 1,06 | 1,03 | ns |
| **Reducing sugar** | 0,57 | 0,28 | 0,51 | 0,42 | ns |

***Table 6.*** *Results from WineScan analysis of the wine samples from the greenhouse trial, differentiated by water stress timing; test for significance was carried out by ANOVA with a significance level of p<0,05: ns = not significant*

### 5.3.2.2. Field Trial

Table 7 shows 9 parameters analysed by WineScan in the wines made from the field trial. Crop-thinning significantly increased ethanol levels and defoliation significantly decreased ethanol compared to the control. Comparing both treatments that were crop-thinned defoliation slightly decreased ethanol but the difference was not significant. Concerning malic acid, the *DefCrop* treatment showed significantly higher values than the *Crop* treatment with the other two treatments showing values in between, not significantly different from the aforementioned two. Similar patterns were observed in regards to pH, where the crop-thinning alone significantly increased and defoliation alone significantly decreased pH levels, while the mixed treatment showed similar values as the control group. In both total acidity and

tartaric acid defoliation alone produced the significantly highest levels and crop-thinning alone the significantly lowest levels. The mixed treatment showed lower levels than the control in both cases even though the difference in regards to total acidity was very small. Glycerol was significantly decreased by defoliation and significantly increased by crop-thinning; the mixed treatment was slightly higher than the control group. Volatile acidity was significantly increased by crop thinning only compared to the other three treatments who showed similar values. Both treatments that were crop-thinned showed similarly higher levels in both fructose and reducing sugars left over from fermentation and in both cases, defoliation was slightly higher than the control group. The differences regarding sugars were not significant, however.

| | **Control** | **DefCrop** | **Def** | **Crop** | ***significance*** |
|---|---|---|---|---|---|
| **Ethanol** | 13,27b | 14,3c | 12,52a | 14,44c | * |
| **Malic acid** | 3,63ab | 3,73b | 3,58ab | 3,43a | * |
| **pH** | 3,01b | 3,04bc | 2,88a | 3,11c | * |
| **Tartaric acid** | 4,17ab | 3,19ab | 4,87b | 2,64a | * |
| **Total acidity** | 9,68b | 9,66b | 10,24b | 8,93a | * |
| **Glycerol** | 7,48b | 7,88b | 6,23a | 8,39c | * |
| **Volatile acidity** | 0,23a | 0,24a | 0,23a | 0,28b | * |
| **Fructose** | 1,17 | 1,78 | 1,19 | 1,73 | ns |
| **Reducing sugar** | 0,76 | 1,47 | 0,86 | 1,46 | ns |

***Table 7.*** *Results from WineScan analysis of the wine samples from the field trial, Control = no treatment, DefCrop = defoliation and crop-thinning, Def = defoliation only, Crop = crop-thinning only; different letters represent statistically significant differences based on ANOVA with * = p<0.05, ns = not significant*

## *5.4. $^{1}$H-NMR-Spectroscopy*

Figure 26 shows a representative $^{1}$H-NMR spectrum of a juice sample obtained from greenhouse grapes. The spectrum is very crowded and complex due to a high degree of signal overlapping. For the sake of simplicity, the NMR spectrum has been divided into 3 main regions: the aliphatic, carbohydrates and aromatic regions. In the aliphatic region, from 0.00 to 3.00 ppm, the signals from non-aromatic amino acids (for example alanine, arginine, valine and leucine) resonate and overlap with the resonances of the organic acids succinate, lactate, acetate, citrate and malate. The carbohydrates, which resonate in the spectral region between 3.01 and 5.50, dominate the spectral landscape in the juice samples. Finally, in the aromatic region, various phenolic compounds together with the alkaloid trigonelline, and the amino acids phenylalanine and tryptophan could be identified. A total of 22 metabolites were identified in the juice samples. A more detailed metabolite assignment is provided in Figure 26. Individual phenolic compounds are very hard to assign since a lot of them overlap in chemical shift and a confined within a very dense, narrow region. Some research (Ali et al. 2011) was able to

assign compounds like monomeric catechin and epicatechin or gallic acid. However, in the NMR data of the present study these identifications were not possible.
Fig.24 shows a representative $^{1}$H-NMR spectrum of a wine sample from the greenhouse trial where a total of 19 metabolites could be assigned. The two ethanol signals show the highest concentration by far. Amino acids valine, alanine, proline and aminobutyric acid could be found in the aliphatic region, as well as organic acids lactate, succinate, citrate and malate. Other alcohols, such as isopropanol in the aromatic region and glycerol and methanol in the carbohydrate region, could also be observed. The carbohydrate region is also the location where tartaric acid was found. Tryptophan, trigonelline, phenylalanine, tyrosine and xanthine could be identified in the aromatic region. The difference between a $^{1}$H-NMR juice spectrum and a $^{1}$H-NMR wine spectrum is mainly defined by the conversion of sugars to alcohols, as well as by an increase in lactate and succinate and a decrease in various non-aromatic amino acids. The aromatic region of the wine sample showed higher concentrations of the assigned metabolites compared to the juice sample and the emergence of a new compound from juice to wine in the aromatic region (xanthine) could also be observed.

A total of 13 baseline-resolved metabolites could be quantified in the juice samples, while 9 metabolites were quantified in the wines. The results are shown in Table 8 and Table 9, respectively.

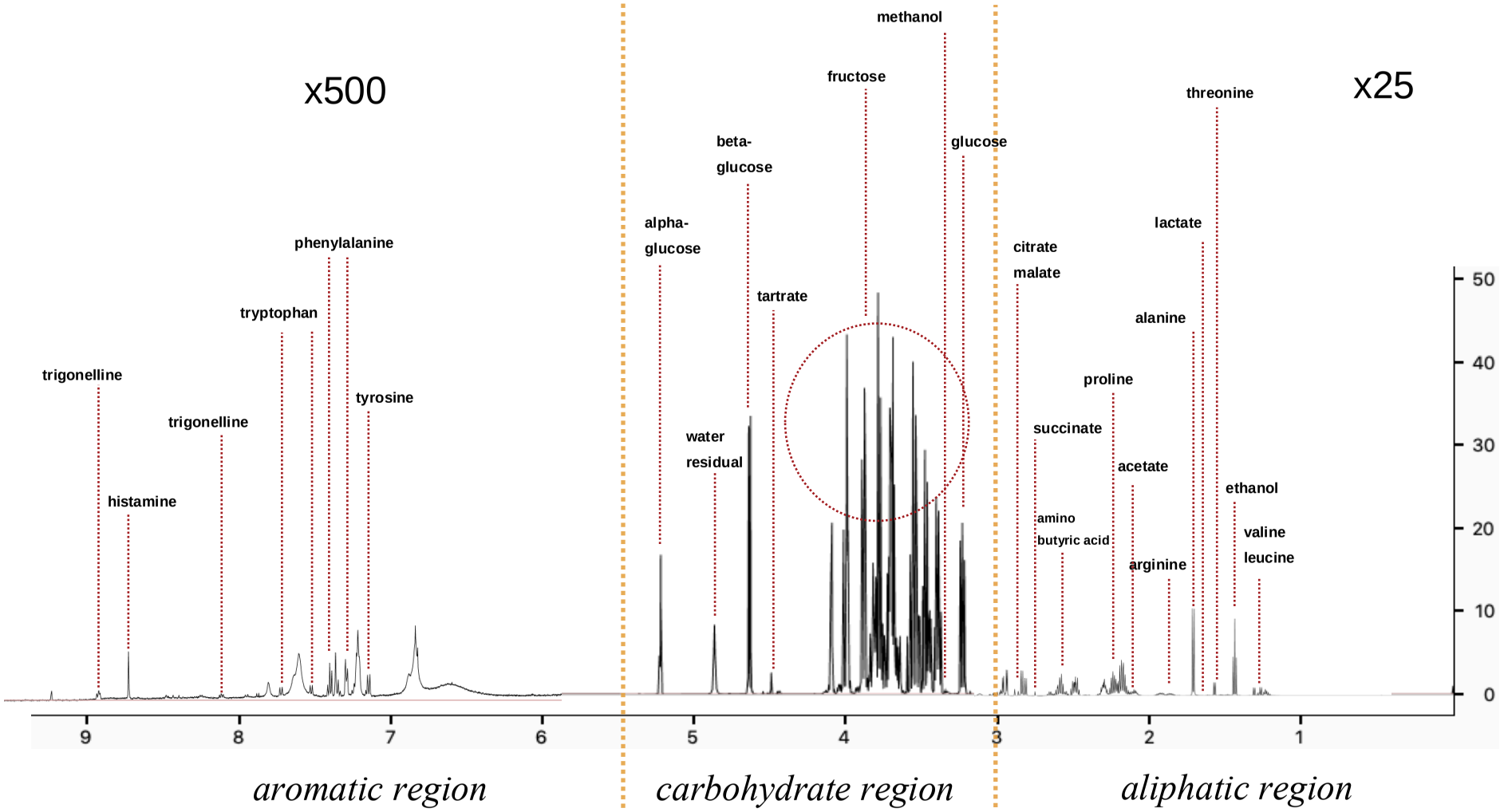


***Figure 24.*** *Example of an NMR spectrum from a juice sample of the greenhouse trial; aliphatic region (0.00 - 3.00 ppm) containing most amino acids, most organic acids and one of the two major ethanol signals, carbohydrate region (3.01 - 5.50 ppm) containing the second major ethanol signal as well as glycerol, methanol and tartaric acid, aromatic region (5.51 - 9.20 ppm) containing phenolic compounds and various amino acids; metabolic assignment was based on previous NMR literature, online NMR spectra databanks and the profiler function of Chenomix NMR Suite (8.4)*

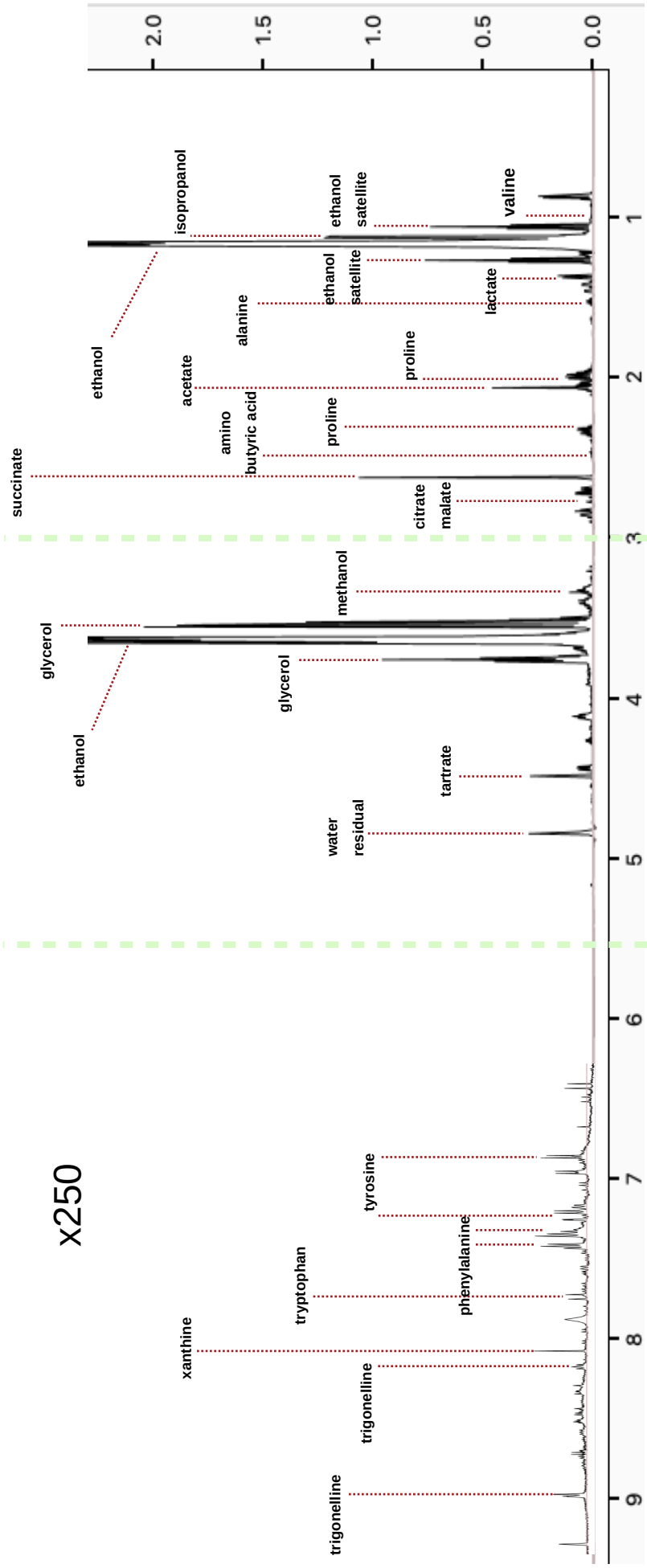


***Figure 25.*** *Example of an NMR spectrum from a wine sample of the greenhouse trial; aliphatic region (0.00 - 3.01 ppm) containing most amino acids, most organic acids and one of the two major ethanol signals, carbohydrate region (3.00 - 5.50 ppm) containing the second major ethanol signal as well as glycerol, methanol and tartaric acid, aromatic region (5.51 - 9.50 ppm) containing phenolic compounds and various amino acids; metabolic assignment was based on previous NMR literature, online NMR spectra databanks and the profiler function of Chenomix NMR Suite (8.4)*

### 5.4.1. Table of Metabolites

#### 5.4.1.1. Juice

Table 8 shows the average relative concentrations of selected metabolites for grape juice with standard deviation. The metabolite selection was based on identifiability in the NMR spectrum as well as relevance in enology. Glucose and fructose were the most abundant compounds with glucose being slightly higher in relative concentration. The field trial was slightly higher in average sugar levels but with lower standard deviations. The third most prevalent compound was tartaric acid, with a slightly higher average in the greenhouse samples. Citric acid on average was more than twice as high in terms of relative concentration in the field samples. The most abundant amino acid was alanine, followed by proline, leucine, threonine, valine and phenylalanine. All amino acids were much more abundant in the greenhouse samples, most of the times by a factor of 2. Ethanol, methanol and succinic acid, three compounds mostly formed during fermentation, were also detected in small amounts and the relative concentrations of all three were higher on average in the field samples.

| Metabolite | average greenhouse | s.d. greenhouse | average field | s.d. field |
|---|---|---|---|---|
| **Alanine** | 50,6 | 5,2 | 29,6 | 4,4 |
| **Citrate** | 6,6 | 1,0 | 15,2 | 0,9 |
| **Ethanol** | 36,2 | 4,3 | 46,8 | 15,3 |
| **Fructose** | 982,3 | 95,9 | 1032,2 | 59,5 |
| **Glucose** | 1141,8 | 122,8 | 1158,5 | 86,5 |
| **Leucine** | 8,3 | 0,9 | 4,4 | 0,6 |
| **Methanol** | 15,1 | 3,1 | 15,4 | 2,6 |
| **Phenylalanine** | 1,3 | 0,6 | 0,7 | 0,5 |
| **Proline** | 29,9 | 10,1 | 11,7 | 4,3 |
| **Succinate** | 1,0 | 0,2 | 1,2 | 0,3 |
| **Tartrate** | 53,9 | 6,6 | 50,6 | 4,5 |
| **Threonine** | 7,0 | 0,6 | 4,0 | 0,5 |
| **Valine** | 4,4 | 0,5 | 2,2 | 0,3 |

***Table 8.*** *Relative concentrations of the baseline-resolved integrated metabolites from NMR measurements of the juice samples; values were divided by $10^6$ for better visualization; s.d. = standard deviation*

#### 5.4.1.2. Wine

Table 9 shows the average values and standard deviations for relative concentrations of the selected metabolites of the wine samples. Ethanol is by far the most abundant compound with higher average in the field samples. The greenhouse, however, showed higher standard deviations in ethanol. The second most prevalent compound was the alcohol glycerol with also higher averages in the field trial. Methanol was very similar between greenhouse and field with higher standard deviation in the greenhouse samples. Tartrate showed similar average values in both experiments, with the field trial having slightly higher levels. Also, the other acids, succinic, citric and acetic, were more abundant in the field samples, in case of citric acid even by a factor of 2. Proline was more than twice as high in the greenhouse samples while phenylalanine was almost twice as high in the field samples.

| Metabolite | average greenhouse | s.d. greenhouse | average field | s.d. field |
|---|---|---|---|---|
| **Acetate** | 3,2 | 1,1 | 4,4 | 1,4 |
| **Citrate** | 1,1 | 0,2 | 2,2 | 0,2 |
| **Ethanol** | 2047,1 | 253,1 | 2139,0 | 181,6 |
| **Glycerol** | 79,2 | 10,2 | 84,8 | 9,1 |
| **Methanol** | 1,8 | 0,3 | 1,9 | 0,2 |
| **Phenylalanine** | 0,04 | 0,01 | 0,1 | 0,01 |
| **Proline** | 5,9 | 1,8 | 2,5 | 0,8 |
| **Succinate** | 11,0 | 1,3 | 12,8 | 1,1 |
| **Tartrate** | 4,0 | 0,3 | 4,0 | 0,3 |

***Table 9.*** *Relative concentrations of the baseline-resolved integrated metabolites from NMR measurements of the wine samples; values were divided by $10^6$ for better visualization; s.d. = standard deviation*

### 5.4.2. PCA Juice

#### 5.4.2.1. Metabolites Table

PCA was performed on the metabolites table of all juice samples. PC1 and PC2 account for 73,78% of total variance (Fig.26). A clear separation between greenhouse and field samples could be observed along PC1, where the scores distribution is mainly driven by amino acids proline, valine, alanine, leucine, threonine and, to a lesser extent, phenylalanine, as well as slightly driven by tartaric acid. Sample distribution along PC2 is mainly driven by fructose, glucose and methanol with the latter two appearing to be tightly correlated. However, no differentiation between experiments can be made based on PC2 even though there is a broad range of respective PC2 scores within the individual experiments themselves. Greenhouse juices are characterized by higher levels of amino acids and tartaric acid, while juices from the field trial are associated with higher levels of ethanol and citric acid. 18 pooled samples, a summation of all juice samples, was included in the PCA in order to visualize the chemical average.

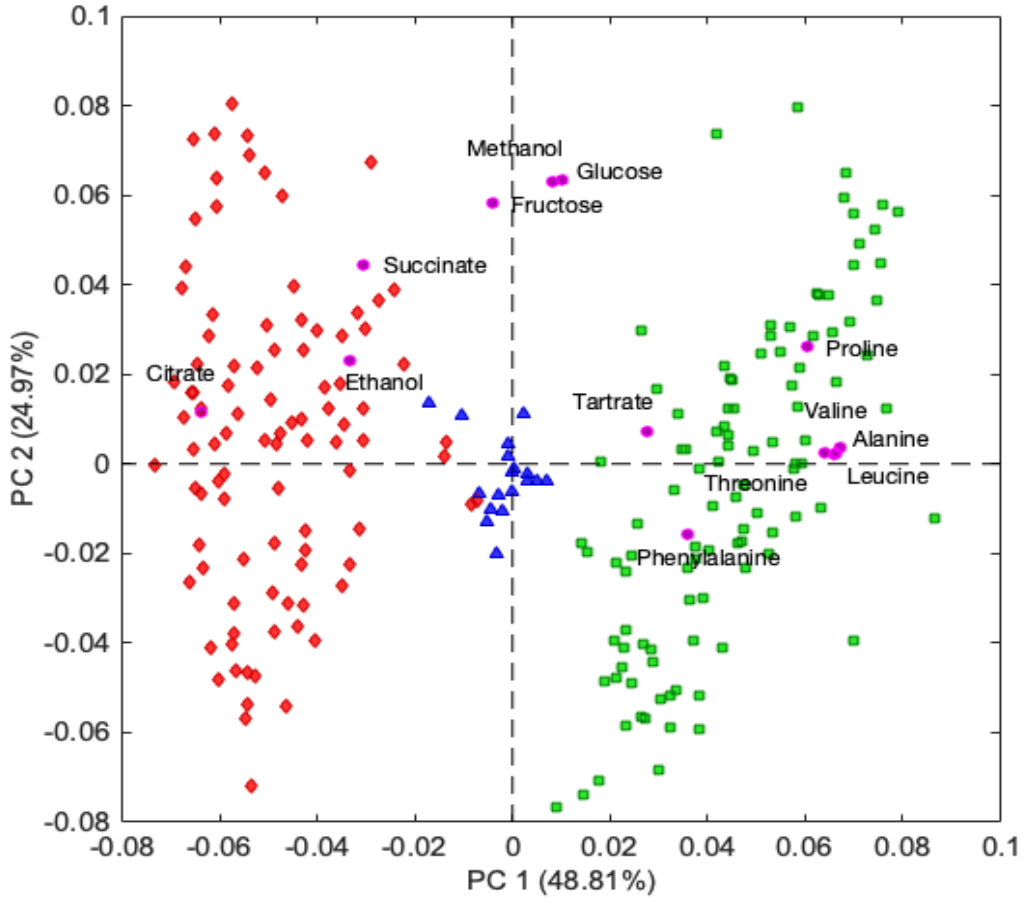


***Figure 26.*** *PCA biplot of the metabolites table of all juice samples; PC1 and PC2 account for 73,78% of total variance; red = field trial, green= greenhouse; blue = pooled samples of all juices representing chemical average*

*Greenhouse*

PCA that was performed on the metabolites table of the greenhouse juice samples. 65,32% of total variance is explained by its two main PCs. The main drivers of PC1 are all measured amino acids except for phenylalanine, which was interestingly not correlated to PC1 in any direction. Methanol, glucose and fructose additionally drive score distribution along PC1 and methanol seems to be tightly correlated to the amino acids threonine and leucine. Scores along PC2 are mainly driven by succinic acid, ethanol and citric acid. Group separation was only possible based on the pruning types (Fig.27) of the plants where single cane vines show higher PC1 scores and therefore higher levels of amino acids (except phenylalanine), sugars and methanol. A few samples diverged from this pattern but an overall tendency can be observed. Regarding water stress timing, no clear group separation can be observed, nor a difference between all stressed plants and the irrigated control group.

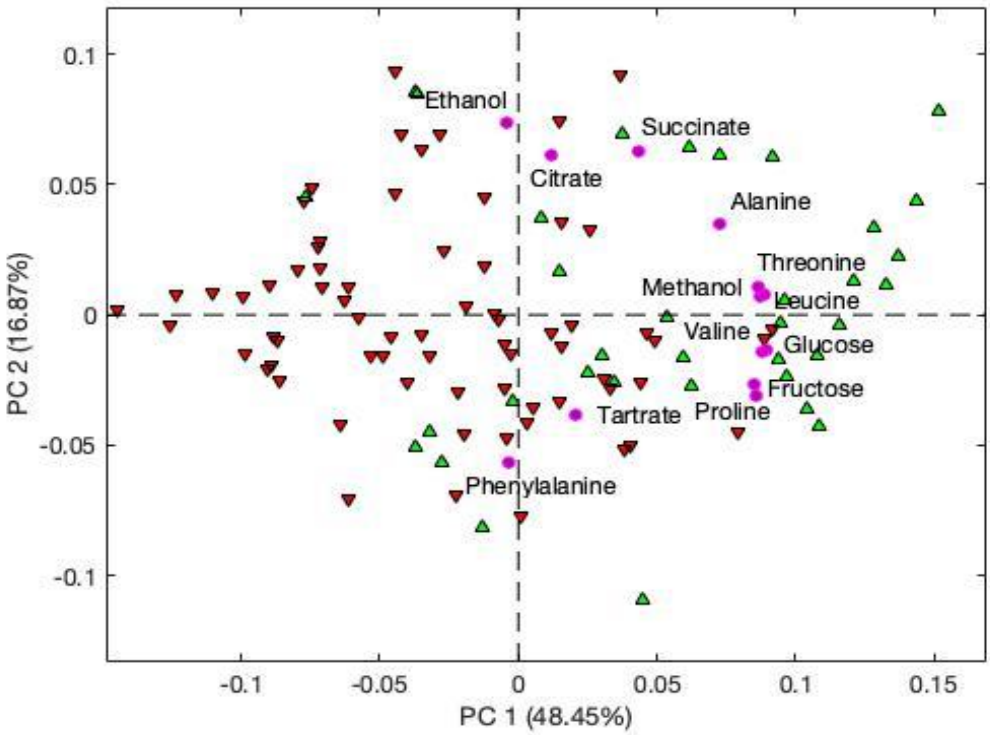


***Figure 27.*** *PCA biplot of the metabolites table of the juice samples from the greenhouse; PC1 and PC2 represent 65,32% of total variance; red = double cane, green = single cane*

*Field Trial*

PCA models performed on the metabolite table of the juice samples from the field trial are displayed in Fig.28 and Fig.29. 61,55 % of total variance is explained by PC1 and PC2. Sample grouping based on crop-thinning is possible along PC1 and is driven mainly by the amino acids (again, except for phenylalanine), sugars and methanol. It can be observed that samples from the crop-thinning treatments tend to show higher levels of these compounds. Even though scores along PC2 did not differentiate the samples based on crop load, separation between samples from defoliated and non-defoliated vines can be observed along PC2. A tendency is apparent that juices from defoliated plants score higher on PC2 and therefore show higher levels of its main drivers, which are all amino acids except proline and tartaric acid. The absence of defoliation results in a tendency to higher levels of mainly succinic acid, citric acid, methanol, ethanol, and sugars. The amino acids threonine, valine, leucine and alanine seem to be correlated in both experiments while phenylalanine exhibits different behaviour. While the other amino acids drive scores distribution along PC1 and PC2 to a similar extent, phenylalanine is more related to scores on PC2 and fairly neutral in regards to PC1. Therefore crop-thinning seems to have less of an impact on phenylalanine than defoliation.

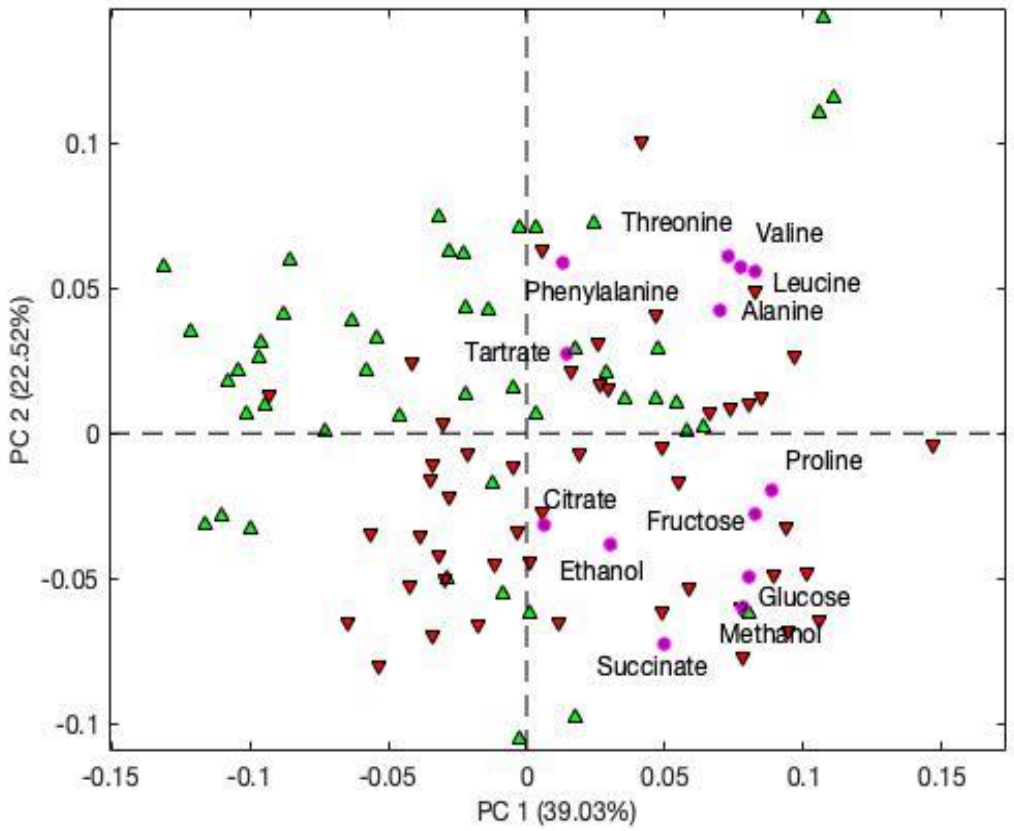


***Figure 28.*** *PCA biplot of the metabolites table of the juice samples from the field trial; PC1 and PC2 represent 61,55% of total variance; green = defoliated, red = full canopy*

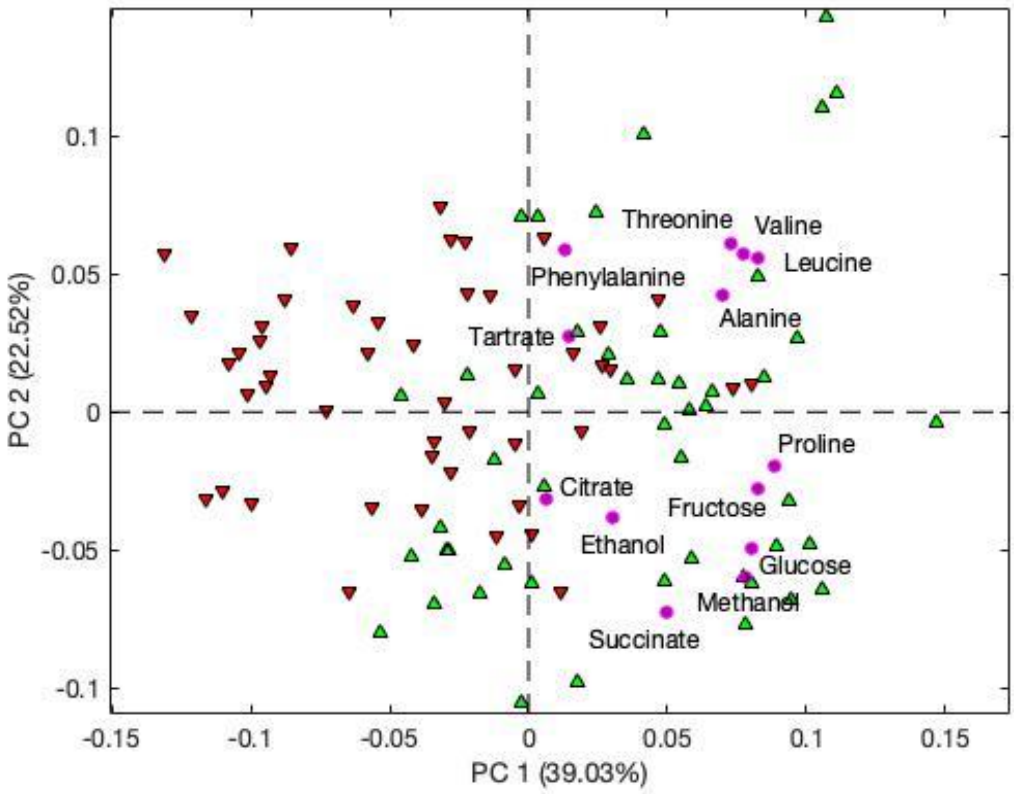


***Figure 29.*** *PCA biplot of the metabolites table of the juice samples from the field trial; PC1 and PC2 represent 61,55% of total variance; green = crop-thinned, red = full crop*

5.4.2.2. Combined Matrix

The NMR data and WineScan (FTIR) data were merged into a new data matrix for additional multivariate analysis. It was conducted separately for juice and wine samples and the juice results are presented in the following section. The wine results will be provided later in the wine section under the same title.

PCA was performed on all juice samples to investigate the variability among them. The results are reported in Fig.30 as a biplot with PC1 and PC2 together accounting for 73,71% of total variance. A clear separation along PC1 can be observed between samples from the greenhouse trial and the field trial. Samples distribution along PC1 is mainly driven by amino acids valine, threonine, alanine, potassium, alpha amino nitrogen and were found to be more abundant in the greenhouse samples while the other drivers of PC1, malic acid, citric acid and total acidity, showed higher levels in the field samples. Grape juice pH and tartaric acid also tend to be higher in the greenhouse samples, while no separation could be made based on ripeness parameters density, sugars or °Brix, which are associated with PC2.

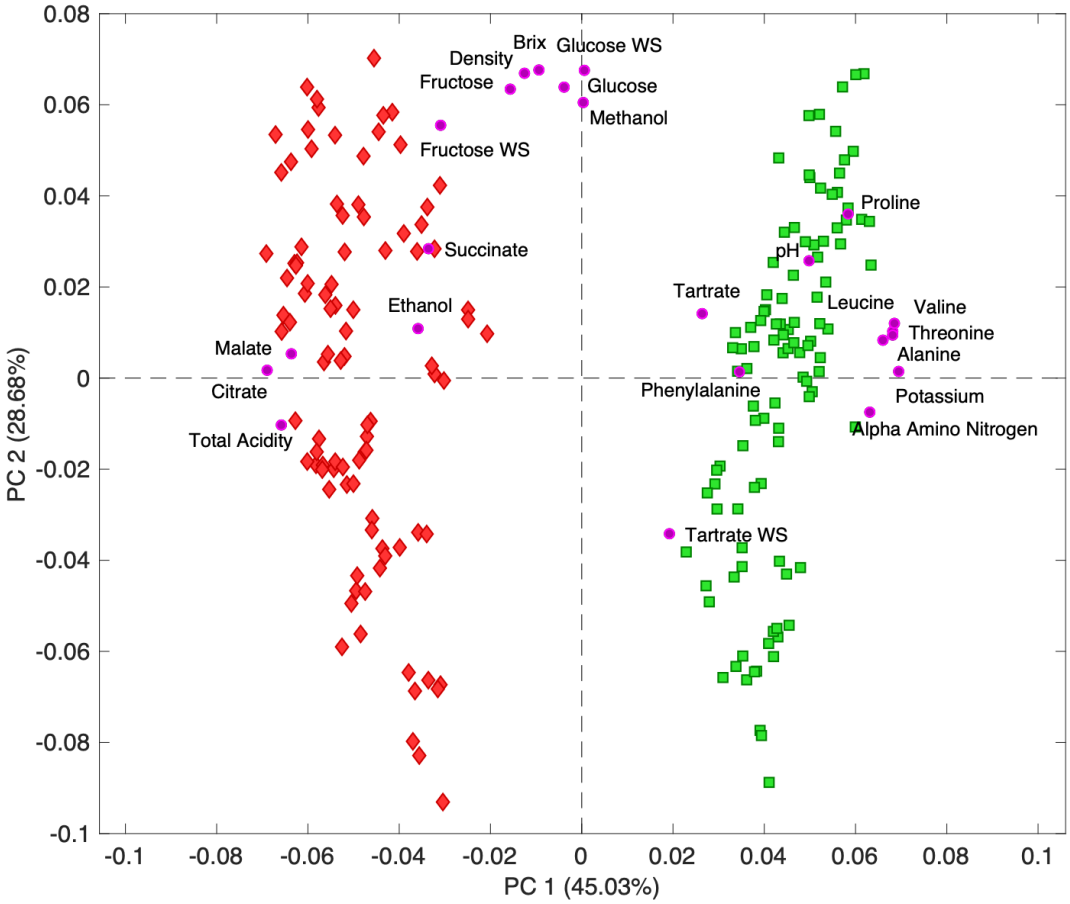


***Figure 30**. PCA biplot of the NMR-FTIR matrix of all juice samples; PC1 and PC2 account for 73,71% of total variance: green = greenhouse, red = field trial; WS = measurement obtained by WineScan*

It is important to note that missing values in the WineScan dataset (see Section 5.3.1.2) that were incorporated into the mixed matrix do not influence the model(s) since PLS_Toolbox replaces missing values with the average of the available values.

*Greenhouse*

PCA was also performed on the combined NMR-NIR matrix of the juice samples from the greenhouse. Fig.31 shows the corresponding biplot with PC1 and PC2 explaining 60,50% of total variance. Distinction between samples based on pruning types reveals a separation along PC1, which is mainly driven by ripeness parameters, such as density and sugars, and all amino acids besides phenylalanine. Juices from single cane vines are found to be more abundant in glucose, fructose, leucine, threonine, proline, valine, alanine and also tended to have higher pH. Similar to the results above, phenylalanine does not show a correlation to the other amino acids. No separation between pruning types along PC2, which is mainly driven by citric and malic acid, is visible. Again, water stress timing does not show any influence on the distribution of samples along any of the PCs. Nor is any clear difference apparent between irrigated control and stressed plants in general.

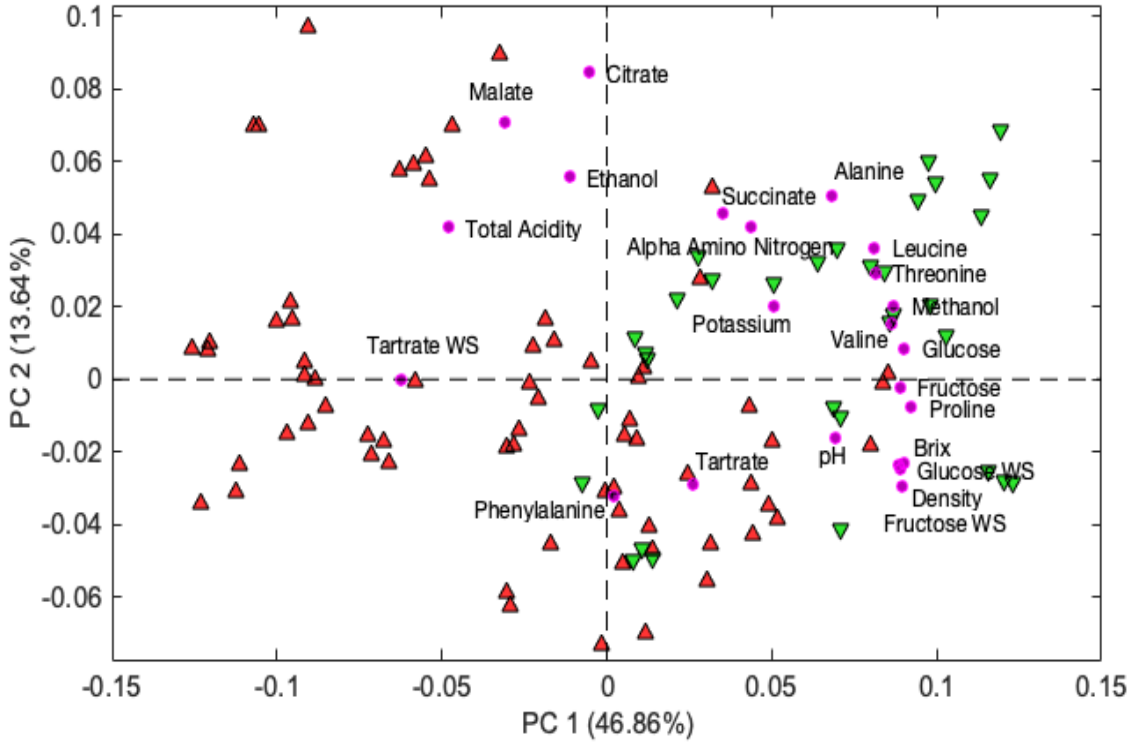


***Figure 31.*** *PCA biplot of the NMR-FTIR matrix of the juice samples from the greenhouse; PC1 and PC2 account for 60,50% of total variance; red = double cane, green = single cane; WS = measurement obtained by WineScan*

*Field Trial*

PCA on the mixed matrix of the juice samples from the field trial revealed a clear trend of sample clustering according to the different treatment groups (Fig.32). PC1 and PC2 account for 56,53% of total variance. In particular juices from the *Crop* group (crop-thinning only) were visibly separated from the other samples along PC1 and show higher abundance glucose, fructose, methanol, succinic acid, malic acid, proline and ripeness indicators such as °Brix and density. The *Def* group, which was only defoliated, shows higher values of total acidity and tartaric acid. The samples from the other two groups generally scored in between the *Def* and the *Crop* group. Separation of samples based on crop-thinning instead of treatment groups gives an even more clear pattern with the juices from crop-thinned plants

showing higher abundance in the aforementioned PC1 drivers glucose, fructose, methanol, succinic acid, proline, °Brix and density, as well as higher levels of alanine, threonine, leucine, valine and malic acid. The corresponding biplot is reported in Fig.33 Despite the visible sample clustering according to treatment groups no clear separation of samples based on defoliation was possible.

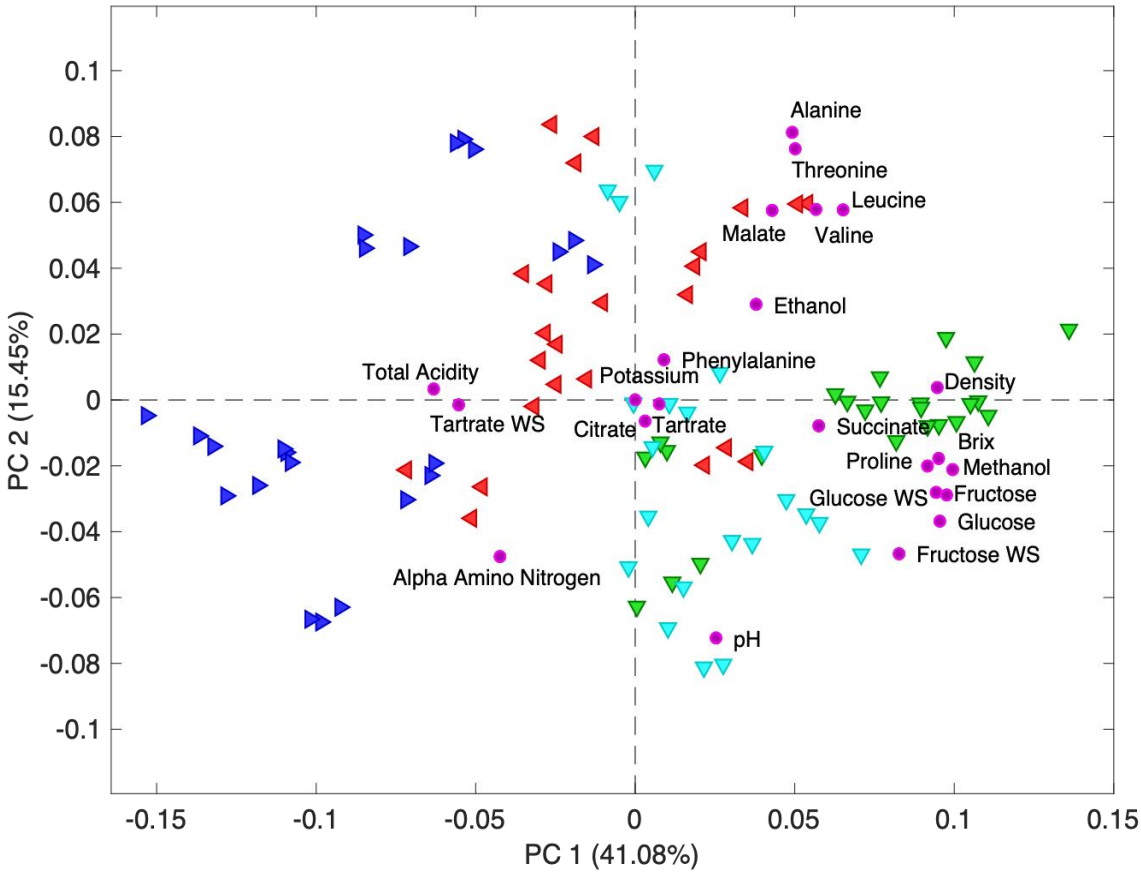


***Figure 32.*** *PCA biplot of the NMR-FTIR matrix of the juices from the field trial; PC1 and PC2 accounted for 56,53% of total variance; green = crop-thinned, turquoise = crop-thinned + defoliated, red = untreated control, blue = defoliated;*
*WS = measurement obtained by WineScan*

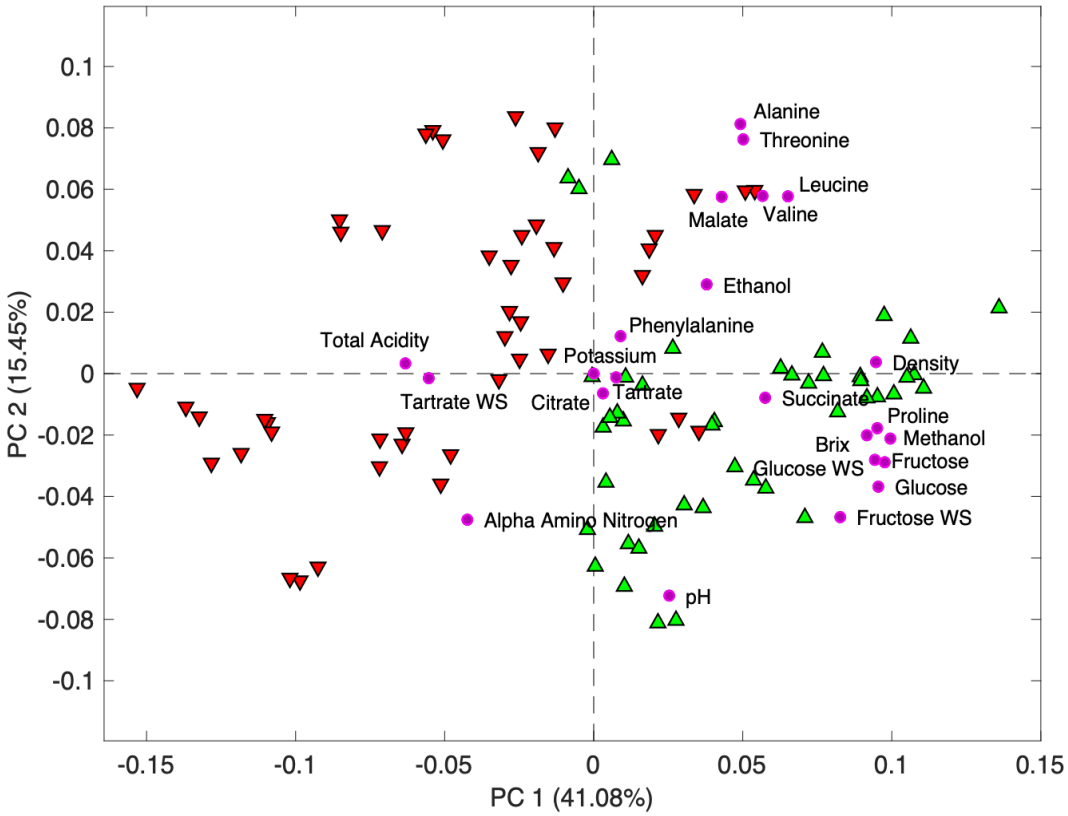


***Figure 33.*** *PCA biplot of the NMR-FTIR matrix of the juices from the field trial; PC1 and PC2 account for 56,53% of total variance: green = crop-thinned, red = full crop load; WS = measurement obtained by WineScan*

## 5.4.3. PCA Wine

### 5.4.3.1. Metabolites Table

Fig.34 shows the biplot of the PCA model on the metabolites table of all wine samples. PC1 and PC2 together account for 76,40% of total variance. A clear separation is observed between greenhouse and field samples along both PC1 and PC2. Phenylalanine, citric acid, acetic acid and succinic acid are the main drivers for the scores distribution along PC1 and are more abundant in the wines from the field trial. Proline is the main driver of the samples distribution on PC2 and is present in higher levels in wines produced from the greenhouse. Ethanol is driving the distribution along both PC1 and PC2 but is more abundant in the field wines. Again, 18 pooled wine samples have been included in the PCA to display the chemical average.

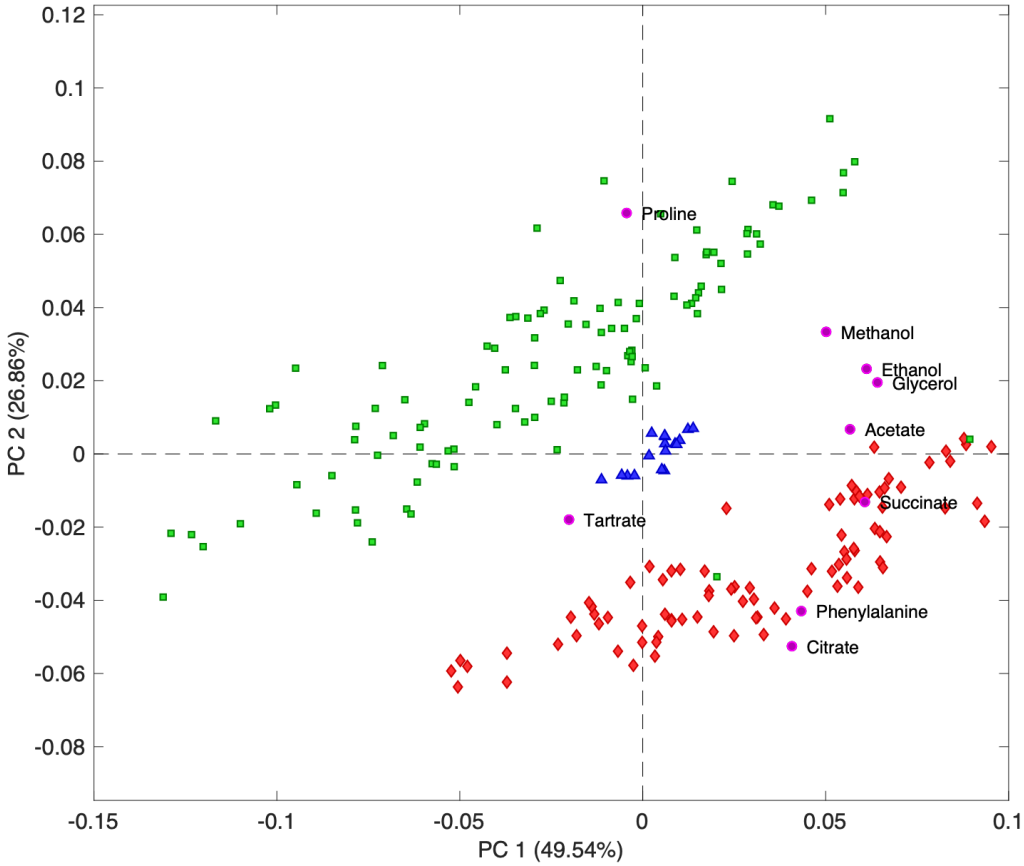


***Figure 34.*** *PCA biplot of the metabolites table of all wine samples; PC1 and PC2 account for 76,40% of total variance; red = field trial, green= greenhouse; blue = pooled samples that represent chemical average*

*Greenhouse*

PCA was performed on the mixed matrix of the wines from the greenhouse but no clear sample clustering was observed based on water stress, water stress timing or pruning type.

*Field Trial*

PCA of the metabolites table of the wines from the field trial is reported in Fig.35. A clear separation according to treatment group is visible. PC1 and PC2 account for 72,53% of systemic variance. Distribution of samples along PC1 is mainly driven by ethanol, methanol, glycerol, succinic acid and proline. These metabolites are found to be more abundant in the *Crop* group and the *DefCrop* group, both of which were crop-thinned. A separation based on crop-thinning is reported in the biplot in Fig.36. PC2 is mainly driven by citric acid and is more abundant in the *DefCrop* group and the *Def* group. No clear separation of samples is observed based on defoliation.

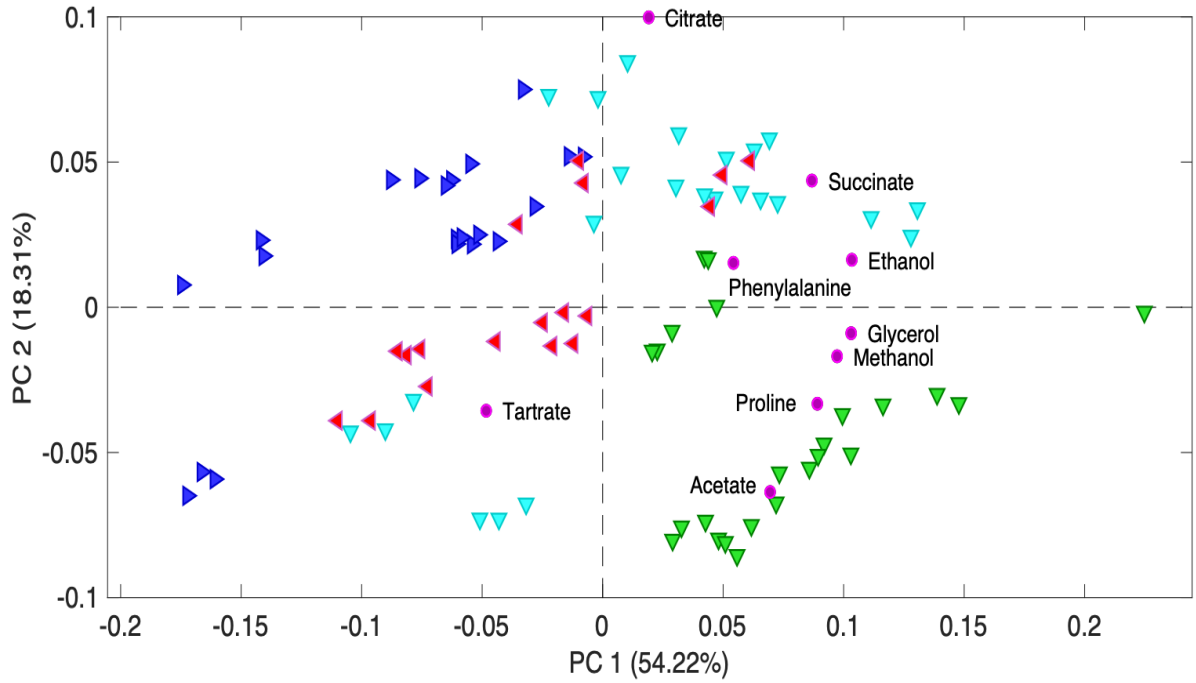


***Figure 35.*** *PCA biplot of the metabolites table of the wines from the field trial; PC1 and PC2 account for 72,53% of total variance; green = crop-thinned, red = untreated control, turquoise = crop-thinned + defoliated, blue = defoliated*

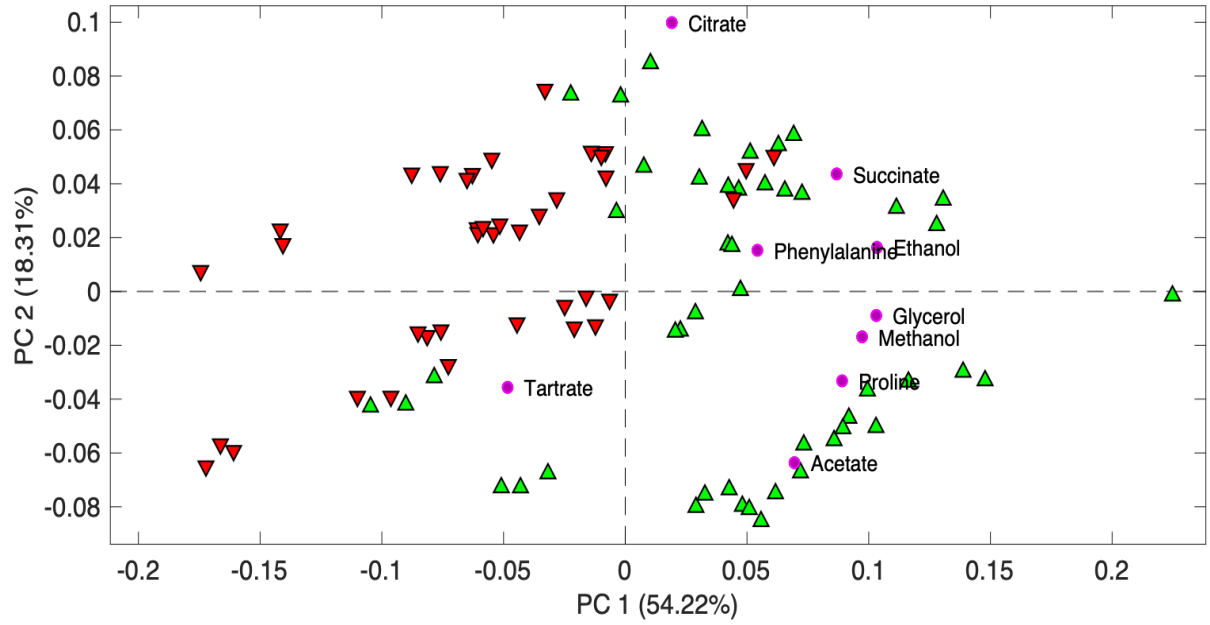


***Figure 36.*** *PCA biplot of the metabolites table of the wines from the field trial; PC1 and PC2 account for 72,53% of total variance; green = crop-thinned, red = full crop load*

5.4.3.2. Combined Matrix

PCA of all wine samples was also performed on the combined matrix of $^{1}$H-NMR and FT-IR data. 72,69% of systematic variance is accounted for by PC1 and PC2. A clear sample distribution is observed according to the type of experiment (Fig.37). PC1 is driven by most measured metabolites, especially ethanol, volatile acidity, reducing sugars, malic acid and total acidity. These compounds are found to be higher in wines produced from the field trial. The wines from the greenhouse are more abundant in proline and show higher levels of pH, both of which are the main drivers of scores distribution along PC2.

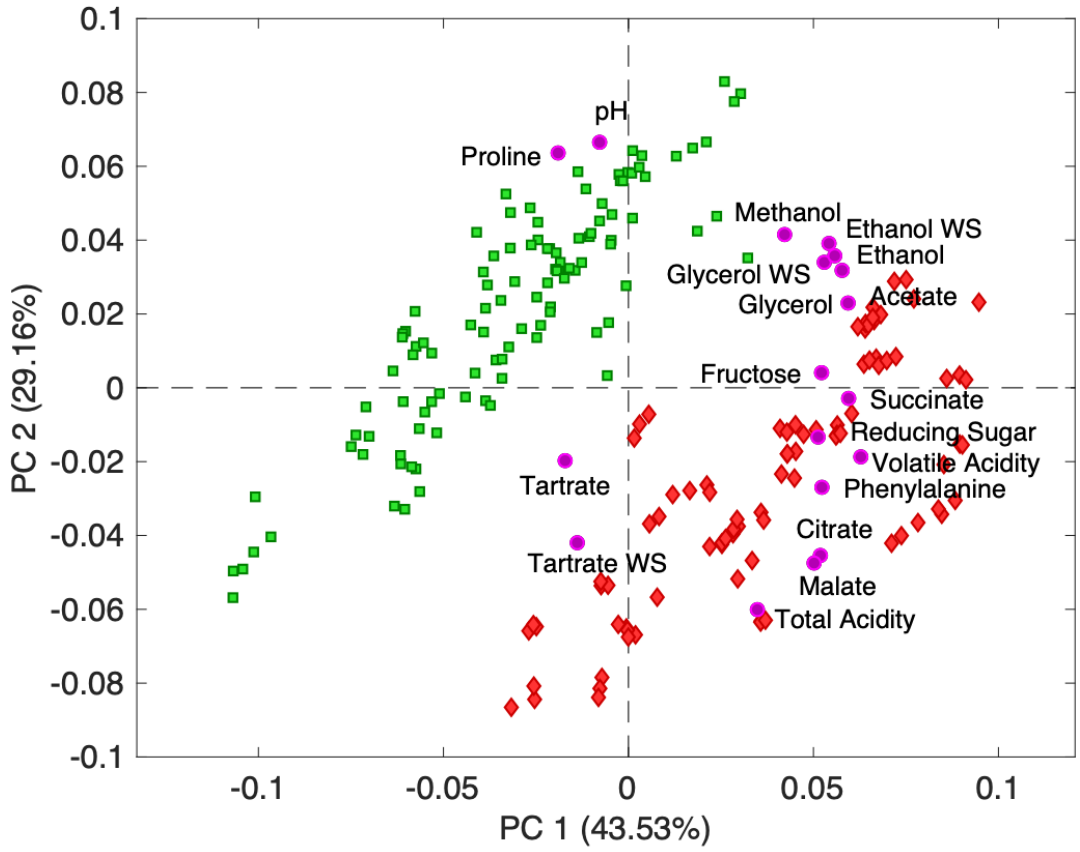


***Figure 37.*** *PCA biplot of the NMR-FTIR matrix of all wine samples; Pc1 and PC2 accounted for 72,69% of total variance; green = greenhouse, red = field trial*

*Greenhouse*

PCA was performed on the NMR-NIR matrix of the wines from the greenhouse. PC1 and PC2 account for 62,48% of total variance. Based on pruning type a separation is visible along PC1 with the samples produced from single cane vines being more abundant in ethanol, glycerol, acetic acid and proline, which are the main drivers of scores distribution along PC1. The biplot is reported in Fig.38. No separation is observed based on water stress or water stress timing.

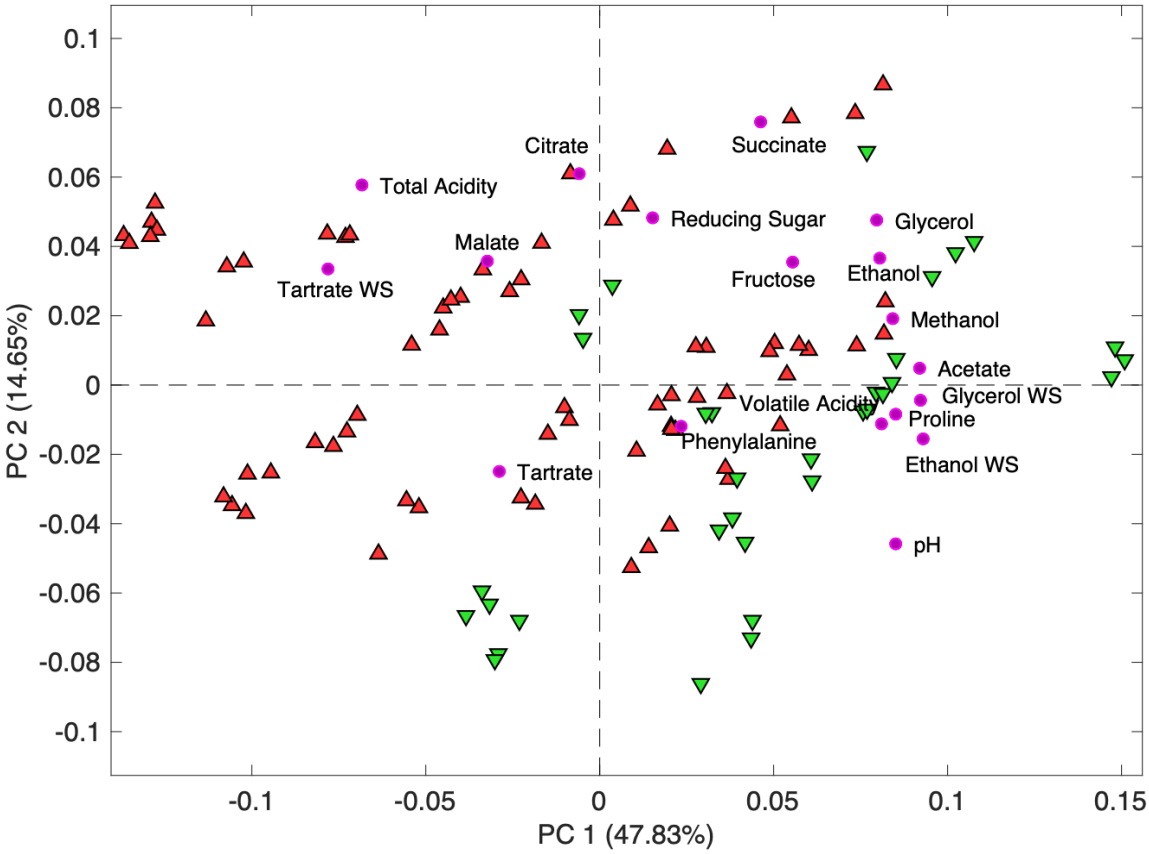


***Figure 38**. PCA biplot of the NMR-FTIR matrix of the wine samples from the greenhouse; PC1 and PC 2 accounted for 62,48% of total variance; green = single cane, red = double cane; WS = measurement obtained by WineScan*

*Field Trial*

PCA was also performed on the combined NMR-NIR matrix of all wines from the field trial. A clear clustering of samples is observed according to treatment group (Fig.40). PC1 and PC2 represent 63,13% of systemic variance. pH is found to be higher in wines from the Crop group, as well as ethanol, acetic acid, glycerol and volatile acidity. Total acidity and tartaric acid are more abundant in the Def group. The biplot in Fig.39 shows separation of samples based on crop-thinning and it is found that wines from crop-thinned plants are more abundant in ethanol, glycerol, volatile acidity, reducing sugars and show higher pH. Wines form non-crop thinned plants show higher levels of tartaric acid and total acidity. Again, sample differentiation based on defoliation did not produce any visible clustering of wine samples.

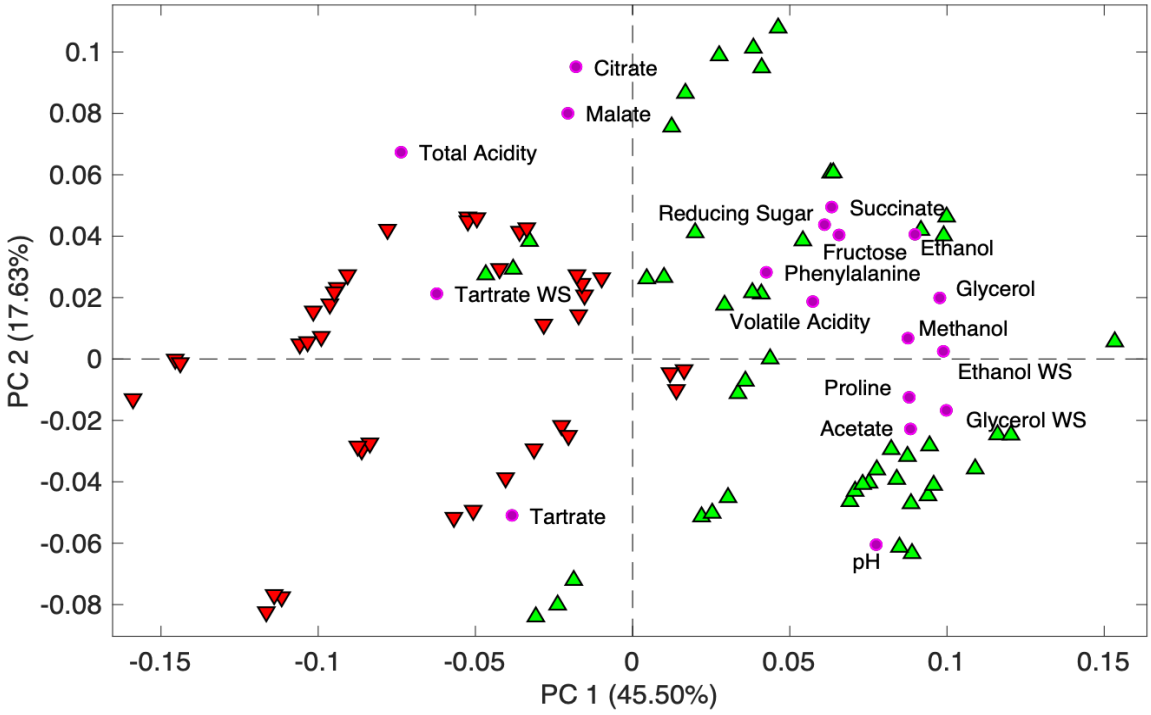


***Figure 39.*** *PCA biplot of the NMR-FTIR matrix of the wines from the field trial; PC1 and PC2 accounted for 63,13% of total variance; green = crop-thinned, red = full crop load; WS = measurement obtained by WineScan*

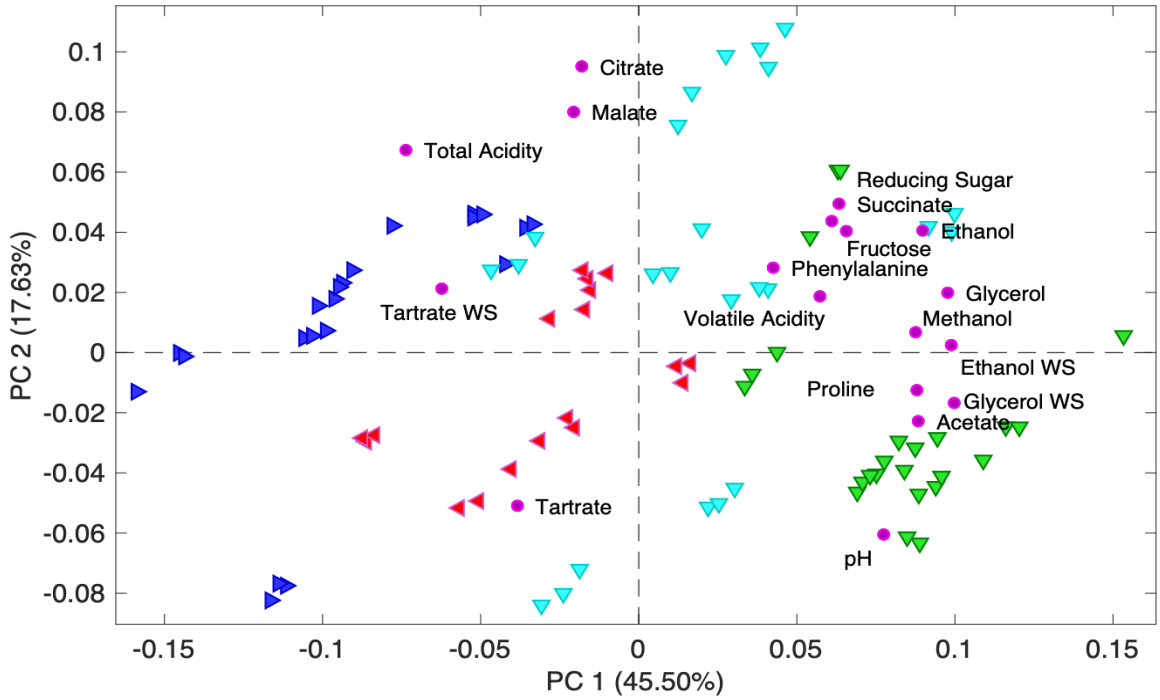


***Figure 40****. PCA biplot of the NMR-FTIR matrix of the wines from the field trial; PC1 and PC2 accounted for 63,13% of total variance; green = crop-thinned, turquoise = crop-thinned + defoliated, red = untreated control, blue = defoliated; WS = measurement obtained by WineScan*

# 6. Discussion

## *6.1. Vegetative Parameters and Yield*

*Greenhouse*

Primary leaf area was less affected by water stress than lateral leaf area, which was reduced by water deficit, especially in single cane vines (Table 2). This reduction in vegetative sink strength in form of lateral leaf area might also be the reason why an increase in primary leaf area was observed in stressed plants, again especially in single cane vines. This increase was also observed in research by Pellegrino et al. (2005) in mild stressed potted grapevines and Jun (1994) in rice. Pellegrino et al. (2005) also found that only severe water stress starts to decrease primary leaf area and therefore that the here applied water stress was either not severe or long enough or too late to affect primary leaf area. The stressed plants compensated for decreased shoot length by a higher number of shoots and a higher number of primary leaves. Even though this led to a similar primary leaf area, total leaf area was lower in all stress treatments through a reduction in lateral leaf area. Primary shoot fresh weight tended to be lower and individual leaf size tended to be smaller in stressed plants, regardless of pruning type. This is in accordance with other research that found reduced internode length as a function of water deficit (Matthews et al. 1987) and is probably based on a reduction in growth, by both decrease in relative water content of the cell and consequential reduction of cell size, as well as through limited CO2 uptake by closure of stomata following water stress (Keller, 2015, p. 276-279). The ratio of leaf area to gram of fruit was decreased by water stress slightly in both pruning types, more in single cane vines than in double cane vines, probably due to the much greater percentage of lateral leaf area in single cane vines (Table 3). It could be argued that plants with longer but less numerous shoots are more sensitive to a decrease in lateral leaf area through water deficit. Yield was unchanged by water stress in single cane vines and increased by water stress in double cane vines. This is surprising since lots of research found decreased yield by water stress (Hardie and Considine 1976, Esteban et al. 1999, Intrigliolo and Castel 2009). It could be speculated that the high temperatures in the greenhouse and high water supply in irrigated vines led to such a high vigour and vegetative growth that it started to impair on fruit growth. Furthermore, as pointed out by Chanishvili et al. (2005), defoliation, or in this case the reduction of lateral leaf area, can lead to enhance photosynthetic capacity in remaining leaves. When comparing the three stress phases in regards to yield (Fig.22), all stress treatments slightly increased yield compared to the irrigated control, implying that the leaf:fruit ratio of constantly irrigated vines having been too high for maximizing yield per plant and that water deficit at the level it was applied in this study can indeed actually reduce vegetative growth in exchange for slightly higher yield. This may also be explained by *Solaris* being a very vigorous cultivar that that tends to excessive vegetative growth when grown in optimal conditions such as a greenhouse (high temperatures, supply with water and nutrients). The most increase in yield was observed in the late stress treatment with more than 1kg more per plant compared to the irrigated control group. Comparing it to the average cluster weight there is a visible trend of decreasing cluster weight in the first two treatments but similar levels between control and late stress treatment (Fig.23). Provided that fruit-set was similar in all plants, which can be assumed in the present study, it can be reasoned that higher cluster weight is based on bigger berry size. Increased berry size through irrigation is reported various studies (Hardie and Considine 1976, Esteban et al. 1999, Intrigliolo

and Castel 2010). This would imply that a decrease in berry weight and size was only caused by early and mid stress and at the time of the late stress phase berry cell expansion was already finished. This of course still leaves the question of higher yield in the late stress treatment. If average cluster weight was similar to the control group, it would seem logical to assume a higher concentration of total soluble solids and total dry matter per berry, while still retaining the same amount of berry water content to account for the higher yield levels in the late stress treatment. This, however, is not reflected in the chemical analysis of juice composition (Table 4). Variations in available nutrients as a reason can be excluded since application of fertilizers was discontinued before the start of the experiment. Since the vines' reaction to water stress varied quite substantially even within groups which could be based on different fruiting and cropping histories from previous experiments, the variation in yield between the late stress group and the control group could also be based on small variations in the genotype between individual plants. The true cause for yield variation has to be considered uncertain at this point and could also be a combination of multiple factors such as plant history, small sample size and differences in vegetative parameters.

*Field Trial*

Defoliation showed a yield-reducing effect, but the effect was small and not significant (Fig.24). However, the general notion of decrease in yield following defoliation is often cited in literature through the mechanism of carbon source limitation (Bennet et al. 2005, Poni et al. 2006, Palliotti et al. 2012, Morano et al. 2016). However, this small effect disappeared and was even reversed a little bit as displayed by the treatment where defoliation and crop-thinning were combined bearing a little bit more fruit than the treatment where defoliation alone was applied. But this effect was also small and not statistically significant (Fig.24). Furthermore, the crop reduction was carried out quite severely with only 8 clusters per vine remaining and since homogenous cluster weight cannot be guaranteed, the yield fluctuations from vine to vine might have been quite high just purely based on different cluster weights. So, the general trend of yield reduction through defoliation was observed, albeit be it small, but it seems to be only relevant with normal levels of crop load. Average cluster weight was increased by all treatment forms compared to the control (Fig.25). Especially the increase based on defoliation alone is an interesting observation, since defoliation is sometimes reported to decrease berry skin weight (Valdivia 2001) and defoliated clusters are exposed to higher temperatures and therefore should have a lower berry water content. Defoliation also increased average cluster weight in the two crop-thinned treatments, although the effect was small and not significant. Even though defoliation can increase photosynthesis in remaining leaves and increase assimilate flux during midday hours (Chanishvili et al. 2005), no increase in solutes has been observed in the defoliation treatment compared to the control treatment (Table 5). Therefore it stands to reason that the additional cluster weight is either based on variations among the plants, which is unlikely since the effect of increased cluster weight was observed in both defoliation treatments compared to their respective controls, or based on a decrease in total water loss by a reduced canopy and consequently more water available for berry cell expansion. Some research, however, found a decrease in berry weight from defoliation (Kliewer 1970) and other research found a decrease in pericarp water content in berries from vines defoliated after veraison (Coombe et al. 1987). Therefore, the reason for higher average cluster weight is not fully understood but a big

influence of the soil-climate-variety-matrix can be assumed. Crop-thinning significantly increased average cluster weight (Fig.25), in agreement with other research (Wolpert et al. 1983, Palliotti and Cartechini 1998, Bubola et al. 2011). Probably because of a higher availability of solubles based on a higher source:sink ratio and a higher influx of potassium with water, which increases when crop load decreases (Hepner and Bravdo 1985).

## *6.2. Comparison of $^{1}$H-NMR Juice Spectrum and $^{1}$H-NMR Wine Spectrum*

The main difference between the juice and wine spectra is the dominance by sugars fructose and glucose and ethanol respectively, which is the very fundamental principle of wine fermentation where glucose and fructose are metabolized by yeast to produce predominantly ethanol and carbon dioxide (Berthels et al. 2004). Changes in amino acids, mores precisely their decreased concentrations in the wine samples, are based on the well-established fact that amino acids are a very important nutrient for yeast metabolism and yeast growth during fermentation (Bell and Henschke 2005).
The wine spectrum also showed much higher levels of the non-volatile organic acids lactic acid and succinic acid, as well as the volatile organic acid acetic acid, all of which are known to be produced during alcoholic fermentation (Thoukis et al. 1965).
The juice spectra were also absent of the higher alcohol isopropanol that could be observed in the wine spectra and is known to be produced during alcoholic fermentation from either sugars or amino acids (Boulton et al. 1998, p.150). Xanthine was also detected in wine spectra and is, according to the yeast metabolome database (ymdb.com), an extremely weak basic organic compound that is found in all living species, including yeasts.
Changes from juice to wine in the phenolic region are very hard to describe and quantify because of the overlapping of phenolic signals in the $^{1}$H-NMR spectrum and should be better studied in context of red wines because of their higher phenolic content and traditionally longer berry skin maceration time. Furthermore, the possibility of combining the 1H-NMR dataset with the FT-IR data set obtained from WineScan was beneficial to the study. It created the possibility to also assess factors that were not derived from NMR (either because of unassignability of spectral peaks such as malic acid or because of factors inaccessible to the present NMR method, such as pH) in a PCA context as well as to investigate the reliability of measurements between FT-IR and NMR by looking at the correlation between the same compound measured by both methods. Measurements by both NMR and FT-IR showed good correlations for fructose and glucose but not for tartaric acid. This can be argued to be due to missing values in the field juice samples (see Section 5.3.1.2).

## *6.3. Juice Composition*

*Differences between experiments*

The PCA plot in Section 5.4.2 showed a clear separation between the two experiments. The greenhouse samples showed a stronger correlation to the levels of measured amino acids, which is also documented in Table 8. The difference is probably based on the fact that the greenhouse plants were well supplied with fertilization in the irrigation water before the start of the experiment, while the field samples had to

extract all nutrients for building amino acids from the soil of the vineyard. Since amino acids are an important nitrogen source for yeast during fermentation (Bell and Henschke 2005) this underlines the benefits of fertilization in winemaking. Tartaric acid was also more correlated with the greenhouse samples. This could be due to the fact that over the entirety of the season, the field samples likely experienced less water stress than the greenhouse house samples and with additional water influx potassium is taken up, which could lead to tartrate precipitation, besides the aforementioned dilution effects of additional water supply. Furthermore, there is research suggesting that higher light exposure and temperature can increase tartaric acid synthesis (Tardaguila et al. 2010) and even though two of the treatments in the field trial were radically defoliated and hence experienced full light exposure this needs to be understood in a vineyard context. Because a greenhouse is by definition designed to receive as much light as possible and usually experiences dramatically higher temperatures than an open field system and decreased turgor and the consequential decreased shading area of leaves experiencing water deficit can be seen as a mild form of defoliation in regard to increased sunlight exposure. Perhaps the temperature is more crucial for the suggested stimulation of additional tartaric acid synthesis or perhaps it is a combination of the factors mentioned above. Levels of citric acid were much more correlated with the field samples, which may be due to a faster degradation by elevated greenhouse temperatures since citric acid level is known to decline over the course of the growing season (Sabir et al. 2010). The integration of the WineScan data into the PCA context revealed additional insight into the correlation of other metabolites to the type of experiment. Malic acid was much higher in the field samples than in the greenhouse (Fig.32). This is in accordance with lots of research saying that malic acid is degraded as a function of increasing temperatures (Lakso and Kliewer 1975, Ruffner 1982, Valdivia 2001, Conde et al. 2007) and greenhouse temperatures were higher and could easily compensate for the effect of two completely defoliated groups in the vineyard, that also experienced increased canopy temperatures. Tartaric acid is more robust in terms of temperature degradation and even though tartaric acid was more correlated with the greenhouse the overall levels for total acidity was more correlated with the field samples (Fig.32), a fact that is also reflected with higher pH levels being correlated with the greenhouse samples. Potassium and alpha amino nitrogen were higher in the greenhouse samples (Fig.32), likely also based on fertilization, similar to the amino acids. In terms of sugars and ripeness parameters such as °Brix and density the experiments could not be separated clearly. Based on the PCA plot in Fig.32 there seems to be a very slight tendency towards higher levels of fructose and density in the field samples, but this is very hard to interpret since the two experiments each received different types of treatments that could have sugar level enhancing effects. The elevated temperatures and constant water supply in the greenhouse provided the tools for higher rate of ripening and better circumstances for sugar accumulation but since it was a water stress experiment irrigation was not applied to all groups at all times which could have photosynthesis suppressing effects and therefore hinder sugar accumulation. The field trial, on the other hand, did not benefit from the same temperatures as the greenhouse but did not experience significant water stress and some groups were designed for very favourable ripening conditions by a high source: sink ratio through crop-thinning. Even though there is no clear conclusion to be made in regards to sugars between the two experiments since they were so different in nature, the slightly higher correlation of ripeness indicators in the PCA, the higher correlation of pH to the greenhouse samples and the fact that the highest overall values for

fructose and glucose were observed in the vineyard would lead to the assumption that the field trial in summation was more favourable for sugar accumulation.

*Greenhouse*

Analysis of the different stress timings did not show any clear differences in any parameters measured in juice. Neither in WineScan (Table 4), nor by $^{1}$H-NMR (data not shown). The PCA graph showed no clear cluster groupings that could be attributed to certain metabolites that were investigated. However, WineScan measurements revealed some tendencies, even though they were not significant. The two last stress treatments tended to lower sugar levels, °Brix and density. This tendency would imply that water deficit during the ripening phase can decrease sugar accumulation. Even though a study by Freeman and Kliewer (1983) found the opposite effect (delayed maturation in irrigated vines), lots of other studies confirmed the results of the present experiment with reduced sugar loading as a consequence of water stress (Hardie and Considine 1976, Esteban et al. 1999, Intrigliolo and Castel 2009, Etcheban et al. 2010). Hardie and Considine (1976) also reported that berries during the lag-phase seemed to be most sensitive to water stress induced delay in ripening. This could not be confirmed here since the treatment group that was water stressed during lag phase (early stress) showed a tendency to higher values than the groups that were stressed later, even no significant differences were observed. The decrease in lateral shoot development and leaf area in stressed groups compared to control also document the first reactions by the plant to water stress (reduced shoot development; see Table 3). The mechanisms suppressing sugar loading are mainly connected to the plant's reaction to water deficit, which is the closure of stomata to reduce water transpiration. This also leads to a reduced $CO_2$ uptake and decreased photosynthetic carbon assimilation and therefore lower sugar production. And this seemed to be most influential during the ripening phase (late stress treatment). Alongside the sugar levels acids are also a ripeness indicator, above all malic acid, which showed tendency to a similar pattern among the groups. Malic acid tended to be slightly lowered in all stress treatments. This could be based on the fact, that water deficit will lead to decreased leaf turgor and therefore more canopy exposition to the sun, which raises temperatures and accelerates the degradation of malic acid. In general, the level of malic acid was low. Apparently, the timing did not have an influence on the severity of that effect, probably because malic acid reaches its maximum value 50-60 days after flowering and is then metabolically consumed according to environmental conditions. If the three stress phases would have been drastically different in weather conditions, a difference in terms of malic acid value according to stress timing could be possible but this was not the case in the present experiment where all ended up at a low level. An increase in malic acid by irrigation was also observed by several other studies (Nadal and Arola 1995, Esteban et al. 1999, Intrigliolo and Castel 2010). Tartaric was unaffected by water stress in general, so the slightly decreased values of total acidity in all stress treatments were probably based on decreased levels of malic and speculatively other organic acids. Parallel changes to acids in pH levels were not observed even though some studies report increased pH levels by irrigation (Freeman and Kliewer 1983, Intrigliolo and Castel 2010) while others found irrigation to have a decreasing effect on pH (Esteban et al. 1999, Bouzas-Cid et al. 2018). However, most of this research was carried out in vastly different environmental conditions and since pH is so complex in its nature with regards to the factors that influence it, it can be assumed that a variety of

variables such as variety, soil-matrix or temperature can shift the outcome in one way or another. The conditions in this study appeared to be such, that pH was not affected by the water stress timings, nor were potassium levels different in juices from stressed plants. Potassium is a very mobile micronutrient and its uptake is, apart from general availability, is also regulated by the amount of water influx into the plant, so it could make sense that the irrigated control group showed higher values, but this was not found in the present study. There is also no evidence to believe that the phenological phase around veraison is more prone to potassium uptake than the other phases. So, it could be argued that the small variations in values are just a statistical coincidence or due to small differences between individual plants. It can be speculated that the different set of time windows, for example including one that reaches further back into the early berry growth phase, or including two or more levels of water stress severity would lead to more profound results but this needs to be confirmed or negated by additional research. One thing that can be confidently stated is that the lack of differences was not based on insufficient water deficit (see Section 4). The levels obtained were quite substantial and a further decrease in water availability could have detrimental effects on plant health and would already be quite far off from a general realistic vineyard scenario. The use of NMR data in a PCA context has the advantage of being able to observe trends, or at least speculate about trends, that would not be so easily accessible in a simple one- or two-dimensional data analysis.

As a side focus of the greenhouse experiment NMR data of two different pruning types and their influence on juice and wine composition were analysed, single cane and double cane pruning. In contrast to water stress timing pruning type showed a visible influence on juice composition. Juices from single cane were more correlated to higher levels of most parameters, above all the ones associated with ripeness, such as sugars, as well as most amino acids and higher levels of pH (Fig.29 and Fig.33). This could be based on a concentration effect or an effect of accelerated ripening through lower crop load. Despite a reduced canopy by fewer leaves and shoots the reduced yield led to a higher leaf area per g fruit in single cane vines, which can be considered an increase in source strength, similar to a crop-thinning treatment. The pruning type investigations were only carried over the course of the NMR data analysis and was not paralleled by WineScan analysis, so further research with both measurement techniques could be beneficial. The lack of clear groupings may also be related to the diverse history of the plants. Even though all the different plant histories were spilt among all treatment groups equally this may have added so much additional variation that potential effects of the stress treatments disappeared.

*Field Trial*

Crop-thinning increased sugar levels to a significantly higher level (see Table 5). This effect is widely reported and accepted as reliable in literature (Petrie and Clingeleffer 2006, Tardaguila et al. 2008, Bubola et al. 2011, Gil et al. 2013). Increased source:sink ratio means more assimilates available for a fewer number of berries and a concentration effect is observed. This was also reflected in °Brix and density. Defoliation can be basically regarded as the exact opposite, increasing the sink:source ratio and reducing the amount of assimilates per berry or cluster. Therefore, it was reasonable to observe significantly lower sugar and ripeness levels for the defoliation treatment. However, other research found that defoliation in fact can in fact lead to higher sugar levels (Hunter et al. 1991, Bubola et al. 2012).

The reason for this discrepancy could be based on the climatic conditions or the plant material used in the various studies. If a very vigorous cultivar allocates too much carbon assimilates for vegetative growth defoliation can theoretically lead to a higher availability of carbon assimilates for the clusters. Moreover, through defoliation of the fruiting zone the fruit temperature could also be raised to a more ideal level of ripening processes or increased sun exposure might decrease water content in berries and therefore also causing a concentration effect. But if the plant does not suffer from an excess of internal canopy shading and an increase in temperature does not improve metabolic performance defoliation should lower the levels of sugars accumulated in the cluster, which was observed in this study. The control group showed values pretty much right in between the defoliation and the crop-thinning levels, nicely illustrating the counteracting effects of the practices in regard to sugars (see Table 5). What is remarkable is that the treatment where both defoliation and crop-thinning was carried out showed similar levels as the crop-thinning treatment therefore implying that the effect of sink reduction outweighs the effect of source reduction and even with a severely reduced canopy sugar levels can be elevated when an intense crop-thinning is additionally performed. However, it is important to note that the crop load reduction in this study was carried out very severely and it still remains unclear at what crop load the observed effect would disappear and reverse towards the sugar levels of the defoliation only treatment. Interpretation of the observed levels of acidity among the treatment groups is difficult since sample preparation issues regarding total acidity, pH, tartaric and malic acid might have blurred the results. The decrease in malic acid that is often reported with defoliation (Poni et al. 2006, Tardaguila et al. 2008, Baiano et al. 2015) was not observed but crop-thinning lowered malic acid content. Based on the fact that crop-thinning alone produced noticeably lower malic acid than crop thinning + defoliation it would stand to reason that defoliation actually had an increasing effect on malic acid levels, which is very unusual because most studies find decreased levels of malic following defoliation or, at most, no effect (Hunter et al. 1991). Questionable values because of aforementioned sample issues are a better explanation of this than a yet unreported effect of increased malic acid through defoliation. The result of crop-thinning alone showing the lowest values for malic acid is supported by selected research (Tardaguila et al. 2012) and could also be speculated to be based on an accelerated metabolic activity and hence malic degradation because of a lower sink strength, but this also needs to be interpreted carefully and preferably repeated and confirmed by additional experiments. Tartaric acid was significantly increased by both defoliation treatments, which could be caused through higher tartaric synthesis by increased light exposure (Kliewer and Schultz 1964) and is also reported in other studies (Palliotti et al. 2012, Uriarte et al. 2012). pH was lowest in the defoliation treatment, highest in the crop-thinning treatment and in between these two for the mixed treatment, suggesting an increasing effect of crop-thinning which is parallel to the lower levels of malic and total acidity, and a decreasing effect of defoliation even though some studies report a lack of influence of defoliation on pH (Tardaguila et el. 2008, Palliotti et al. 2012) while other research supports the pH increasing characteristics of crop-thinning (Palliotti and Cartechini, 1998, Kok 2011). The ratio between sink and source is very complex and even though not all expected effects were observed (malic acid) some of the results are in agreement with previous literature. It is hard to quantify the result of the interaction between source reducing practices such as defoliation and sink reducing practices like crop-thinning but there is evidence that in some aspects, like for example sugars, the crop-thinning treatment has far more impact

than defoliation while in other regards like pH the influence might be more equally distributed between the treatments. Integration of NMR data also revealed some cluster groupings in regard to additional metabolites, such as amino acids (see Fig.34). Kliere and Ough (1970) found that crop- thinning greatly increased levels of arginine and proline and found that proline is highly correlated with ripeness parameters. Arginine was not measured in the present study but both of the other two results could be confirmed, with crop-thinning being correlated with higher levels of proline, as well as threonine, leucine, valine and alanine. Phenylalanine did not show the same reaction to crop-thinning. The reason for elevated amino acid values could be also based on the same theory of increased source:sink ratio and the consequential concentration effect, as described with sugars above. Defoliated vines were more correlated with lower levels of amino acids, especially proline. This is in agreement with the theory that proline is associated with ripeness status (Kliere and Ough 1970) and defoliated vines could experience a decrease or delay in maturity, based on circumstances (as mentioned on the topic of sugars above). There also seems to be a slight correlation between defoliation and higher levels of citric acid, which could be explored in future experiments.

## *6.4. Wine Composition*

*Greenhouse*

The differences in sugar level of the juices were reflected in differences in alcohol level in wines made from the juices, therefore implying a successful fermentation (approx. 47% of sugar were converted to ethanol, calculation data not shown). Malic acid also retained its pattern among the groups with the control showing the highest values, but tartaric acid changed slightly in the sense, that there was a weak tendency to the lowest values in control and early stress wines. Total acidity was similar in all groups and the small differences can be reasoned to be based on the differences of malic acid. Glycerol, another alcohol produced during fermentation, was slightly higher in the control group. pH remained similar in all groups, parallel to the juices, because malolactic fermentation was inhibited by sulphur additions. Volatile acidity was similar among the groups, and the slight variation among treatments are probably best explained by a difference in air exposure during processing, rather than by physiological processes of the grapevine. Regarding stress timings the characteristics of the juices carried over into wine for the most part, besides a slight change in tartaric acid. The wines in general showed much higher levels of proline than the wines from the field trial, probably because of the aforementioned advance in maturity as well as a better supply of nutrients through fertilization.

*Field Trial*

The wines from the field trial showed similar characteristics as the juices. The differences in ethanol expectedly showed the same pattern as the sugars in juices (in this experiment 48% of the sugars were turned into ethanol; calculation data not shown), with the two crop-thinning treatments having the highest values and the defoliation treatment the lowest. Wines from crop-thinning showed a little higher level of residual sugars. Same as in juice analysis, the crop-thinning treatment showed the lowest levels for malic acid but the highest levels were observed in the combined treatment, unlike in juices. This could be explained by the mentioned measurement issues. Tartaric acid showed similar patterns as in the

juices with reduced values in all treatments, which is expected after fermentation, but the combined treatment showed the biggest reduction. Either this is also due to a measurement issue in the juice value, or an increased tartrate precipitation occurred in the wines of the combined treatment, possibly by higher potassium levels. Glycerol and volatile acidity were highest in the two crop-thinning treatments and lowest in the defoliation only treatment, probably based on the higher sugar availability that directly impacts the amount of glycerol and acetic acid produced. Overall and similar to the juices, the defoliation only treatment showed the lowest maturity status, with the lowest pH, highest acidity and lowest sugar levels. The crop-thinning showed the highest maturity status. Therefore it can be stated, that crop load reduction has an effect towards increased ripening and concentration, defoliation has an effect towards delay or decreased ripening with lower levels of ripeness indicators and higher retention of the main organic acids, and the combination of the two effects go in favour of the crop-thinning characteristics, therefore concluding that crop-thinning has superior impact on juice and wine composition. However, it is important to note, that these statements are made solely in regard to the circumstances of the present study and have to be evaluated separately for other experiments under different conditions.

# 7. Conclusion

Water stress did not produce different grape juice compositions based on different timings during the phenological cycle. A huge single plant variation was observed most likely due to both differences in pruning and cropping history in previous years. More uniform plant material should be used and a higher plant number per treatment would also increase the chances for getting significant different levels among the treatments. Further research is needed to confirm this insensitivity of berry composition to time-variable water deficit or to reveal the factors that were missed in this study if such a correlation genuinely exists. Research in this manner with different varieties and different geographical locations, climatic conditions and soil characteristics might also be beneficial to gain more conclusive insight about the influence of water stress during different phases of the growing season. Crop-thinning was confirmed to be a reliable way to increase sugar content and advance ripening. Defoliation caused a decrease in sugar accumulation and ripeness parameters, therefore delaying or decreasing the process of maturation. The two treatments had opposite effects in most cases, sometimes with a similar level of impact (pH) and sometimes with a much bigger influence of the crop-thinning practice (sugar levels). Crop-thinning produced lots of results that agreed with previous research while defoliation did not always confirm past results, implying a big influence of climatic conditions, soil and grape variety. The nature of the source: sink relationship is very complex, and it might be beneficial to enhance future studies of this kind by complementary measurements of additional parameters tied to the ripening process, such as stomatal conductance, carbohydrate composition of leaves or phloem flux. NMR was found to be a useful tool in the search for correlations and patterns among samples that might not be easily accessible by conventional analytical methods but nevertheless a good correlation was observed between NMR derived results and WineScan data. Future research of water deficit by NMR-based metabolomics is highly encouraged because of the level of detailed information that can be obtained from a grape juice or wine sample by NMR. As research progresses and more metabolites found in juice and wine can be assigned to their NMR spectra new insights could potentially be unlocked that could lead to more fundamental knowledge of the relationship between the circumstances in which the grapevine thrives and the characteristics of the fruit it produces.

# 8. References

Adt, I., Kohler, A., Gognies, S., Budin, J., Sandt, C., Belarbi, A. et al. (2010). FTIR spectroscopic discrimination of Saccharomyces cerevisiae and Saccharomyces bayanus strains. Canadian Journal of Microbiology, 56(9), 793-801.

Agatonovic-Kustrin, S. (2013). The Use of Fourier Transform Infrared (FTIR) Spectroscopy and Artificial Neural Networks (ANNs) to Assess Wine Quality. Modern Chemistry & Applications, 1(4).

Ali, K., Maltese, F., Toepfer, R., Choi, Y. H., & Verpoorte, R. (2011). Metabolic characterization of Palatinate German white wines according to sensory attributes, varieties, and vintages using NMR spectroscopy and multivariate data analyses. Journal of Biomolecular NMR, 49(3-4), 255-266.

Amargianitaki, M., & Spyros, A. (2017). NMR-based metabolomics in wine quality control and authentication. Chemical and Biological Technologies in Agriculture, 4(1).

Arnold, R. A., & Bledsoe, A. M. (1990). The Effect of Various Leaf Removal Treatments on the Aroma and Flavor of Sauvignon blanc Wine. American Journal of Enology and Viticulture
American Journal of Enology and Viticulture, 41(1), 74-76.

Aru, V., Sørensen, K. M., Khakimov, B., Toldam-Andersen, T. B., & Balling Engelsen, S. (2018). Cool-Climate Red Wines-Chemical Composition and Comparison of Two Protocols for $^{1}$H-NMR Analysis. Molecules, 23(1).

Ashenfelter, O., & Storchmann, K. (2016). Climate Change and Wine: A Review of the Economic Implications. Journal of Wine Economics, 11(1), 105-138.

Baiano, A., De Gianni, A., Previtali, M. A., Del Nobile, M. A., Novello, V., & de Palma, L. (2015). Effects of defoliation on quality attributes of Nero di Troia (Vitis vinifera L.) grape and wine. Food Research International, 75, 260-269.

Baiano, A., Terracone, C., Longobardi, F., Ventrella, A., Agostiano, A., & Del Nobile, M. A. (2012). Effects of different vinification technologies on physical and chemical characteristics of Sauvignon blanc wines. Food Chemistry, 135(4), 2694-2701.

Bates, B.C., Kundzewicz, Z.W., Wu, S. and Palutikof, J.P. (2008) Climate Change and Water. Technical paper of the Intergovernmental Panel on Climate Change, IPCC Secretariat. Intergovernmental Panel on Climate Change, Geneva.

Bauer, R., Nieuwoudt, H., Bauer, F. F., Kossmann, J., Koch, K. R., & Esbensen, K. H. (2008). FTIR spectroscopy for grape and wine analysis. Analytical Chemistry, 80(5), 1371-1379.

Bell, S. and Henschke, P. A. (2005), Implications of nitrogen nutrition for grapes, fermentation and wine. Australian Journal of Grape and Wine Research, 11: 242-295.

Bennett, J. (2005). Influence of defoliation on overwintering carbohydrate reserves, return bloom, and yield of mature Chardonnay grapevines. American Journal of Enology and Viticulture, 56(4), 386-393.

Bergqvist, J., Dokoozlian, N., & Ebisuda, N. (2001). Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the Central San Joaquin Valley of California. American Journal of Enology and Viticulture, 52(1), 1-7.

Bertamini, M. A. S. S. I. M. O., Zulini, L. U. C. A., Muthuchelian, K., & Nedunchezhian, N. (2006). Effect of water deficit on photosynthetic and other physiological responses in grapevine (Vitis vinifera L. cv. Riesling) plants. Photosynthetica, 44(1), 151-154.

Berthels, N., Corderootero, R., bauer, F., Thevelein, J., & Pretorius, I. (2004). Discrepancy in glucose and fructose utilisation during fermentation by wine yeast strains. FEMS Yeast Research, 4(7), 683-689.

Bevilacqua, M., Bro, R., Marini, F., Rinnan, Å., Rasmussen, M. A., & Skov, T. (2017). Recent chemometrics advances for foodomics. TrAC Trends in Analytical Chemistry, 96, 42-51.

Bevin, C. J., Fergusson, A. J., Perry, W. B., Janik, L. J., & Cozzolino, D. (2006). Development of a Rapid “Fingerprinting” System for Wine Authenticity by Mid-infrared Spectroscopy. Journal of Agricultural and Food Chemistry, 54(26), 9713-9718.

Birkner, N. & Wang, Q. (2019): How an FTIR Spectrometer Operates (online document, accessed July 31st,2019)https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_(Physical_and_Theoretical_Chemistry)/Spectroscopy/Vibrational_Spectroscopy/Infrared_Spectroscopy/How_an_FTIR_Spectrometer_Operates

Bittman, S., & Simpson, G. M. (1989). Drought Effect on Leaf Conductance and Leaf Rolling in Forage Grasses. Crop Science, 29(2), 338.

Bonada, M., Jeffery, D. W., Petrie, P. R., Moran, M. A., & Sadras, V. O. (2015). Impact of elevated temperature and water deficit on the chemical and sensory profiles of Barossa Shiraz grapes and wines. Australian Journal of Grape and Wine Research, 21(2), 240-253.

Boulet, J. C., Williams, P., & Doco, T. (2007). A Fourier transform infrared spectroscopy study of wine polysaccharides. Carbohydrate Polymers, 69(1), 79-85.

Boulton, R. B., Singleton, V. L., Bisson, L. F., & Kunkee, R. E. (1998). Principles and Practices of Winemaking. Boom Koninklijke Uitgevers.

Bouzas-Cid, Y., Falqué, E., Orriols, I., & Mirás-Avalos, J. M. (2018). Effects of irrigation over three years on the amino acid composition of Treixadura (Vitis vinifera L.) musts and wines, and on the aromatic composition and sensory profiles of its wines. Food Chemistry, 240, 707-716.

Bowen, P., Bogdanoff, C., Usher, K., Estergaard, B., & Watson, M. (2011). Effects of Irrigation and Crop Load on Leaf Gas Exchange and Fruit Composition in Red Winegrapes Grown on a Loamy Sand. American Journal of Enology and Viticulture, 62(1), 9-22.

Bowen, P., Bogdanoff, C., Estergaard, B., Marsh, S., Usher, K., Smith, C., & Frank, G. (2005). Geology and wine 10: Use of geographic information system technology to assess viticulture performance in the Okanagan and Similkameen Valleys, British Columbia. Geoscience Canada, 32(4).

Braconi, D., Bernardini, G., Millucci, L., & Santucci, A. (2018). Foodomics for human health: current status and perspectives. Expert Rev Proteomics, 15(2), 153-164.

Bravdo, B., Hepner, Y., Loinger, C., Cohen, S., & Tabacman, H. (1985). Effect of Irrigation and Crop Level on Growth, Yield and Wine Quality of Cabernet Sauvignon. American Journal of Enology and Viticulture 36(2), 132-139.

Bubola, M., Peršurić, Đ., & Ganić, K. K. (2011). Impact of cluster thinning on productive characteristics and wine phenolic composition of cv. Merlot. Journal of food agriculture & environment, 9(1), 36-39.

Bubola, M., Peršurić, Đ., Kovačević Ganić, K., Karoglan, M., & Kozina, B. (2012). Effects of fruit zone leaf removal on the concentrations of phenolic and organic acids in Istrian Malvasia grape juice and wine. Food Technology and Biotechnology, 50(2), 159-166.

Burattini, E., Cavagna, M., Dell'Anna, R., Malvezzi Campeggi, F., Monti, F., Rossi, F. et al. (2008). A FTIR microspectroscopy study of autolysis in cells of the wine yeast Saccharomyces cerevisiae. Vibrational Spectroscopy, 47(2), 139-147.

Capozzi, F., & Bordoni, A. (2013). Foodomics: a new comprehensive approach to food and nutrition. Genes & Nutrition, 8(1), 1-4.

Caspari, H. W., Lang, A., & Alspach, P. (1998). Effects of girdling and leaf removal on fruit set and vegetative growth in grape. American Journal of Enology and Viticulture, 49(4), 359-366.

Castellarin, S.D., Pfeiffer, A., Sivilotti, P.., Degan, M., Peterlunger, E., & Di Gaspero, G. (2007). Transcriptional regulation of anthocyanin biosynthesis in ripening fruits of grapevine under seasonal water deficit. Plant, Cell & Environment, 30(11), 1381-1399.

Chanishvili, S. S., Badridze, G. S., Barblishvili, T. F., & Dolidze, M. D. (2005). Defoliation, photosynthetic rates, and assimilate transport in grapevine plants. Russian Journal of Plant Physiology, 52(4), 448-453.

Chapman, D., Roby, G., Ebeler, S., Guinard, J., & Matthews, M. (2005). Sensory attributes of Cabernet Sauvignon wines made from vines with different water status. Australian Journal of Grape and Wine Research, 11(3), 339-347.

Chapman, D. M., Thorngate, J. H., Matthews, M. A., Guinard, J. X., & Ebeler, S. E. (2004). Yield effects on 2-methoxy-3-isobutylpyrazine concentration in cabernet sauvignon using a solid phase microextraction gas chromatography/mass spectrometry method. Journal of Agricultural and Food Chemistry, 52(17), 5431-5435.

Clausen, M. R., Pedersen, B. H., Bertram, H. C., & Kidmose, U. (2011). Quality of sour cherry juice of different clones and cultivars (Prunus cerasus L.) determined by a combined sensory and NMR spectroscopic approach. Journal of Agricultural and Food Chemistry, 59(22), 12124-12130.

Conde, C., Silva, P., Fontes, N., Dias, A.C.P., Tavares, R.M., Sousa, M.J., et al. (2007) Biochemical Changes throughout Grape Berry Development and Fruit and Wine Quality. Food, 1, 1-22.

Coombe, B. G., Bovio, M., & Schneider, A. (1987). Solute Accumulation by Grape Pericarp Cells: V. Relationship to berry size and the effects of defoliation. Journal of Experimental Botany, 38(11), 1789-1798.

Coombe, B. G. (1995). Growth Stages of the Grapevine: Adoption of a system for identifying grapevine growth stages. Australian Journal of Grape and Wine Research, 1(2), 104-110.

Correira, M. J., Chaves, M. M. C., & Perreira, J. S. (1990). Afternoon Depression In Photosynthesis in Grapevine Leaves—Evidence for a High Light Stress Effect. Journal of Experimental Botany, 41(4), 417-426.

Costa, J. M., Ortuño, M. F., Lopes, C. M., & Chaves, M. M. (2012). Grapevine varieties exhibiting differences in stomatal response to water deficit. Functional Plant Biology, 39(3), 179-189.

Cuny, M., Vigneau, E., Le Gall, G., Colquhoun, I., Lees, M., & Rutledge, D. N. (2008). Fruit juice authentication by 1H NMR spectroscopy in combination with different chemometrics tools. Analytical and Bioanalytical Chemistry, 390(1), 419-427.

Currle, O., Bauer, O., Hofäcker, W., Schumann, F., & Frisch, W. (1983). Biologie der Rebe. Neustadt an der Weinstrasse: Meininger.

Diago, M.-P., Vilanova, M., & Tardaguila, J. (2010). Effects of Timing of Manual and Mechanical Early Defoliation on the Aroma of Vitis vinifera L. Tempranillo Wine. American Journal of Enology and Viticulture, 61, 382-391.

Edelmann, A., Diewok, J., Schuster, K. C., & Lendl, B. (2001). Rapid method for the discrimination of red wine cultivars based on mid-infrared spectroscopy of phenolic wine extracts. Journal of Agriculture and Food Chemistry, 49(3), 1139-1145.

Edelmann, A., Diewok, J., Baena, J. R., & Lendl, B. (2003). High-performance liquid chromatography with diamond ATR–FTIR detection for the determination of carbohydrates, alcohols and organic acids in red wine. Analytical and Bioanalytical Chemistry, 376(1), 92-97.

Edelmann, A., & Lendl, B. (2002). Toward the Optical Tongue: Flow-Through Sensing of Tannin−Protein Interactions Based on FTIR Spectroscopy. Journal of the American Chemical Society, 124(49), 14741-14747.

Esteban, M. A., Villanueva, M. J., & Lissarrague, J. R. (1999). Effect of irrigation on changes in berry composition of Tempranillo during maturation. Sugars, organic acids, and mineral elements. American Journal of Enology and Viticulture, 50(4), 418-434.

Etchebarne, F., Ojeda, H., & Hunter, J. J. (2010). Leaf: fruit ratio and vine water status effects on Grenache Noir (Vitis vinifera L.) berry composition: water, sugar, organic acids and cations. South African Journal of Enology and Viticulture, 31(2), 106-115.

Falcetti, M., & Scienza, A. (1989). Influence de la densité de plantation et du porte-greffe sur la production et la qualité du moût de Pinot blanc et de Chardonnay cultivés en Italie dans le Trentin. OENO One, 23(3), 151-164.

European Commission. (2009). Regions 2020: The climate change challenge for European regions. Background document to commission staff working document SEC(2008) 2868 final. Brussels, Belgium: European Commission, Directorate General for Regional Policy.

Fereres, E., Goldhamer, D. A., & Sadras, V. O. (2012). Yield response to water of fruit trees and vines: guidelines. FAO Irrigation and Drainage Paper, 66), 460-485.

Fiehn, O. (2002). Metabolomics - The link between genotypes and phenotypes. Plant Molecular Biology 48(2),155-171.

Figueiredo-González, M., Simal-Gándara, J., Boso, S., Martínez, M. C., Santiago, J. L., & Cancho-Grande, B. (2012). Anthocyanins and flavonols berries from Vitis vinifera L. cv. Brancellao separately collected from two different positions within the cluster. Food Chemistry, 135(1), 47-56.

Fontes, J. E. D. N., Rodriguez-Caturla, M. Y., Sant'Ana, A. S., Lopes, T. I. B., & Marsaioli, A. J. (2018). Foodomics and storage monitoring of three meat cuts by 1 H NMR. Concepts in Magnetic Resonance Part A, 47A(2), e21474.

Freeman, B. M., & Kliewer, W. M. (1983). Effect of irrigation, crop level and potassium fertilization on Carignane vines. II. Grape and wine quality. American Journal of Enology and Viticulture, 34(3), 197-207.

Friedel, M., Patz, C.-D., & Dietrich, H. (2013). Comparison of different measurement techniques and variable selection methods for FT-MIR in wine analysis. Food Chemistry, 141(4), 4200-4207.

Fu, Y., Lim, L. T., & McNicholas, P. D. (2009). Changes on enological parameters of white wine packaged in bag-in-box during secondary shelf life. Journal of food science, 74(8), C608-C618.

Gil, M., Esteruelas, M., González, E., Kontoudakis, N., Jiménez, J., Fort, F. et al. (2013). Effect of two different treatments for reducing grape yield in Vitis vinifera cv Syrah on wine composition and quality: berry thinning versus cluster thinning. Journal of Agricultural and Food Chemistry, 61(20), 4968-4978.

Grandizoli, C. W. P. D. S., Campos, F. R., Simonelli, F., & Barison, A. (2014). Grape juice quality control by means of $^{1}$H Nmr spectroscopy and Chemometric analyses. Química Nova, 37(7), 1227-1232.

Hardie, W. J., & Considine, J. A. (1976). Response of grapes to water-deficit stress in particular stages of development. American Journal of Enology and Viticulture, 27(2), 55-61.

Hepner, Y., & Bravdo, B. (1985). Effect of crop level and drip irrigation scheduling on the potassium status of Cabernet Sauvignon and Carignane vines and its influence on must and wine composition and quality. American journal of enology and viticulture, 36(2), 140-147.

Hernandez-Orte, P., Concejero, B., Astrain, J., Lacau, B., Cacho, J., & Ferreira, V. (2014). Influence of viticulture practices on grape aroma precursors and their relation with wine aroma. Journal of the Science of Food and Agriculture, 95(4), 688-701.

Hunter, J. J., De Villiers, O. T., & Watts, J. E. (1991). The effect of partial defoliation on quality characteristics of Vitis vinifera L. cv. Cabernet Sauvignon grapes. II. Skin color, skin sugar, and wine quality. American Journal of Enology and Viticulture, 42(1), 13-18.

Hunter, J. J. & Visser, J. H. 1988. The effect of partial defoliation, leaf position and developmental stage of the vine on the photosynthetic activity of vitis vinifera L. cv cabernet sauvignon. South African Journal of Enology & Viticulture, 9(2), 9-15.

Hunter, J. J. & Visser, J. H. 1990. The effect of partial defoliation on growth characteristics of vitis vinifera L. cv. cabemet sauvignon I. vegetative growth. South African Journal of Enology & Viticulture, 11(1), 18-25.

Ibáñez, C., Valdés, A., García-Cañas, V., Simó, C., Celebier, M., Rocamora-Reverte, L. et al. (2012). Global Foodomics strategy to investigate the health benefits of dietary constituents. Journal of Chromatography A, 1248, 139-153.

Intergovernmental Panel on Climate Change (2013). Climate Change 2013: The Physical Science Basis. Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press.

Intrieri, C., Filipetti, I., Allegro, G., Centinari, M., & Poni, S. (2008). Early defoliation (hand vs mechanical) for improved crop control and grape composition in Sangiovese (Vitis vinifera L.). Australian Journal of Grape and Wine Research, 14(1), 25-32.

Intrigliolo, D. S., & Castel, J. R. (2010). Interactive effects of deficit irrigation and shoot and cluster thinning on grapevine cv. Tempranillo. Water relations, vine performance and berry and wine composition. Irrigation Science, 29(6), 443-454.

Intrigliolo, D. S., & Castel, J. R. (2009). Response of grapevine cv. 'Tempranillo' to timing and amount of irrigation: water relations, vine growth, yield and berry and wine composition. Irrigation Science, 28(2), 113-125.

James, T. L. (1998). Fundamentals of NMR. Online Textbook: Department of Pharmaceutical Chemistry, University of California, San Francisco, 1-31.

Jun, L., & Province, Z. (1994). Effects on growth and yield of rice by control of tillering through induced mild drought stress. Proceedings from The development, testing and application of crop models simulating the potential of production of rice. SARP Research Proceedings DLO-WAU-IRRI of the International Workshop on the Simulation of Potential Production in Rice, 57-61.

Jung, Y., Lee, J., Kwon, J., Lee, K. S., Ryu, D. H., & Hwang, G. S. (2010). Discrimination of the geographical origin of beef by (1)H NMR-based metabolomics. Journal of Agricultural and Food Chemistry, 58(19), 10458-10466.

Kadioglu, A., & Terzi, R. (2007). A dehydration avoidance mechanism: leaf rolling. The Botanical Review, 73(4), 290-302.

Keller, M. (2015). The science of grapevines: anatomy and physiology. Academic Press.

Keller, M., Mills, L. J., Wample, R. L., & Spayd, S. E. (2005). Cluster thinning effects on three deficit-irrigated Vitis vinifera cultivars. American Journal of Enology and Viticulture, 56(2), 91-103.

Keller, M., Smithyman, R. P., & Mills, L. J. (2008). Interactive effects of deficit irrigation and crop load on Cabernet Sauvignon in an arid climate. American Journal of Enology and Viticulture, 59(3), 221-234.

Kennedy, J. A., Hayasaka, Y., Vidal, S., Waters, E. J., & Jones, G. P. (2001). Composition of grape skin proanthocyanidins at different stages of berry development. Journal of Agricultural and Food Chemistry, 49(11), 5348-5355.

Kessler, W. (2006). Multivariate Datenanalyse. John Wiley & Sons.

Khakimov, B., Gürdeniz, G., & Engelsen, S. B. (2015). Trends in the application of chemometrics to foodomics studies. Acta Alimentaria, 44(1), 4-31.

Kliewer, M., & Bledsoe, A. (1986,). Influence of hedging and leaf removal on canopy microclimate, grape composition, and wine quality under California conditions. In Symposium on Grapevine Canopy and Vigor Management, XXII IHC 206 (pp. 157-168).

Kliewer, W. M. (1970). Effect of time and severity of defoliation on growth and composition of'Thompson Seedless' grapes. American Journal of Enology and Viticulture, 21(1), 37-47.

Kliewer, W. M., & Antcliff, A. J. (1970). Influence of defoliation, leaf darkening, and cluster shading on the growth and composition of Sultana grapes. American Journal of Enology and Viticulture, 21(1), 26-36.

Kliewer, W. M., & Schultz, H. B. (1964). Influence of environment on metabolism of organic acids and carbohydrates in Vitis vinifera. II. Light. American Journal of Enology and Viticulture, 15(3), 119-129.

Kliewer, W. M., & Torres, R. E. (1972). Effect of controlled day and night temperatures on grape coloration. American Journal of Enology and Viticulture, 23(2), 71-77.

Kliewer, W. M., & Lider, L. A. (1970). Effects of day temperature and light intensity on growth and composition of Vitis vinifera L. fruits. Journal of the American Society of Horticultural Science, 95, 766-769.

Kliewer, W. M., & Ough, C. S. (1970). The effect of leaf area and crop level on the concentration of amino acids and total nitrogen in" Thompson Seedless" grapes. Vitis, 9, 196-206.

Koda, M., Furihata, K., Wei, F., Miyakawa, T., & Tanokura, M. (2012). Metabolic Discrimination of Mango Juice from Various Cultivars by Band-Selective NMR Spectroscopy. Journal of Agricultural and Food Chemistry, 60(5), 1158-1166.

Kok, D. (2011). Influences of pre-and post-veraison cluster thinning treatments on grape composition variables and monoterpene levels of Vitis vinifera L. cv. Sauvignon Blanc. J Food Agric Environ, 9(1), 22-26.

Kramer, P. J., & Boyer, J. S. (1995). Water Relations of Plants and Soils. Academic Press.

Krapp, A., & Stitt, M. (1995). An evaluation of direct and indirect mechanisms for the “sink-regulation” of photosynthesis in spinach: Changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady-state transcript levels after cold-girdling source leaves. Planta, 195(3), 313-323.

Kriedemann, P. E. (1977). Vineleaf photosynthesis. Proceedings from International Symposium on the Quality of the Vintage, 14-21.

Kupina, S. A., & Shrikhande, A. J. (2003). Evaluation of a Fourier transform infrared instrument for rapid quality-control wine analyses. American Journal of Enology and Viticulture, 54(2), 131-134.

Lachenmeier, D. (2007). Rapid quality control of spirit drinks and beer using Multivariate Data Analysis of Fourier Transform Infrared Spectra. Food Chemistry, 101, 825-832.

Lakso, A. N., & Kliewer, W. M. (1975). The influence of temperature on malic acid metabolism in grape berries: I. Enzyme responses. Plant Physiology, 56(3), 370-372.

Lee, J. E., Hwang, G. S., Van Den Berg, F., Lee, C. H., & Hong, Y. S. (2009). Evidence of vintage effects on grape wines using 1H NMR-based metabolomic study. Analytica chimica acta, 648(1), 71-76.

León, C., Cifuentes, A., & Valdés, A. (2018). Foodomics Applications. Proceedings from Comprehensive Analytical Chemistry, (82), 643-685.

López-Rituerto, E., Savorani, F., Avenoza, A., Busto, J. H., Peregrina, J. M., & Engelsen, S. B. (2012). Investigations of La Rioja terroir for wine production using 1H NMR metabolomics. Journal of Agricultural and Food Chemistry, 60(13), 3452-3461.

Martínez-Lüscher, J., Morales, F., Delrot, S., Sánchez-Díaz, M., Gomés, E., Aguirreolea, J. et al. (2013). Short- and long-term physiological responses of grapevine leaves to UV-B radiation. Plant Science, 213, 114-122.

Masoum, S., Malabat, C., Jalali-Heravi, M., Guillou, C., Rezzi, S., & Rutledge, D. N. (2007). Application of support vector machines to 1 H NMR data of fish oils: methodology for the confirmation of wild and farmed salmon and their origins. Analytical and bioanalytical chemistry, 387(4), 1499-1510.

Matthews, M. A., Anderson, M. M., & Schult, H. R. (1987). Phenologic and growth responses to early and late season. Vitis, 26, 147-160.

Matthews, M. A., Ishii, R., Anderson, M. M., & O'Mahony, M. (1990). Dependence of wine sensory attributes on vine water status. Journal of the Science of Food and Agriculture, 51(3), 321-335.

Mazzei, P., Francesca, N., Moschetti, G., & Piccolo, A. (2010). NMR spectroscopy evaluation of direct relationship between soils and molecular composition of red wines from Aglianico grapes. Analytica Chimica Acta, 673(2), 167-172.

McDowell, N. G. (2011). Mechanisms Linking Drought, Hydraulics, Carbon Metabolism, and Vegetation Mortality. Plant Physiology, 155(3), 1051-1059.

Miller, D. P., & Howell, G. S. (1998). Influence of vine capacity and crop load on canopy development, morphology, and dry matter partitioning in Concord grapevines. American Journal of Enology and Viticulture, 49(2), 183-190.

Moreno, D., Valdés, E., Uriarte, D., Gamero, E., Talaverano, I., & Vilanova, M. (2017). Early leaf removal applied in warm climatic conditions: Impact on Tempranillo wine volatiles. Food Research International, 98, 50-58.

Nadal, M., & Arola, L. (1995). Effects of limited irrigation on the composition of must and wine of Cabernet Sauvignon under semi-arid conditions. Vitis, 34(3), 151-154.

Naumann, D., Helm, D., & Labischinski, H. (1991). Microbiological characterizations by FT-IR spectroscopy. Nature, 351(6321), 81-82.

Nemkov, T., Hansen, K. C., Dumont, L. J., & D'Alessandro, A. (2016). Metabolomics in transfusion medicine. Transfusion, 56(4), 980-993.

Nicholson, J. K., Lindon, J. C., & Holmes, E. (1999). 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica, 29(11), 1181-1189.

Nieuwoudt, H. H., Prior, B. A., Pretorius, I. S., Manley, M., & Bauer, F. F. (2004). Principal component analysis applied to Fourier transform infrared spectroscopy for the design of calibration sets for glycerol prediction models in wine and for the detection and classification of outlier samples. Journal of Agriculture and Food Chemistry, 52(12), 3726-3735.

Noronha, H. L. S. D. (2010). The effect of high-temperature on sugar transport in grape cells (Master dissertation). University of Minho, Portugal.

O. Downey, M., Dokoozlian, N., & Krstic, M. (2006). Cultural Practice and Environmental Impacts on the Flavonoid Composition of Grapes and Wine: A Review of Recent Research. American Journal of Enology and Viticulture, 57, 257-268.

Oliver, S. G., Winson, M. K., Kell, D. B., & Baganz, F. (1998). Systematic functional analysis of the yeast genome. Trends in biotechnology, 16(9), 373-378.

Oms-Oliu, G., Odriozola-Serrano, I., & Martín-Belloso, O. (2013). Metabolomics for assessing safety and quality of plant-derived food. Food Research International, 54(1), 1172-1183.

Otto, M. (2016). Chemometrics: statistics and computer application in analytical chemistry. John Wiley & Sons.

Ough, C. S., & Nagaoka, R. (1984). Effect of cluster thinning and vineyard yields on grape and wine composition and wine quality of Cabernet Sauvignon. American journal of enology and viticulture, 35(1), 30-34.

Palliotti, A., & Cartechini, A. (1998, August). Cluster thinning effects on yield and grape composition in different grapevine cultivars. In XXV International Horticultural Congress, Part 2: Mineral Nutrition and Grape and Wine Quality 512, 111-120.

Palliotti, A., Gardi, T., Berrios, J. G., Civardi, S., & Poni, S. (2012). Early source limitation as a tool for yield control and wine quality improvement in a high-yielding red Vitis vinifera L. cultivar. Scientia horticulturae, 145, 10-16.

Palliotti, A., Silvestroni, O., & Petoumenou, D. (2009). Photosynthetic and photoinhibition behavior of two field-grown grapevine cultivars under multiple summer stresses. American Journal of Enology and Viticulture, 60(2), 189-198.

Patz, C.-D., Blieke, A., Ristow, R., & Dietrich, H. (2004). Application of FT-MIR spectrometry in wine analysis. Analytica Chimica Acta, 513(1), 81-89.

Pellegrino, A., Lebon, E., Simonneau, T. & Wery, J. (2005). Towards a simple indicator of water stress in grapevine (Vitis vinifera L.) based on the differential sensitivities of vegetative growth components. Australian Journal of Grape and Wine Research, 11(3), 306-315.

Pereira, G. E., Gaudillere, J.-P., Leeuwen, C. V., Hilbert, G., Maucourt, M., Deborde, C. et al. (2006). 1H NMR metabolite fingerprints of grape berry: Comparison of vintage and soil effects in Bordeaux grapevine growing areas. Analytica Chimica Acta, 563(1-2), 346-352.

Petrie, P., & Clingeleffer, P. (2006). Crop thinning (hand versus mechanical), grape maturity and anthocyanin concentration: outcomes from irrigated Cabernet Sauvignon (Vitis vinifera L.) in a warm climate. Australian Journal of Grape and Wine Research, 12(1), 21-29.

Picone, G., Trimigno, A., Tessarin, P., Donnini, S., Rombolà, A. D., & Capozzi, F. (2016). (1)H NMR foodomics reveals that the biodynamic and the organic cultivation managements produce different grape berries (Vitis vinifera L. cv. Sangiovese). Food Chemistry, 213, 187-195.

Pieri, P., & Fermaud, M. (2004, June). Effects of defoliation on temperature and wetness of grapevine berries. Proceedings from VII International Symposium on Grapevine Physiology and Biotechnology 689 (pp. 109-116).

Pimentel, G., Burton, K. J., Vergères, G., & Dupont, D. (2018). The role of foodomics to understand the digestion/bioactivity relationship of food. Current Opinion in Food Science, 22, 67-73.

Pinu, F. R., Edwards, P. J. B., Jouanneau, S., Kilmartin, P. A., Gardner, R. C., & Villas-Boas, S. G. (2014). Sauvignon blanc metabolomics: grape juice metabolites affecting the development of varietal thiols and other aroma compounds in wines. Metabolomics, 10(4), 556-573.

Poni, G., Intrieri, C., & del Campo, M. G. (2001). Vine performance and leaf physiology of conventionally and minimally pruned Sangiovese grapevines. Vitis: Journal of Grapevine Research, 40(3), 123-130.

Poni, S., Gatti, M., Bernizzoni, F., Civardi, S., Bobeica, N., Magnanini, E. et al. (2013). Late leaf removal aimed at delaying ripening in cv. Sangiovese: physiological assessment and vine performance. Australian Journal of Grape and Wine Research, 19(3), 378-387.

Poni, S., Casalini, L., Bernizzoni, F., Civardi, S., & Intrieri, C. (2006). Effects of Early Defoliation on Shoot Photosynthesis, Yield Components, and Grape Composition. American Journal of Enology and Viticulture, 57, 397-407.

Ramos, A., Neves, A. R., & Santos, H. (2002). Metabolism of lactic acid bacteria studied by nuclear magnetic resonance. Proceedings from Lactic acid bacteria: Genetics, metabolism and applications, 249-261. Springer.

Ricci, A., Olejar, K. J., Parpinello, G. P., Kilmartin, P. A., & Versari, A. (2015). Application of Fourier Transform Infrared (FTIR) Spectroscopy in the Characterization of Tannins. Applied Spectroscopy Reviews, 50(5), 407-442.

Ringnér, M. (2008). What is principal component analysis. Nature Biotechnology, 26(3), 303.

Risco, D., Pérez, D., Yeves, A., Castel, J. R., & Intrigliolo, D. S. (2014). Early defoliation in a temperate warm and semi-arid Tempranillo vineyard: vine performance and grape composition. Australian Journal of Grape and Wine Research, 20(1), 111-122.

Roberts, R. W., Reynolds, A. G., & Savigny, C. D. (2007). Composition and Wine Sensory Attributes of Chardonnay Musqué from Different Viticultural Treatments. International Journal of Fruit Science, 7(2), 57-83.

Roby, G., Harbertson, J. F., Adams, D. A., & Matthews, M. A. (2004). Berry size and vine water deficits as factors in winegrape composition: anthocyanins and tannins. Australian Journal of Grape and Wine Research, 10(2), 100-107.

Rochfort, S., Ezernieks, V., Bastian, S. E. P., & Downey, M. O. (2010). Sensory attributes of wine influenced by variety and berry shading discriminated by NMR metabolomics. Food Chemistry, 121(4), 1296-1304.

Romero, P., Fernández-Fernández, J. I., & Martinez-Cutillas, A. (2010). Physiological thresholds for efficient regulated deficit-irrigation management in winegrapes grown under semiarid conditions. American Journal of Enology and Viticulture, 61(3), 300-312.

Roussel, S., Bellon-Maurel, V., Roger, J.-M., & Grenier, P. (2003). Authenticating white grape must variety with classification models based on aroma sensors, FT-IR and UV spectrometry. Journal of Food Engineering, 60(4), 407-419.

Sabir, A., Kafkas, E., & Tangolar, S. (2010). Distribution of major sugars, acids, and total phenols in juice of five grapevine (Vitis spp.) cultivars at different stages of berry development. Spanish Journal of Agricultural Research, 8(2), 425-433.

Samuelsson, L. M., Förlin, L., Karlsson, G., Adolfsson-Erici, M., & Larsson, D. G. (2006). Using NMR metabolomics to identify responses of an environmental estrogen in blood plasma of fish. Aquat Toxicol, 78(4), 341-349.

Santesteban, L. G., & Royo, J. B. (2006). Water status, leaf area and fruit load influence on berry weight and sugar accumulation of cv. ‘Tempranillo’ under semiarid conditions. Scientia Horticulturae, 109(1), 60-65.

Santos, T. P., Lopes, C. M. A., Rodrigues, M., Souza, C. R. D., Silva, J. R., Maroco, J. et al. (2005). Effects of partial root-zone drying irrigation on cluster microclimate and fruit composition of field-grown Castelão grapevines. Vitis, 44, 117-125.

Santos, M., Fonseca, A., Fraga, H., Jones, G. V., & Santos, J. A. (2019). Bioclimatic conditions of the Portuguese wine denominations of origin under changing climates. International Journal of Climatology 2019, 1-15.

Schievano, E., Peggion, E., & Mammi, S. (2010). 1H nuclear magnetic resonance spectra of chloroform extracts of honey for chemometric determination of its botanical origin. Journal of Agriculture and Food Chemistry, 58(1), 57-65.

Schultz, H. R. (2016). Global Climate Change, Sustainability, and Some Challenges for Grape and Wine Production. Journal of Wine Economics, 11(1), 181-200.

Sivilotti, P., Bonetto, C., Paladin, M., & Peterlunger, E. (2005). Effect of soil moisture availability on Merlot: from leaf water potential to grape composition. American Journal of Enology and Viticulture, 56(1), 9-18.

Smart, R. E. (1985). Principles of grapevine canopy microclimate manipulation with implications for yield and quality. A review. American Journal of Enology and Viticulture, 36(3), 230-239.

Son, H.-S., Hwang, G.-S., Kim, K. M., Kim, E.-Y., van den Berg, F., Park, W.-M. et al. (2009). 1H NMR-Based Metabolomic Approach for Understanding the Fermentation Behaviors of Wine Yeast Strains. Analytical Chemistry, 81(3), 1137-1145.

Spayd, S. E., Tarara, J. M., Mee, D. L., & Ferguson, J. C. (2002). Separation of sunlight and temperature effects on the composition of Vitis vinifera cv. Merlot berries. American Journal of Enology and Viticulture, 53(3), 171-182.

Staff, S. L., Percival, D. C., Sullivan, J. A., & Fisher, K. H. (1997). Fruit zone leaf removal influences vegetative, yield, disease, fruit composition, and wine sensory attributes of Vitis vinifera L. 'Optima' and 'Cabernet franc'. Canadian journal of plant science, 77(1), 149-153.

Sturges, S., & Brown, M. (1975). Polypsychopharmacy. Bulletin of the Menninger Clinic, 39(3), 274-279.

Tardaguila, J., Blanco, J. A., Poni, S., & Diago, M. P. (2012). Mechanical yield regulation in winegrapes: comparison of early defoliation and crop thinning. Australian Journal of Grape and Wine Research, 18(3), 344-352.

Tardáguila, J., Diago, M. P., de Toda, F. M., Poni, S., & Vilanova, M. (2008). Effects of timing of leaf removal on yield, berry maturity, wine composition and sensory properties of cv. Grenache grown under non irrigated conditions. OENO One, 42(4), 221-229.

Tardaguila, J., Diago, M.-P., Martinez de Toda, F., Poni, S., & Vilanova, M. (2008). Effects of timing of leaf removal on yield, berry maturity, wine composition and sensory properties of CV. grenache grown under non irrigated conditions. Journal international des sciences de la vigne et du vin, 42, 221-229.

Tardaguila, J., Martinez de Toda, F., Poni, S., & Diago, M.-P. (2010). Impact of early leaf removal on yield and fruit and wine composition of “Vitis vinifera L.” Graciano and Carignan. American Journal of Enology and Viticulture, 61, 372-381.

Tardaguila, J., Petrie, P. R., Poni, S., Diago, M. P., & de Toda, F. M. (2008). Effects of mechanical thinning on yield and fruit composition of Tempranillo and Grenache grapes trained to a vertical shoot-positioned canopy. American Journal of Enology and Viticulture, 59(4), 412-417.

Tarter, M. E., & Keuter, S. E. (2005). Effect of rachis position on size and maturity of Cabernet Sauvignon berries. American Journal of Enology and Viticulture, 56(1), 86-89.

Thoukis, G., Ueda, M., & Wright, D. (1965). The formation of succinic acid during alcoholic fermentation. American Journal of Enology and Viticulture, 16(1), 1-8.

Trimigno, A., Marincola, F. C., Dellarosa, N., Picone, G., & Laghi, L. (2015). Definition of food quality by NMR-based foodomics. Current Opinion in Food Science, 4, 99-104.

Tsiafoulis, C. G., Papaemmanouil, C., Alivertis, D., Tzamaloukas, O., Miltiadou, D., Balayssac, S. et al. (2019). NMR-Based Metabolomics of the Lipid Fraction of Organic and Conventional Bovine Milk. Molecules, 24(6).

Tyagi, B., Chudasama, C. D., & Jasra, R. V. (2006). Determination of structural modification in acid activated montmorillonite clay by FT-IR spectroscopy. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 64(2), 273-278.

Uriarte, D., Picón, J., Mancha, L. A., Blanco, J., Prieto, M. H., Moreno, D., ... & Intrigliolo, D. S. (2010, August). Early defoliation of ‚Tempranillo' grapevines in semi-arid terroirs of Spain. Proceedings of XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on the 931, 299-306.

Valdivia, G., & Francisco, J. (2001). Efecto del vigor y del grado de madurez de las bayas sobre la fracción polifenólica de las semillas del cv. Cabernet Sauvignon en el valle del Maipo (Master dissertation). Escuela de Agronomía, Facultad de Ciencias Agronómicas. Universidad de Chile.

Versari, A., Boulton, R., & Thorngate, J. (2004). The Prediction of the Color Components of Red Wines Using FTIR, Wine Analyses, and the Method of Partial Least Squares. Proceedings from ACS Symposium Series: Red Wine Color, 53-67. Washington, DC: American Chemical Society.

Versari, A., Parpinello, G. P., Scazzina, F., & Rio, D. D. (2010). Prediction of total antioxidant capacity of red wine by Fourier transform infrared spectroscopy. Food Control, 21(5), 786-789.

Viant, M. R., Rosenblum, E. S., & Tjeerdema, R. S. (2003). NMR-based metabolomics: a powerful approach for characterizing the effects of environmental stressors on organism health. Environmental science & technology, 37(21), 4982-4989.

Vilanova, M., Diago, M. P., Genisheva, Z., Oliveira, J. M., & Tardaguila, J. (2012). Early leaf removal impact on volatile composition of Tempranillo wines. Journal of the Science of Food and Agriculture, 92(4), 935-942.

Weekley, A. J., Bruins, P., Sisto, M., & Augustine, M. P. (2003). Using NMR to study full intact wine bottles. Journal of Magnetic Resonance, 161(1), 91-98.

Wenter, A., Zanotelli, D., Montagnani, L., Tagliavini, M., & Andreotti, C. (2018). Effect of different timings and intensities of water stress on yield and berry composition of grapevine (cv. Sauvignon blanc) in a mountain environment. Scientia Horticulturae, 236, 137-145.

Winkler, A. J. (1969). Effect of vine spacing in an unirrigated vineyard on vine physiology, production and wine quality. American Journal of Enology and Viticulture, 20(1), 7-15.

Wishart, D. S. (2016). Emerging applications of metabolomics in drug discovery and precision medicine. Nature reviews Drug discovery, 15(7), 473.

Wold, S., Esbensen, K., & Geladi, P. (1987). Principal component analysis. Chemometrics and intelligent laboratory systems, 2(1-3), 37-52.

Wold, S., Sjöström, M., & Eriksson, L. (2001). PLS-regression: a basic tool of chemometrics. Chemometrics and intelligent laboratory systems, 58(2), 109-130.

Wolpert, J. A., Howell, G. S., & Mansfield, T. K. (1983). Sampling Vidal blanc grapes. I. Effect of training system, pruning severity, shoot exposure, shoot origin, and cluster thinning on cluster weight and fruit quality. American Journal of Enology and Viticulture, 34(2), 72-76.

Zamski. (1996). Photoassimilate Distribution Plants and Crops Source-Sink Relationships. CRC Press.

Zarrouk, O., Fortunato, A., & Chaves, M. M. (2018). Crop Responses to Available Soil Water. Proceedings from Encyclopedia of Sustainability Science and Technology, 1-27. Springer New York.

Zarrouk, O., Brunetti, C., Egipto, R., Pinheiro, C., Genebra, T., Gori, A., Lopes C.M., Tattini, M. & Chaves, M. M. (2016). Grape ripening is regulated by deficit irrigation/elevated temperatures according to cluster position in the canopy. Frontiers in Plant Science, 7, 1640.

Zhang, A., Sun, H., Wang, P., Han, Y., & Wang, X. (2012). Modern analytical techniques in metabolomics analysis. The Analyst, 137(2), 293-300.