UNIVERSITÀ DEGLI STUDI DI PADOVA

School of Agricultural Sciences and Veterinary Medicine

Second-cycle degree in Italian Food and Wine

Analyzing the Impact of Leaf Removal Timing and Intensity as Adaptive Strategies for Climate Change on Pinot noir in Burgundy

Internship supervisor: Benjamin Bois
Supervisor: Prof. Franco Meggio

Author: Tsiory Tiavina Rajaonary
Student n°: 2040546

ACADEMIC YEAR: 2023-2024

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ABSTRACT

With the rise in global temperatures, the wine industry faces significant challenges in maintaining grape quality. Elevated sugar levels in grapes are leading to wines with higher alcohol content and reduced acidity. This is particularly challenging in Burgundy, where such imbalances compromise the uniqueness of wines, clashing with traditional standards and consumer preferences. In response, the University of Bourgogne engaged in a project from 2020 to 2023, on apical leaf removal as a strategy to mitigate the effects of climate change. This technique involves the removal of the uppermost leaves from grapevines, an action that aims to lower sugar content and water stress by reducing the leaf's transpiration area, all while preserving the grapes' essential acidity levels.

This study encompassed experimental trials with Pinot noir and Chardonnay grape varieties. It compared the impact of moderate and severe apical leaf removal; applied at two critical fruit development stages — bunch closure and 10 days after mid-veraison — against control groups with no leaf removal. The results revealed that severe apical leaf removal postponed mid-veraison by an average of 5 days and resulted in grapes with lower sugar and pH levels at harvest than those in the control group. This technique also led to more negative $\delta^{13}C$ values, indicating a decrease in water stress in the vines. However, it concurrently resulted in reduced assimilable nitrogen and potential impacts on yield.

Apical leaf removal emerges as a promising approach to producing high-quality grapes in an era of climatic change. Yet, further research is vital to optimize this method and fully understand its long-term impact on sustainable grape cultivation.

Key words: Burgundy, Climate Change, Leaf removal, Pinot noir, Leaf to Fruit Ratio, Exposed Leaf Area

INTRODUCTION

Climate change has become a focal point for extensive global research in across various sectors. As in viticulture, maintaining grapevine production require adaptation to climate change. Thus, numerous projects are conducted to study the impact of climate change on vineyards and the potential adaptations that may arise. Responding to this challenge while meeting consumer expectations has become essential for the industry stakeholders.

From April to September 2023, I had the opportunity to be part of the *Stratagème* project, at the University of Bourgogne, led by the Chamber of Agriculture in partnership with the Interprofessional Burgundy Wine Bureau (BIVB), and other estates such as Domaine Louis Latour. The project aims to assess adaptive practices in response to climate change within the Burgundian vineyard.

This report investigates the management of the exposed leaf area (ELA) and its impact on agronomic characteristics on Pinot N.. Different intensity of bunch thinning and apical leaf removal were implemented to delay and limit sugar accumulation in grapes while maintaining acidity. Similar studies have been conducted in Italy but not much in French vineyards, thus allowing us to establish a reference for the Burgundian vineyard region.

How can we implement apical leaf removal practices in Burgundian vineyards to mitigate the impact of climate change while maintaining the quality and characteristics of the wine?

To address this research question, we will first explore the Burgundian *terroir*, accompanied by a comprehensive literature review that assesses the impact of climate change on vineyards and potential adaptation strategies. Following this, we will present the methodology employed in our experiment. Lastly, we will discuss the results obtained from this study, providing an in-depth analysis and interpretation of the findings.

1. The Burgundian *Terroir*

1.1. Bourgogne wine producing region

A historic wine region shaped during the Middle Ages by monks, Burgundy is one of the most important French wine regions, although only representing 4% of it. (Chabin, 1995) Bourgogne is located in the eastern part of France. The Bourgogne wine region extends over 230 km from the North, Auxerre, to the South, Macon. It is divided into two distinctive areas: the slope and the eastern frontage. The slope consists of the Chablis and Grand Axerrois vineyards, while the eastern frontage forms an almost straight line and includes the Côte de Nuits, the Côte de Beaune, the Côte Chalonnaise and the Maconnais vineyards. (Figure 1.1.1)

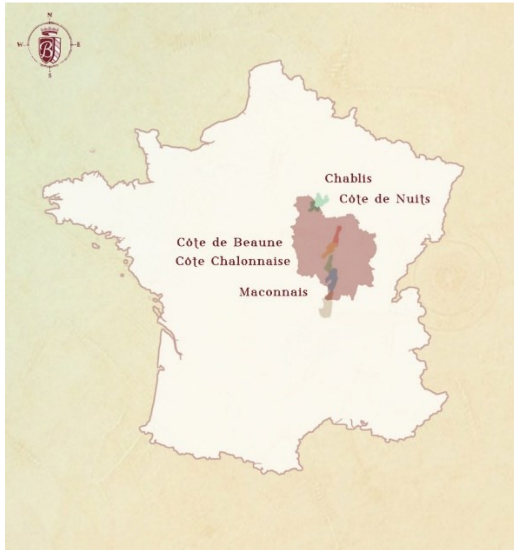


Figure 1.1 1: Map of the Burgundian wine region, France

(Source: burgundywinecompany.com)

The uniqueness of this region is its ability to produce an extensive range of wines, each characterized by distinct *terroirs,* locally called *"climats"* – specific local conditions that impart unique qualities. Indeed, Burgundian *climats* are the origin of the vineyard's division illustrating perfectly the concept of "terroir"[1]. Thus, Bourgogne wines are based on the terroir concept, with four levels: regional appellations, village appellations, premier crus, and grand crus at the top of the structure, which are necessarily, recognized *climats* of excellence, schematized in Figure 1.1.2. Although, since the 18th century, this system for hierarchizing burgundy wines by location has undergone several changes to establish Burgundy as the land of terroir wines. These mutations have been driven by various economic and commercial revolutions, including the fiscal and political crisis of the 18th century and the wave of wine globalization in the early 21st century that led to a readjustment of the valorization of burgundy wines. Moreover, viticulture crisis have also contributed to the reevaluation of the quality of Burgundy wines. (Garcia et al., 2022)

[1] Terroir is a concept that relate a product (wine) with the specific location where is has been produced. The flavor of the wine is the results of the interactions of various parameters (soil, climate, plant material, vineyard training, winemaking, …). A complete definition of terroir has been proposed in 2010 by the OIV (Resolution OIV/VITI 333/2010)

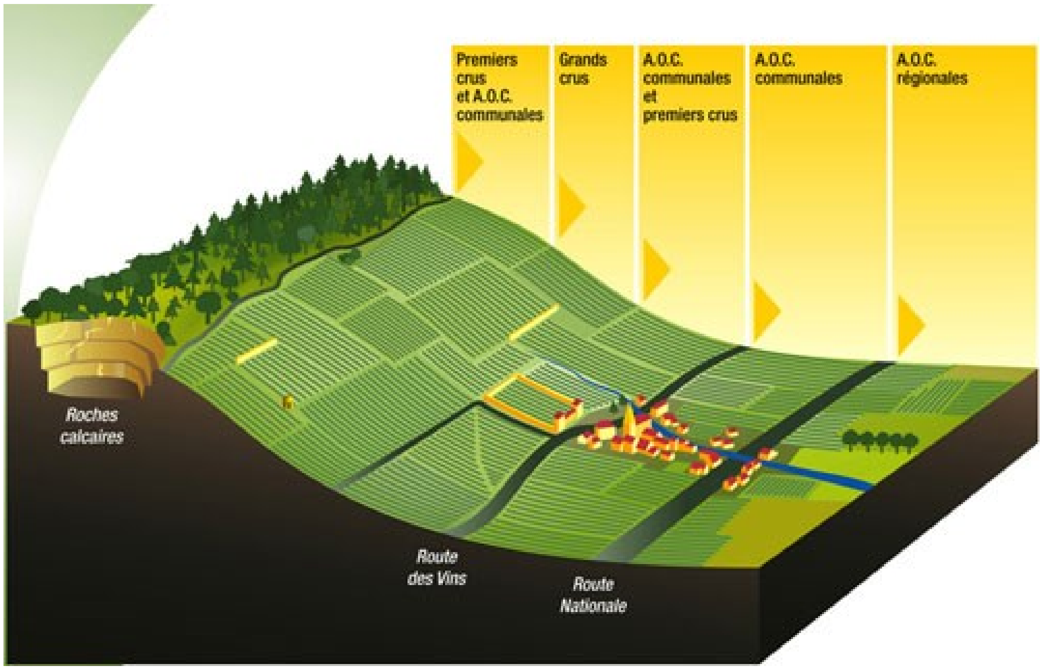


Figure 1.1 2: An example of appellation repartitions in Côte de Nuits

(Source: eliewine.com)

Furthermore, this notion of *climats* (terroirs) is directly linked to the geological context of Burgundy. The soils of Burgundy are originate mostly from calcareous or marls bedrocks which soils rich in limestones, clay and slits develop. Soils depth depends on the position on the slope but is often below one meter. Premier *climats,* Grand cru and Premier cru exhibit usually higher content in gravels (about 25%) that village and regional appellations. (Mériaux et al., 1981)

Burgundy's climate is oceanic with semi-continental tendency with cold winters, hot summers, and evenly distributed rainfall throughout the year, with violent thunderstorms occasionally. The average temperature in summer and winter is 19.6°C and 1.6°C, respectively and an average rainfall equivalent to 775mm per year.[2] Figure 1.1.3 The vineyards, primarily facing east on the slopes, benefit from the diversity of microclimates influenced by the number and orientation of valleys, leading to a variety of terroirs.

The combination of these climatic conditions and the geology of Burgundy gives rise to some inimitable wines. (Chabin, 1995)

[2] www.regions-of-france.com

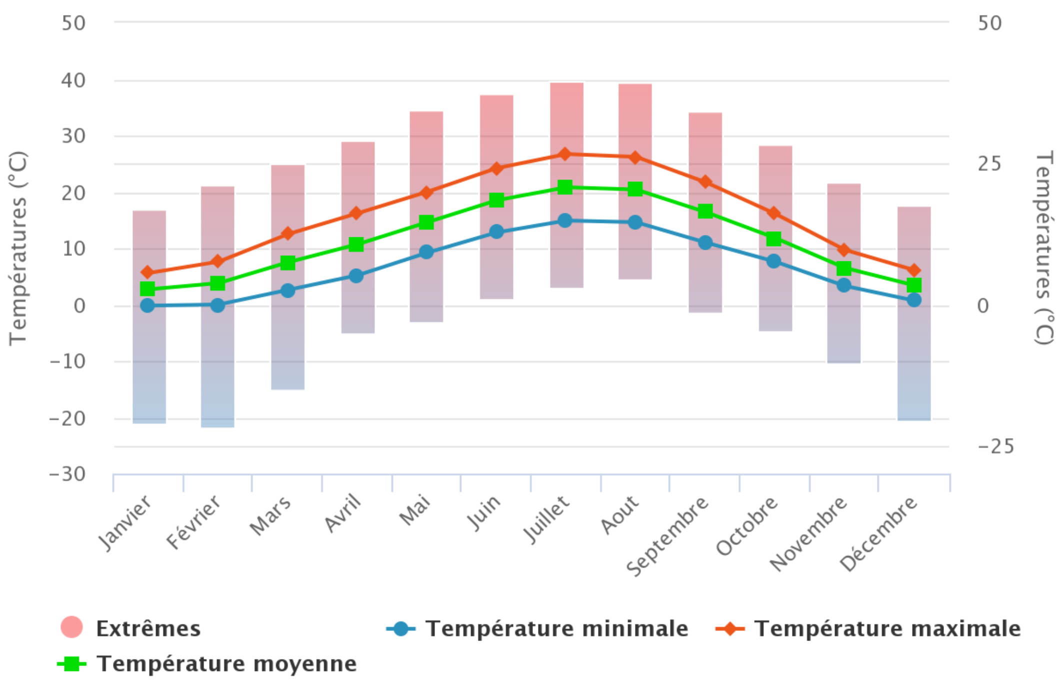


Figure 1.1 3: Temperature in Dijon-Longvic in 1990-2020 period

(Source: infoclimat.fr)

1.2. Burgundian wines

1.2.1. The grape varieties

With nearly 86 AOC (Appellation d'Origine Contrôlée) designations, the great majority of Bourgogne wine are produced from the majestic grape varieties: Pinot N. and Chardonnay, representing 40% and 49%, respectively, of the cultivated area. Aligoté, Gamay and other varieties such as Sauvignon are also grown.[3]

Pinot noir is an old grape variety descended from *Vitis vinifera*. This grapevine is resistant to winter frosts but it is sensitive to spring frosts since it buds early. Furthermore, at the end of its ripening cycle, the variety is vulnerable to temperature. Pinot noir forms a packed cylindrical-conical cluster of small oval shaped berries, which makes it prone to rots towards harvest if the weather is humid.(Coates, 2008)

Pinot noir has lower levels of tannins and anthocyanins, compared to Cabernet-Sauvignon or Syrah varieties, resulting in a ruby-red colour, a lighter body and a softer mouthfeel. The wine has an elegant structure and complex aromas of summer red fruits and spice.

Chardonnay is originally from Burgundy, it is a vigorous variety. It buds later than Pinot noir but it is sensitive to spring frosts as well. *Coulure* is also an issue for this variety. The formed cluster

[3] vins-bourgogne.fr/vins-et-terroirs/nos-cepages-nos-couleurs

has a winged-cylindrical shape with small berries; it is relatively compact so is less prone to grey rot. [4](Coates, 2008)

Chardonnay is renowned for expressing a refined minerality and richness, with a gold color, fresh and fruity notes along with flavors ranging from crisp green apple to creamy lemon, often with nuanced notes of oak and a long, clean finish. Thus, the best Côte d'Or white Burgundies are vinified in wood.

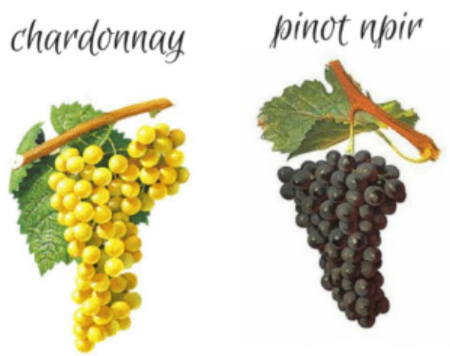


Figure 1.2 1: The dominant varieties in Burgundy: Chardonnay and Pinot noir

(Source: fr.rjscraftwinemaking.com/craft-and-cork)

1.2.2. Viticulture and viniculture methods

The training method used in the region, according to the "Cahier des Charges de l'AOC Bourgogne", is either spur pruning (Cordon de Royat) or cane pruning (Guyot). (Coates, 2008)

The spacing between the rows should not exceed 2.5 m and the height of the trellised foliage is at least equal to 0.6 time the row spacing. Additionally, the vines on the Côte are generally planted at a high density of 10000 vines per hectare. This high-density planting is a hallmark of the region. The maximum average plot load is set at 11,000 kg/ha for white wines, and 10,000 kg/ha for red and rosé wines, much lower for some AOC village, Premier and Grand crus; the yield is set at 68 hL/ha for white wines and 60 hL/ha for red and rosé wines.

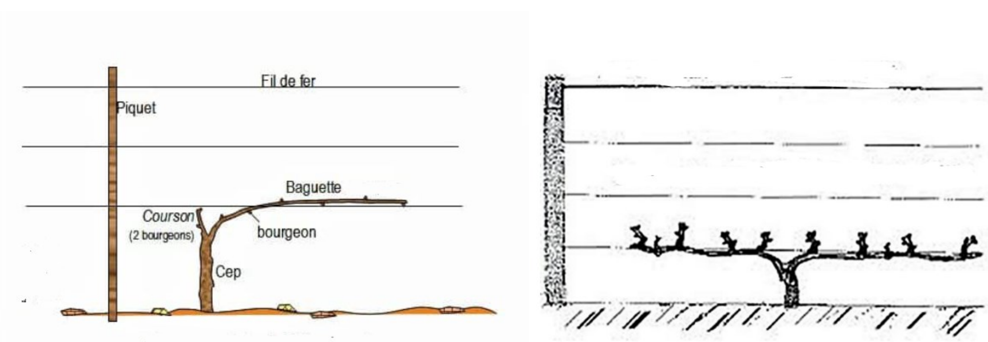


Figure 1.2 2: Training systems: Guyot (left) and Cordon de Royat (right)
(Source: dico-du-vin.com, cetab bio)

[4]burgundy-report.com/discover-burgundy/03-the-vines-of-burgundy/#chardonnay

The quality of the wine is predominantly from the fruit, but the *savoir-faire* of winemakers in Burgundy plays a big part in producing its prestigious wines. (Chabin, 1995) In fact, the winemaker's expertise has a great influence over the character of the wine, for instance in adapting to each vintage. In addition, since each estate has its long-standing tradition, there are various methods to produce high quality Burgundy wines.

Antoher practice in Burgundy is the chaptalization. This latter is permitted to a maximum level that would increase the alcohol content by 2°. The winemaker is free on when and how he adds his sugar.

Figure 1.2 3: A typical Burgundy wine label

(Source: wine-searcher.com)

1.3. Organizational profile

The study was conducted at the Université de Bourgogne mainly at the Centre de Recherches de Climatologie (CRC) and at the Domaine Viticole de l'Université de Bourgogne, located in Dijon and Marsannay-la-Côte, respectively, in the Burgundy region in France. The project was implemented at the experimental estate of the University of Bourgogne. In France, the University of Bourgogne is the only university to have an experimental estate.[5]

[5] francebleu.fr/infos/agriculture-peche/quelles-experiences-scientifiques-sont-menees-aujourd-hui-dans-les-vignes-de-bourgogne-8772839

Figure 1.3: Aerial view of operating buildings in 1962 (left) and in 2017 (right)

(Source: iuvv-domaine-viticole.u-bourgogne.fr)

The 3 ha estate of the university is a bequest from Jean-Baptiste Hippolyte Lucotte, a wine-grower and owner, in 1918. Today the university owns 3.6 ha spread over the municipalities of Marsannay-la-Côte, Couchey and Dijon; managed by Camille Bossuat, oenologist and directed by Pr. Hervé Alexandre, oenology professor at the Institute Jules Guyot. Thus, the estate allows researchers and university professors to use its plant materials and products for their activities, and it serves as a place of application for specialized courses in vine and wine sciences.[6]
During the 6 months of my internship, I gained valuable insights into their operations and contributed to various projects aligned with their mission to administer experiments to study regional and national problems with collaboration of other organizations.

2. Literature Review

2.1. Climate change

2.1.1. Global climate change

According to the IPCC the atmosphere and oceans are warming, snow and ice cover are decreasing, and the sea levels and GHG concentrations are rising because of human activity.(Torquebiau, 2016) The average global temperature in 2022 was about 1.15°C above the pre-industrial levels (1850-1900) average used as a baseline for the Paris Agreement on climate change. Since pre-industrial times, the concentration of carbon dioxide (CO2) has risen by 40%, for the most part because of emissions from fossil fuels and agriculture (about 24%).[7] Figure 2.1.1 Agriculture is certainly the human activity that is the most dependent on the climate. The problems and challenges faced by farmers due to climate change are more frequent and even more serious.
The sixth Assessment Report of the IPCC, the Climate Change 2021, reported that global mean surface temperature relative to pre-industrial levels is expected to increase well over the 2°C warming threshold (for human and ecosystems sustainability) in the status quo GHG emissions scenario with temperatures potentially increasing by 6°C in the worst-case scenarios by the year 2100. While a warming planet is nearly certain, the IPCC projects more extremes in weather patterns (i.e., increased incidence of flood or drought events).[8]

[6] iuvv-domaine-viticole.u-bourgogne.fr
[7] public-old.wmo.int/en/media/press-release/past-eight-years-confirmed-be-eight-warmest-record
[8]https://www.ipcc.ch/report/sixth-assessment-report-working-group-i/

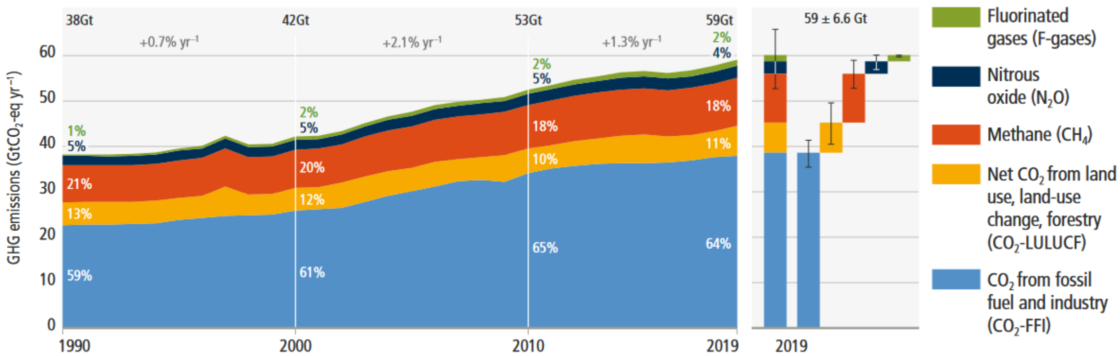


Figure 2.1 1: Global net anthropogenic GHG emissions 1990-2019

(Source: IPCC WGIII)

The latest report on climate change in Europe, by the World Meteorological Organization shows that Europe has been warming two times as much as the global average since the 1980s. In 2022, Europe was approximately 2.3°C above the pre-industrial levels.(R.Shukla et al., 2022) In addition, in the same year, many countries in western and south-western Europe, including France, had their warmest year on record; precipitation was below the average across much of the region. France had its driest January to September, with important impacts on energy production and agriculture.[9] This latter will have to evolve to adapt to climate change and biodiversity loss, while also mitigating their impacts, in order to cope with the significant economic, social and environmental challenges of the 21st century and sustainably meet the needs of a predominantly urban population of approximately nine billion by 2050.(Torquebiau, 2016)

2.1.2. Climate change in Burgundy

In winegrowing, harvest start dates records are the proof of these increased temperatures as they are maintained in many European wine regions for centuries; specifically, the long-homogenized Beaune series documents harvest start dates since 1354. It shows that harvest dates in Burgundy, where approximately stable until recent warming trends over the past 30 to 40 years, have led to an advance of the harvest start date by almost 15 days when compared to the historic average. Figure 2.1.2 Grapes were harvested on the 28th September from 1354 to 1987, on the other hand they were picked on the 15th September between 1988 and 2018. (Labbé et al., 2019) Moreover, the increased temperature also had a significant influence on the dates of grapevine phenological stages of mid-bud break, mid-flowering and mid-veraison of Pinot noir. They have advanced from 10 to 15 days between 1970 and the end of the 20th century. (Bois, 2013)

[9] public-old.wmo.int/en/media/press-release/climate-change-impacts-scar-europe-increase-renewables-signals-hope-future

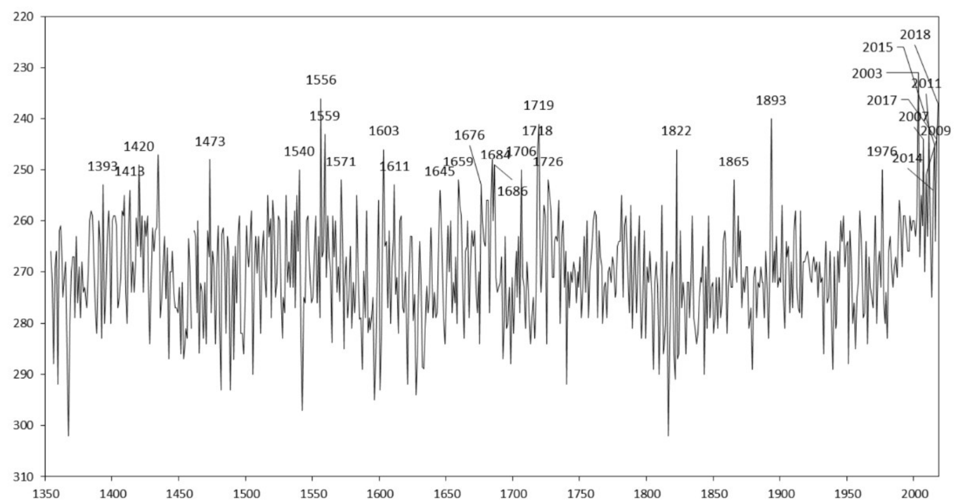


Figure 2.1 2: Long-homogenized Beaune series of harvest dates

(Source: Labbé et al, 2018)

Although, Burgundy region shows a little movement in alcohol levels compared to other wine regions[10] showed in Figure 2.1.3. The levels of warming reveal that, traditionally, alcohol levels hovered around 12-12.5%, have in recent times clocked at 13.5-14.5% [11], which can lead winemakers to cease chaptalizing. Hence, today producers are faced with a delicate situation in maintaining the typicity of wines: further increasing alcohol levels risk unbalanced wines, while further advancing the date of harvest risks the production of wines that have reached technical maturity but not phenolic.(Leeuwen and Darriet, 2016)

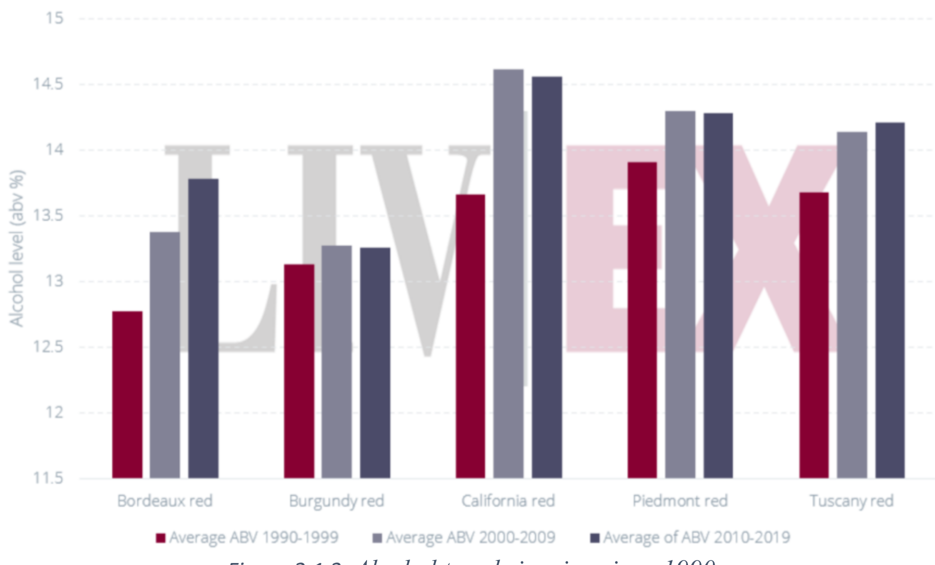


Figure 2.1 3: Alcohol trends in wine since 1990

(Source: liv-ex.com/)

[10] liv-ex.com/2021/06/alcohol-levels-wine-rising-proof

[11] https://winehog.org/burgundy-and-alcohol-levels-what-the-label-say-36116/

2.2. Impacts of climate change on viticulture

With the onset of climate change, vineyards around the world are experiencing changes that pose both challenges and opportunities. Rising temperatures can lead to earlier bud breaks and harvests, potentially altering phenological rhythm of vineyards. Worsen hydric stress and alterations in the Leaf-to-Fruit Ratio further complicate vine management, affecting grape maturation and quality. Although, some regions may benefit from a warmer climate, which allows the ripening of grapes that was then challenging, others might suffer from excessive heat, disrupting the balanced maturation of sugars, acids, and tannins in grapes.

2.2.1. Impacts on the phenology of grapevine

Grapevines are developing through key stages as budburst, flowering, veraison and harvest; these stages are indeed influenced by temperature, which is a factor affecting both the growth and the quality of the grape. Hence, extreme low and extreme high temperature can both damage the grapevines. If extreme low temperatures may cause freezing injury, damage buds and therefore reduce yields and quality, on the other hand high temperatures can cause early veraison, damage the skin of the grape and defective flavor development during berry growth. (Mullins et al., 1992) In addition, in the maturation stage, high temperature may advance ripening and causing a rapid decomposition of acid and an increase in sugar levels in grapes resulting in higher alcohol and lower acid levels in wines. (Duchêne and Schneider, 2005) Therefore, the warming trend is a major issue in wine production as it shifts the phenology of grapevines. (Jones and Davis, 2000; Ruml et al., 2016)

Numerous studies have shown the consequences of climate change on the phenology of vines. In their studies, Xu et al. demonstrate that due to higher temperature, in the 2030s, flowering will happen 8 days earlier and 12 days earlier as of veraison. (Xu et al., 2012) Moreover, as mentioned previously, in Burgundy, harvest dates were shifted from 28 September, from 1354 to 1987, to 15 September between 1988 and 2018. (Labbé et al., 2019) In the Bordeaux region, by 2050, flowering and harvest dates are predicted to be advanced by 15 and 25 days, respectively, and by 30 and 45 days, respectively, at the end of the century.(Leeuwen and Darriet, 2016) Figure 2.2.1

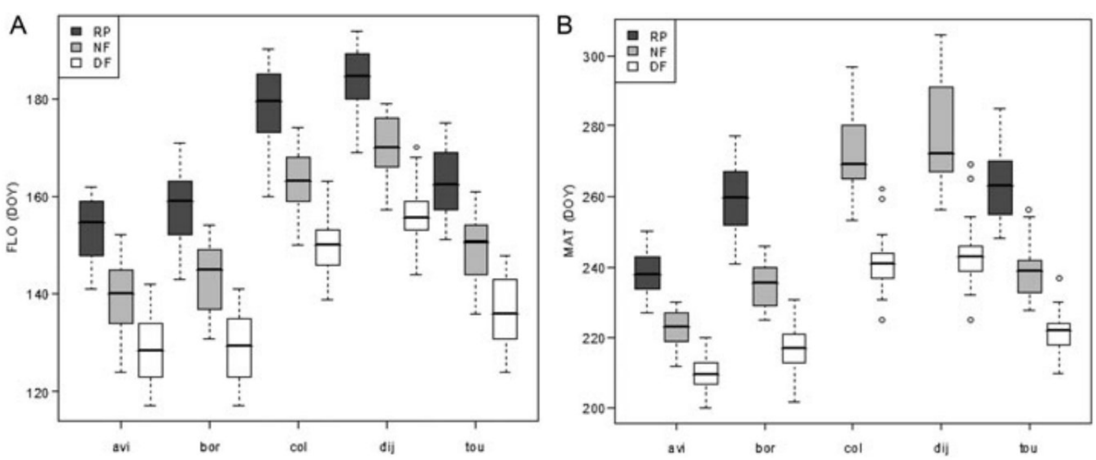


Figure 2.2 1: Modeled mean flowering (A) and harvest dates (B) for Merlot in Avignon (avi), Bordeaux (bor), Colmar (col), Dijon (dij) and Toulouse (tou). RP=Recent Past (1971-2000), NF=Near Future (2020-2050) and DF= Distant Future (2070-2100)

(Source: Leeuwen and Darriet, 2016)

2.2.2. Impacts on hydric stress

Climate change might change rainfall patterns and the rise of temperature, leading to higher evaporative demand in various parts of the world has intensified drought conditions, that mighty induce severe hydric stress.(Schultz, 2017) In fact, when summer rainfall does not counterbalance water loss through evapotranspiration, vineyards face more severe water deficit as the growing period progresses.(Keller et al., 2016) Therefore, for growers, challenges can arise in ensuring grapevines receive an optimal amount of water throughout their growth cycle.

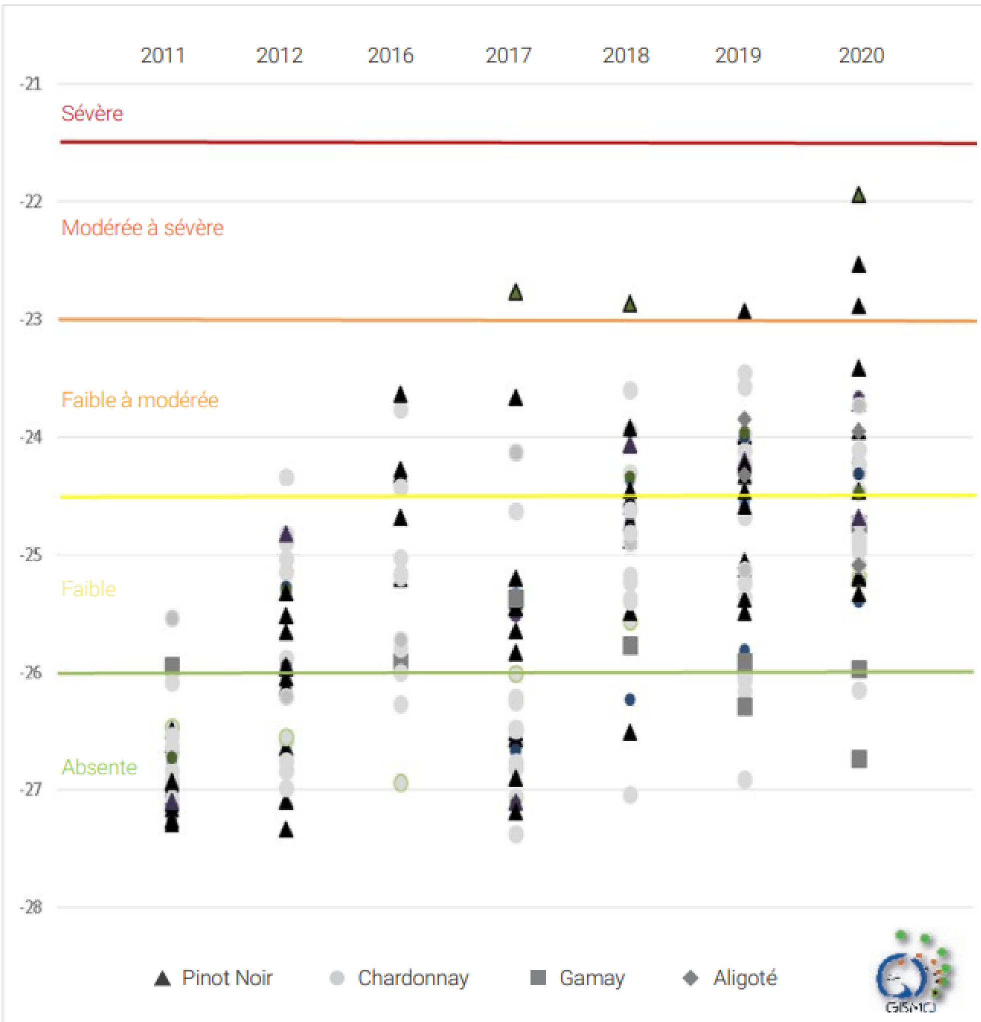


Figure 2.2 2: Comparison of water stress of different millesimes before harvest in Burgundy. Y-axis: Threshold values of the δ13C <-26= absence, -25 to -26= weak, -24 to -25= weak to moderate, -23 to -24= moderate to severe and >-23= severe

(Source: extranet.bivb.com/technique-et-qualite/publications-techniques/plaquettes-techniques/les-cahiers-du-pole-technique-et-qualite-4-les-effets-du-changement-climatique-en-bourgogne,1976,16045.html, 2021, GISMO platform, Biogéosciences)

The $\delta^{13}C$ has been monitored for 10 years since 2011 in Burgundy for a large number of plots. Figure 2.2.2 illustrates the increased temperature and modified precipitation patterns in Burgundy. In fact, according to the BIVB, 2020 was undoubtedly the hottest year since the 20th century in the Côte de Nuits.[12]

Severe water stress during the growing stage can inhibit shoot growth and photosynthesis and negatively influence yield and berry composition and compromise wine quality.(Keller et al., 2016; Leeuwen et al., 2009; Marciniak et al., 2013) Nevertheless, mild water stress can enhance red wine quality because berry size is reduced resulting in less acidity level in grapes .(Leeuwen et al., 2009; van Leeuwen and Destrac-Irvine, 2017) Thus, hydric stress has a direct impact on grape quality.

One method to determine the levels of water deficit is the carbon isotope discrimination. Thereby, an index called $\delta^{13}C$ resulting from the ratio of ^{13}C to ^{12}C in grape sugar, can be used to demonstrate the hydric stress endured by vines during ripening stage. The $\delta^{13}C$ is interpreted compared to a standard with a range of -27 ‰ (no water deficit) to -20 ‰ (severe water deficit stress). (Leeuwen et al., 2009)

2.2.3. Impacts on phenolic compounds

Phenolic compounds in grapes, including tannins, anthocyanin, and flavonoids, are responsible for the color, taste and mouthfeel of wine, thereby playing an essential role in its quality and characteristics. These compounds are sensitive to environmental conditions, such as temperature and water availability, which pose an important challenge to the winemakers in maintaining the traditional characteristics of their wines. In fact, Mori et al. found in their study, in 2007, that the total anthocyanin content decreased considerably under high temperature (35°C) treatment. In addition, “night coolness” index is often allied to the aromatic traits of the grape variety and the colour of wine.(Deloire et al., 2004) However, mild water stress can lead to increased concentration of phenolic compounds and can improve wine quality, as mentioned previously in 2.2.2. section, as of the case of Bordeaux. (Leeuwen and Darriet, 2016; Van Leeuwen and Destrac-Irvine, 2017)

[12] extranet.bivb.com/technique-et-qualite/publications-techniques/plaquettes-techniques/les-cahiers-du-pole-technique-et-qualite-4-les-effets-du-changement-climatique-en-bourgogne,1976,16045.html?

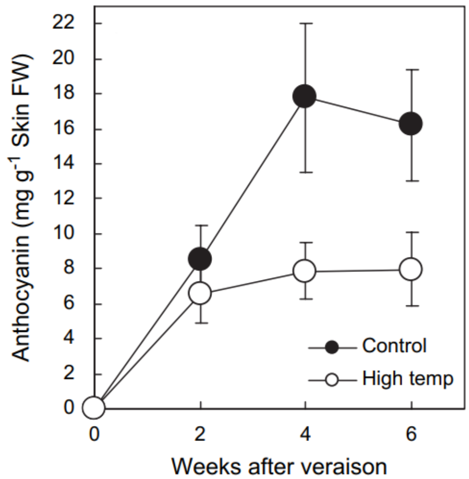


Figure 2.2 3: Effects of temperature on anthocyanin accumulation in the skin at 25°C (control) and 35°C (High temp)

(Source: Mori et al 2007)

2.2.4. Impacts on sugar and acidity Levels

Sugar and acidity balance contributes highly to the one's perception of the quality of a product including wine. (Coombe et al., 1980) This balance is crucial in ripeness criterion and in winemaking because sugar levels are the precursors to alcohol in wine (Coombe et al., 1980) and they contribute to the sweetness and body of the wine;(Jordão et al., 2015) and acidity including tartaric and malic acids, gives structure and freshness to the wine.(Volschenk et al., 2017) Due to the warmer temperatures, ripening of grapes tend to occur earlier resulting in a disproportion of the sugar and acidity levels.(Mira de Orduña, 2010)

The rising temperature promotes leaf photosynthetic activity and tends to increase grape sugar accumulation but its effects on final sugar content are relatively small according to Coombe. However higher temperature (30°C) increase suspended solid concentrations (> 24-25 Brix) are not due to photosynthesis but more likely to concentration by evaporation.(Keller, 2010)

With higher temperature, total acidity is affected: main grape acid and tartaric acid are relatively stable; but malic acid levels decrease with higher temperatures.(van Leeuwen and Destrac-Irvine, 2017) This decrease of acidity levels are generally correlated with higher grape pH leading to significant changes in musts and wines. In a study done by B. Ganichot in 2002, in southern France, the total acidity decreased from 6 to 4 g l^{-1} H_2SO_4 and the pH increased from 3 to 3.3 between 1980 and 2001. Must pH values higher than 4 are now attained in hot climates, this increases the risk of spoilage. (Mira de Orduña, 2010)

To put briefly, to address climate change in viticulture, adaptation is necessary. As a long-term adaptation, relocating vineyards further north to higher altitudes with more suitable climatic conditions can be considered. Nevertheless, this strategy would affect severely the economy of the abandoned region, as it is an extreme solution. Additionally, changing genetic materials such as grape varieties, clones and rootstocks that are more resistant to increased temperatures is another potential strategy. Pruning late can potentially delay vine phenology resulting in wines with lower alcohol content. Irrigation can be a solution for managing vine water supply in regions where the practice is allowed. Finally, canopy management is also an effective way in manipulating the evapo-transpiration of the vine, reducing grape sugar content while maintaining acidity.(Santos et al., 2021a, 2021b)

2.3. Canopy management a potential viticulture adaptation to climate change

Canopy management is an intriguing practice in viticulture aimed at mitigating the effects of climate change. This practice consist of the adjustment of the exposed leaf surface, thereby altering the leaf to fruit ratio. In fact, when leaves are removed, the total leaf area available for photosynthesis decreases, limiting the synthesis and accumulation of carbohydrates in the berries, consequently delaying the ripening stage. (Caccavello et al., 2017; Poni et al., 2008; Santos et al., 2021a)

Notably, when leaf removal was applied at veraison in cv. Istrian Malvasia the Brix levels increased by 1°. (Palliotti et al., 2014) Additionnaly, according to Santos et al., reducing exposed leaf area to less than 0.75 m^2/kg after fruit set can delay the time from flowering to veraison by 5 days. In another study, it was found that severe leaf removal carried out on Riesling prior to veraison delayed ripening by approximately 2 weeks compared to control vines.(Stoll et al., 2010)

Moreover, the impacts of leaf removal on yield, cluster number, cluster weight or berry weight were not significant according to some authors. (Bledsoe et al., 1988) Thus, the timing and the severity of leaf removal method provide an effective strategy to regulate its impacts on grapevines. While this practice is simple and yields promising results, it is time consuming and comes with significant labor costs.

Although researchers have explored the application of this method in various cultivars, such as Sauvignon blanc, Aglianico, Chasselas etc., in different wine regions, there is currently no reference for its application in Burgundian Chardonnay and Pinot N.. This report discusses about the application of leaf removal apical at different stages and severities within the Burgundy region.

3. Materials and Methods

3.1. Plant material and experimental layout

The experiment was carried out on two vineyard plots located in Marsannay-la-Côte (Burgundy region, France) over the 2020, 2021, 2022 and 2023 seasons; a plot of Chardonnay and Pinot N., but the focus of this report is the plot of Pinot N..

The parcel of Pinot N., named Champforey, is distinguished by a clay-silty texture with a medium limestone gravel content. *Vitis vinifera* L. cv. Pinot N. clone #115 were planted in 2015, and are grafted onto SO4 rootstock. They are planted at 1 m x 1 m inter-row and intra row, with a trunk height of 0.45 m and trained to the single Guyot and the single Guyot Poussard methods, 2 buds per spur and 6 to 8 buds per cane. Chemical herbicides or perstcides are not used, the soil is tilled three or four times during the vegetative period and shoots are trimmed mechanically. As a preventative measure, normal leaf removal on the sunrise side of the canopy can be performed in June depending on the climatic conditions and disease pressure of a given year.

The trial compares 6 treatments of bunch thinning with apical leaf removal at different levels of severity and timing. (I) two bunch thinning treatments at levels selected based on estimated Leaf to Fruit Ratio (LFR) both at bunch closure (hereafter, these treatments will be named as moderate bunch thinning, BT- and severe bunch thinning, BT+). (II) three leaf removal treatments that left 40 or 20 cm of the leaves at bunch closure and 20 cm 10 days after mid-veraison (hereafter, these treatments will be named as moderate leaf removal, LR-, severe leaf removal, LR+ and late leaf removal LLR, respectively). Finally, (III) a control (no bunch thinning or leaf removal). Bunch thinning treatments were not applied in 2021 due to insufficient yields, caused by late spring frost.

The experimental layout was designed in 2020 and repeated over the three other vintages. The external rows of the parcel and that of the vines of each sub-plots were kept as buffer zones, this is done to diminish influence from neighboring parcels and sub-plot treatment, buildings, the road and nature. Hence, the layout consists of 7 repetitions/ blocks, which are composed of 6 treatment sub-plots spatially randomized. Within each block, all six treatments were implemented according to their respective sub-plots, consisting of 21 grapevines, 3 adjacent panels of 7 vines. Measurements were taken from the middle row of the 3 adjacent panels, e.g for the first 3 panels, 2, 3, 4, measurements were taken from row 3. Thus, the values used in this work were taken from row 3, 6 and 9.

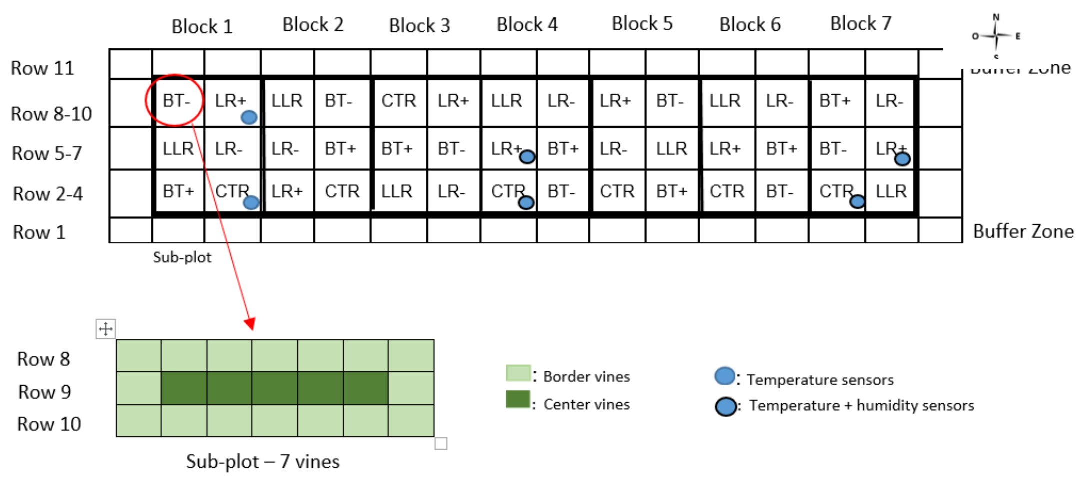


Figure 3.1: Experimental design

3.2. Vineyard Measurements

3.2.1. Microclimate

In order to observe the microclimate within each treatment, a follow-up of the temperature and of the relative humidity was realized in 2020, 2022 and 2023. Therefore, temperature sensors were placed in the cluster, while the other ones were placed at the height of the bunch, at approximately 50 cm from the ground, with or without humidity sensors in radiation shields. In 2023, in total 12 temperature sensors were placed on the plot, 6 in the cluster and 6 at the height of the bunch; and 4 humidity sensors. They were installed on the CTR and LR+ treatments. (Figure 3.2.1)

Figure 3.2 1: Temperature and humidity captors at the height of the bunch (left) and in the cluster (right)

3.2.2. Exposed leaf area and leaf to fruit ratio

An excessive leaf to fruit ratio (LFR) can overshadow grape clusters, affecting their ripening process, leading to uneven maturation, reduced fruit quality, and imbalanced sugar and acid concentrations in the grapes. Additionally, a reduction in LFR can delay the onset veraison leading to grapes with lower sugar content while not significantly altering their acidity levels.(Parker et al., 2014) Such changes can influence the grape's composition (Kliewer and Dokoozlian, 2005), or the escalation of more herbaceous notes in both grapes and wines (Van Leeuwen and Darriet, 2016)

Before the application of the different treatments, on the 4th of July, the number of clusters per vine of all sub-plots were counted. Porosity measurements were taken on the sub-plots of BT+/- treatments in order to determine the levels of the treatment based on the LFR based on the Exposed Leaf Area (ELA) and estimated yield. For the porosity measurements, pictures of the measurement vines (middle vines) were taken with a brown cardboard background and later on cropped at approximately 75 cm, from the lower wire to the canopy height. The cropped pictures were then analyzed using an RStudio script to obtain the vine's porosity. The idea is to count the pixels of each picture and isolate the visible NDVI (Normalized Difference Vegetation Index) of each pixel to distinguish green pixels, therefore leaves, from the pixels of the cardboard background, therefore the holes in the canopy i.e. vine's porosity. A vegetation index is derived as the normalized difference between red and green bands of the picture. As pictures exhibit a binomial distribution (a group of low vegetation index values of the brown cardboard and the trunk vs a group with higher values of the leaves and other green organs). The local minimum between the two distribution modes is calculated to set a threshold below which pixels are considered as gaps in the vegetation, which proportion (porosity) is used to calculate ELA. The following formula of ELA was used for the calculation: (Murisier, 1996) where the foliage height, width and spacing between vines are provided in meter.

$$ELA = \frac{2 * foliage\ height + foliage\ width}{spacing\ between\ vines}(1 - porosity)$$

The LFR was then calculated using the formula $LFR = \frac{ELA}{yield}$. The yield was obtained from the number of clusters counted per vine and the average of the past 3 years' yields. The initial LFR estimations (based in 2020 by rule of thumbs from average Pinot N. cluster weight at harvest in Bourgogne, and from 2021 using cluster weights measured in the previous years of the experiment on the plot) indicated that about 4 and 6 clusters should be removed per vine for BT- and BT+ respectively. indicated that 4 and 6 clusters should be removed per vine for BT- and BT+, respectively.
This process was done for each treatment to determine the final LFR using the yields at harvest.

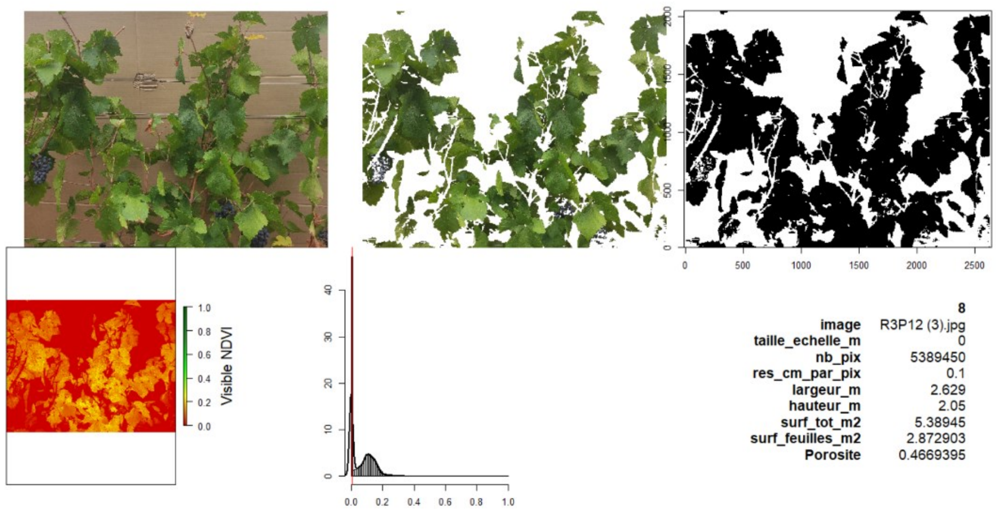


Figure 3.2 2: Porosity photos, from left to right: cropped picture and R output

3.2.3. Nitrogen state

N-tester measurements were taken before and after implementation of the late severe apical leaf removal treatments (the 3rd and 7th of July). 30 measurements were taken on each experimental sub-plot of 30 randomly selected leaves from the lower part of the canopy of the 5 measurement vines. (Figure 3.2.3) The N-tester measures the absorbance of light through each leaf in order to provide an index of its chlorophyll content corresponding to the plant's nitrogen levels. During the second N-tester field data collection, field observations were recorded regarding the number of sunburnt leaves per vine in order to gain insight into the vines' expression of hydric stress levels leading up to harvest.

Figure 3.2 3: Absorbance measurement in the field using N-tester

Table 1: Threshold for interpretation of the chlorophyll index on Pinot N. (Verdenal et al., 2023)

N-tester Index	<420	420-460	460-540	540-570	>570
Nitrogen Status	Very low	Low	Normal	High	Very high

3.2.4. Mid-Veraison Estimations

Starting on July 24th, veraison was observed every 5 days to follow up its evolution to determine the mid-veraison date. Veraison estimations were done visually using the veraison class and percentage of riped berries (Table 2) until mid-veraison. 3 random clusters were chosen for each of the 4 or 5 measurement vines and the percentage of the berries that had attained veraison were determined. After mid-veraison was reached, the estimations were continued until almost 100% veraison with the 50 berries selected for the pooled block maturity controls (see section 3.2.5). Here, in order to calculate the percentage of berries which had attained the veraison, each berry was examined. The veraison estimation data from the two methods was pooled and analyzed to create a veraison graph and determine the date of mid-veraison. The mid-veraison date, expressed in Julian day, was obtained using linear interpolation.

3.2.5. Maturity controls

Maturity analyses were conducted on the five measurement vines of each experimental panel (except for block 7) once per week from mid-veraison until harvest in order to track the accumulation of sugar and the evolution of other parameters including berry weight, pH, total acidity, tartaric and malic acid and nitrogen.

Blocks 1 and 2, 3 and 4 and 5 and 6 were pooled for the maturity controls (except harvest where each block was treated separately) to minimize the number of berries removed per vine while maintaining sufficient statistical power. 50 berries were selected randomly (25 from the measurement vines of each panel) to gain a representative sample of each of the pooled blocks.

Table 2: Veraison class and percentage of riped berries

Class	% of veraison
0	0
5	5±5
15	15 ± 5
25	25 ± 5
35	35 ± 5
45	45 ± 5
55	55 ± 5
65	65 ± 5
75	75 ± 5
85	85 ± 5
95	95 ± 5
100	100

Figure 3.2 4: Riped berries estimations

The berries were recounted and weighed to obtain the average berry weight and then pressed by hand. Approximately 20 ml of the pressed juice were centrifuged at 10000 rpms and 4°C for 5 minutes in 50 ml Falcon tubes. 15 ml of centrifuged juice was analyzed using the FOSS WinescanTM FT-IR spectrophotometer. The device simultaneously measures sugar, potential alcohol, pH, total acidity, malic acid, YAN (Yeast Assimilable Nitrogen) organic and inorganic nitrogen and tartaric acid content among other parameters. (Figure 3.2.5)
The remaining juice in the Falcon tubes were frozen for further useful analyses.

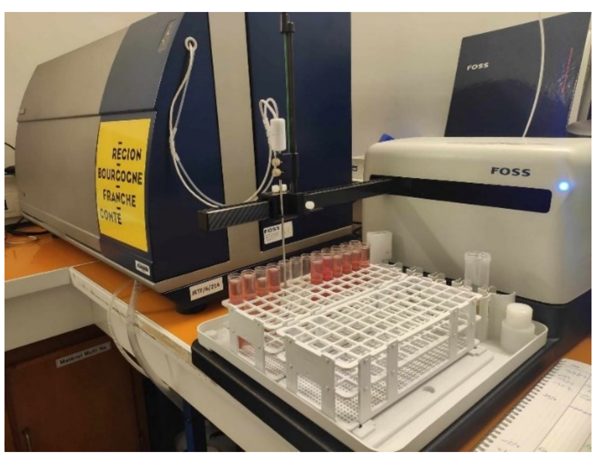


Figure 3.2 5: Maturity controls using FOSS WinescanTM FT-IR

3.2.6. Sunburn damage

Sunburn damage, resulting from the exposure of berries to intense sunlight and high heat, can significantly impact yield at harvest. To assess this, a PhD student conducted counts the day before the harvest for previous years and for this season, aiming to study the effects of various treatments on this phenomenon and investigate potential associations with the microclimate and the grape's hydric status. Each grape cluster was therefore evaluated, and a percentage of sunburn damage was assigned to it.

3.2.7. Harvest

The harvest took place on September 12 2023. Each measurement vine was harvested separately, with the harvested clusters being counted and weighed. These measurements provided data on the number of clusters and yield per vine, as well as the average weight of clusters. Additionally, 200 berries were randomly selected from each panel for analysis of their compositions, following the same procedures used for the maturity controls.

The pulp was conserved for phenolic compounds analysis (see 3.2.9 section).

3.2.8. Hydric stress

In October, $\delta^{13}C$ analyses were conducted on the frozen grape juice at harvest in order to quantify the hydric state of the vine. Each sample, contained within an Eppendorf tube, was unfrozen, homogenized with a vortex and then centrifuged at 5000 rpm for 10 minutes at room temperature. For the analysis, each sample was prepared in duplicate: 5 µL of the centrifuged juice was pipetted into a tin capsule and dried in an oven at 60°C for at least 12 hours, and the tin capsules were folded. The samples were analyzed at the GISMO platform using GC Isolink II, Isotope Ration MS. (Figure 3.2.6)

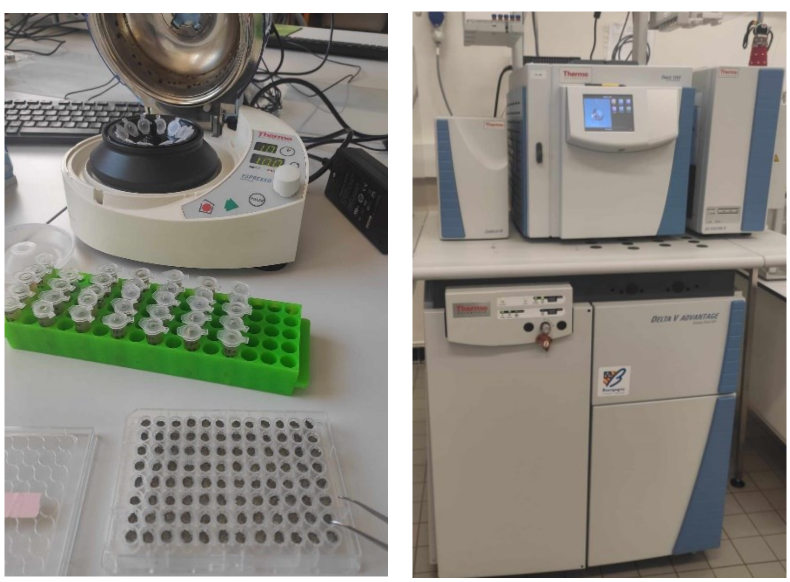


Figure 3.2 6: Sample preparation and GC Isolink II, Isotope Ration MS machine

Table 3: Threshold values of the δ13C of grape must to interpret water stress (Van Leeuwen et al., 2023)

Hydric Stress	Absence	Weak	Weak to moderate	Moderate to severe	Severe
$\delta^{13}C$	< -26	-25 to -26	-24 to -25	-23 to -24	> -23

3.2.9. Phenolic compositions

For several days in October, phenolics analysis were conducted on Pinot N. Each of the 42 bags of 200 berries at harvest were weighed to get 10 g samples of only skin and seeds, they were prepared in 50 ml Falcon tubes. 20 mL of a 1 % hydrochloric acid solution in methanol were added to each tube and then grinded and blended and completed to 45 mL with 1 % hydrochloric acid solution.

For the first extraction, each sample was centrifuged at 5000 rpm and 10°C for 10 minutes with the liquid portion being poured into a Pyrex balloon. Then, the remaining solid portion was completed to 45 mL with 1 % hydrochloric acid solution, homogenized, submitted to an ultrasound bath for 15 minutes, centrifuged again at 5000 rpm and 10°C for 10 minutes and poured into their respective Pyrex balloon to complete the 2nd extraction. This process was done one more timesfor a total of three extractions with the 1 % hydrochloric acid solution.

After the extractions completed, each Pyrex balloon was subjected to a concentration process using a Buchi Rotavapor which spun the Pyrex balloon in a bath of 30°C by increasing progressively the speed of the revolution. This process evaporated the methanol into the upper part of the device which was chilled by a closed circuit of water flowing at 10°C and condensed leaving behind only the phenolics that had been extracted by the hydrochloric acid. This process took on average 45 minutes per sample. (Figure 3.2.7)

The samples were then diluted with approximately 100 mL of distilled water and separated into two 50 mL Falcons in order to conduct the phenolics analysis in duplicate.

Total phenolics analyses were conducted using a spectrometer in relation to a sample of GAE (Gallic Acid Equivalent) for calibration. Each sample was diluted by 100 mL of distilled water per 1 mL of the previously diluted sample, homogenized and passed through the UV-Vis spectrophotometer in quartz cuvette.

Total anthocyanin analyses were conducted using the Y15 machine. 2 mL of each sample was directly pipetted into the machine's sample slots and the machine conducted the analyses using a reagent. The analysis was duplicated and the concentration was obtained.

Total tannin analyses by butanolysis were conducted using an acid reagent in order to depolymerize the tannins after having been further diluted by 50 mL of distilled water per 1 mL of sample in a Pyrex tube. 2 mL of this diluted sample was completed with 6mL reagent. Then, the mixture was divided into two: one for the control, which is directly poured in disposable cuvettes and and measured at 550nm absorbance (Abs_A); the other half was sealed with a Teflon stopper, immersed in a water bath for 30 minutes, and then placed in ice-cold water to stop the reaction. Finally, the absorbance was read at 550nm (Abs_B). (Figure 3.2.7) The concentration was calculated with the following formula:

$$Concentration(g/l) = (\text{AbsB} - \text{AbsA}) * 0.1736 * \text{dilution factor}$$

Figure 3.2 7: Extraction with the Buchi Rotavapor and measurement of the absorbance of total tannins

3.3. Statistical Analysis

In our study, we analyzed the collected data using the R statistical processing program through the Rstudio interface. To determine whether our study methods significantly impacted our measurements, we conducted ANOVA (Analysis of Variance) tests or Kruskal-Wallis tests to compare means. We chose ANOVA when the Bartlett test confirmed the equality of variances and the Shapiro test confirmed the normality of residuals. If these two conditions were not met, we employed the non-parametric Kruskal-Wallis test. A p-value lower than 0.05 indicated a significant effect, leading us to perform post hoc tests to identify differences between groups. Depending on the test's assumptions being met, we used either the parametric Tukey-HSD test or the non-parametric Fisher-LSD test, which helped to assign different letters to significantly different groups.

4. Results and Discussion

4.1. Leaf to fruit ratio

In Figure 4.1.1, the exposed leaf area shows a significant difference among the defoliated modalities, as we know that different severities were applied. Since the CTR, BT+ and BT- did not undergo any defoliation, they do not have difference in their average exposed leaf area in 2022 and 2023. This is however different in 2020 where CTR and BT- are different from each other. This is probably because 2020 was the first year of the trial's implementation, which might explain the observed bias.

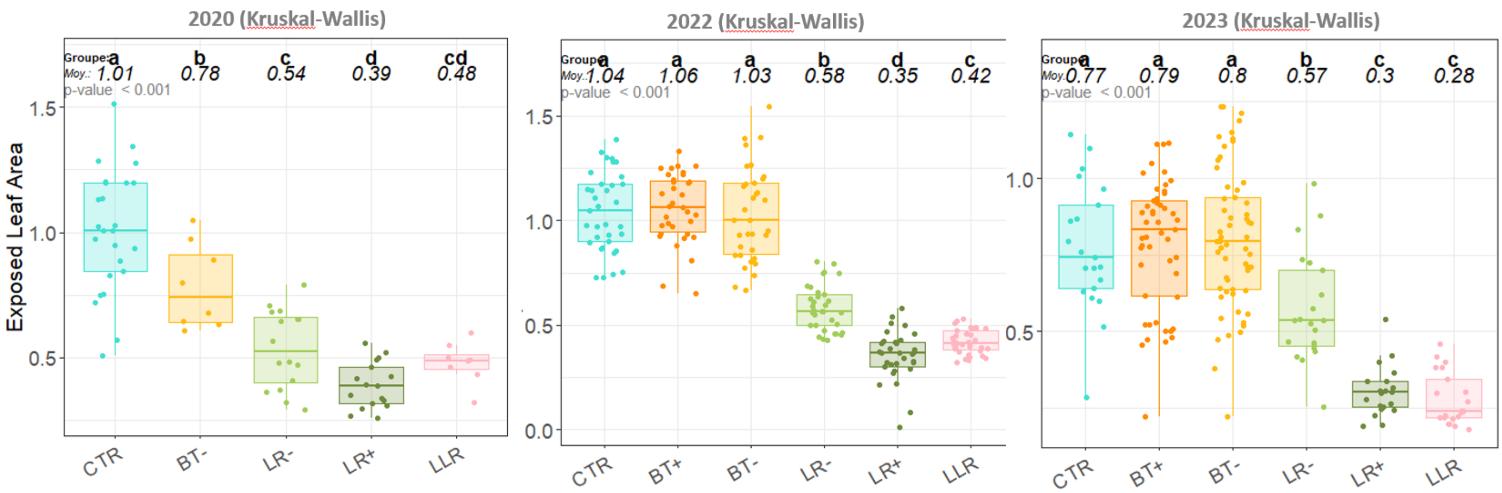


Figure 4.1 1: Exposed Leaf Area at harvest expressed in (m^2/m^2 ground)

In 2023, we observed that LLR and LR+ do not have significant difference since the same severity was applied to both but at different timing. Additionally, throughout the season, we did not observe significant regrowth in the foliage of the LR-, LR+, and LLR treatments, so only one session was required for the implementation of apical leaf removal.

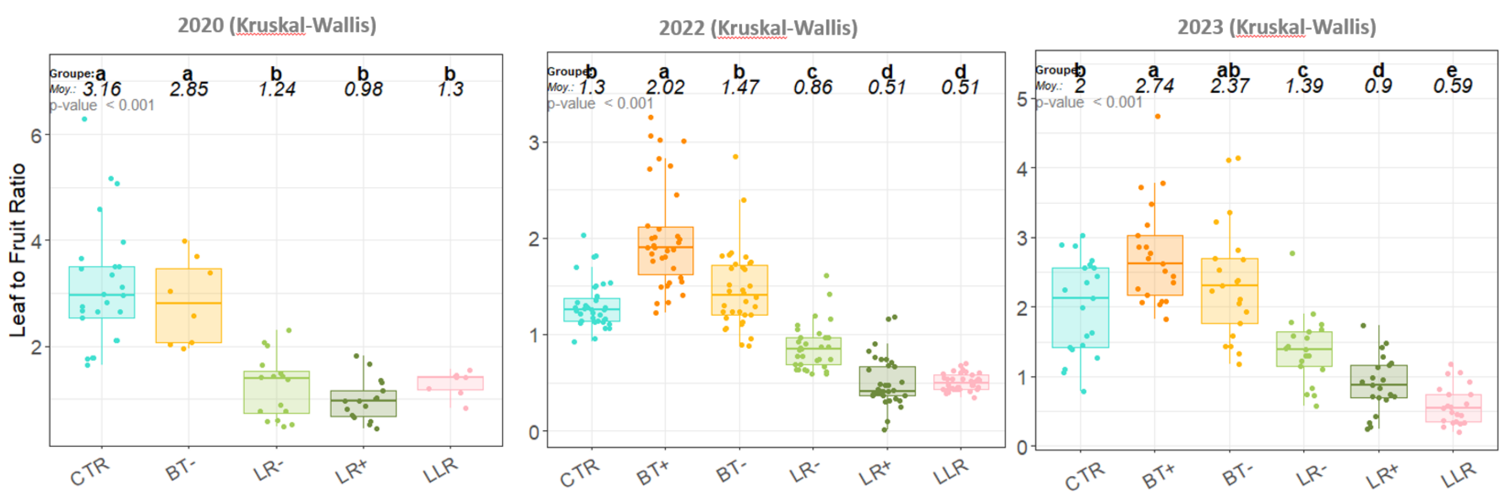


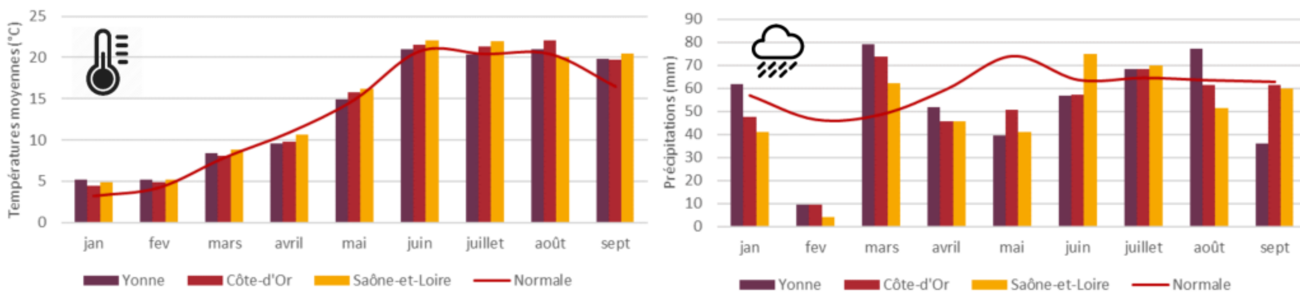
Figure 4.1 2: LFR at harvest expressed in (m^2/kg)

Moreover, for the leaf to fruit ratio, defoliated vines have low values compared to CTR, more or less two times less than the CTR in 2022 and 2023. However, in 2023, LR+ and LLR seem to have a statistical difference. The reason is probably that different operators did the applications. According to the literature, reducing exposed leaf area to less than 0.75 m^2/kg after fruit set can delay the time from flowering to veraison, which is the coherent with the findings of 2022. (Santos et al., 2021a) Additionaly, managing the leaf to fruit ratio through the reduction of leaf area does not appear to have been offset by regrowth in the inter-nodes over the course of the season.

The correlation of the LFR and sugar content, pH, tartaric acid (AT) and the sugar/ AT ratio can be found in Appendix A.1.

4.2. Climate

The weather throughout the year was quite unpredictable in Burgundy: a gentle winter, a varied spring with an observation of a high water deficit, and a summer filled with extremes of heat, refreshing rains, and even a hailstorm. Harvesting began in late August among a heatwave that was unusually intense for that time of year.[13] The Pinot N. grapes also took advantage of the September sun to reach optimal ripeness.[14]



(a) (b)

Figure 4.2 1: Comparison of the average temperature (a) and precipitations (b) by department for 2023 with the norm

Source: (Meteo France/BIVB)

As of the microclimate on the plot, Figure 4.2.2 demonstrates no significant difference in air temperature at any time of the day. However, it shows that the control grapevines experienced a higher temperature compared to the grapevines with leaf removal treatments. Hence, this suggests that the air was warmer around the control vines.

Inversely, the temperature was higher around the grape clusters in the LR+ group this phenomenon might be due to more sunlight reaching the grape clusters in the LR+ group because of the severe leaf removal, which created a microclimate with higher temperatures.

However, the fact that the air temperature didn't change much overall, even with potential for lower high temperatures in the LR+ group, could be due to the vines' thermal regulation through increased evaporation. This is because, with more water available to the LR+ vines (as they had less leaf area to lose water), they could evaporate more water, which cools the plants down. On the other hand, the CTR vines, with more leaf area, might use more water and be less able to cool themselves through evaporation when water is scarce, leading to less temperature regulation by the latent heat of evaporation.

[13] vins-bourgogne.fr/millesime-2023-en-bourgogne-au-dela-des-esperances

[14] info-beaune.com/articles/2023/11/13/4765/vins-de-bourgogne-climatologie-du-millesime-2023/

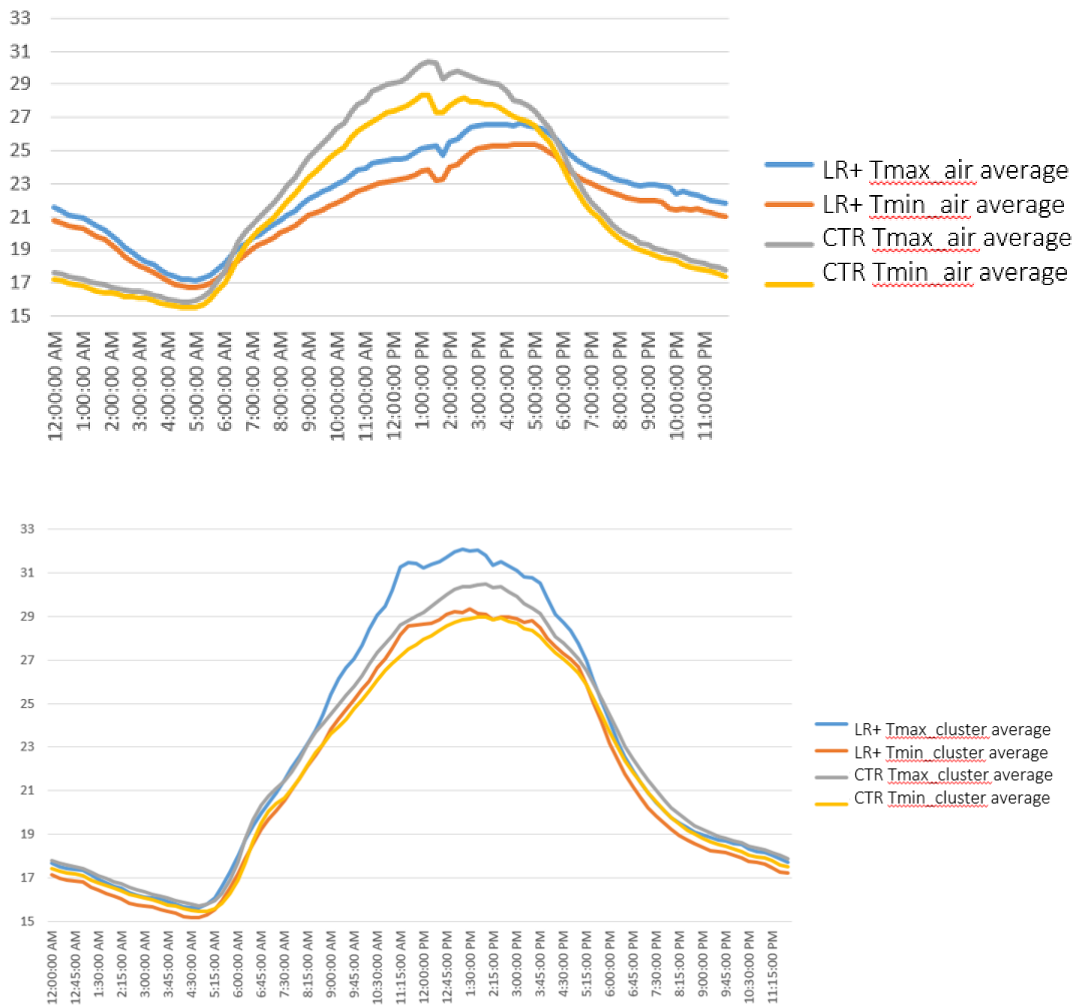


Figure 4.2 2: Average temperature of the air (first) and in the cluster (second) by hour from July to September

4.3. Veraison dynamics

We collected veraison data starting two weeks post cluster closure and continued for a month. This allowed us to observe the veraison kinetics and determine the mid-veraison date using Rstudio. We included data from all vintages except for 2021 due to missing of some information (this will be the case for some data analysis).

Our initial hypothesis posited an earlier mid-veraison date for the BT- treatment and a delayed one for the LR- and LR+ modalities. The veraison dynamics, as shown in Figure 4.3.1, revealed significant differences in the progression of veraison across the dates among the modalities. Notably, the differences were more pronounced between the removed leaf modalities (LR- and LR+) compared to BT-, CTR, and LLR. It is important to mention that the LLR treatment was implemented after mid-veraison, which explains why its pattern is similar to the control.

Moreover, as indicated in Figure 4.3.2, the mid-veraison date for the LR+ treatment is consistently later across the three years studied. On average, mid-veraison for the LR+ treatment occurs 4 days later than the control. Meanwhile, the LR- treatment's mid-veraison date aligns with the rest of the modalities even though its dynamics are generally significantly different from BT- and BT+ but not from CTR and LLR. Additionally, the BT- and BT+ treatment do not show notable difference from the LLR and CTR modalities regarding mid-veraison dates.

LFR does indeed have an impact on the onset of veraison, and apical defoliation helps to delay it. In fact, severe defoliation (LR+) has delayed the mid-veraison date by about 4 days which could lead to a delay of maturation. However, the BT- and BT+ treatments did not result to an earlier onset of veraison as we had assumed.
These findings are consistent with other studies on the topic which show that a reduction in Leaf-to-Fruit Ratio can delay veraison(van Leeuwen and Destrac-Irvine, 2017). In fact, as mentioned previously, according to Santos et al., in an overview from the H2020 Clim4Vitis action, the time span between flowering and veraison can be extended by around five days by reducing the canopy area to less than 0.75 m^2/kg right after fruit set. However, these findings contradict the results of the experiment conducted at the Louis Latour estate, where no significant difference was observed (Lesaffre et al., 2023). Nonetheless, this result should be considered cautiously since only a single veraison count was conducted over the veraison period.

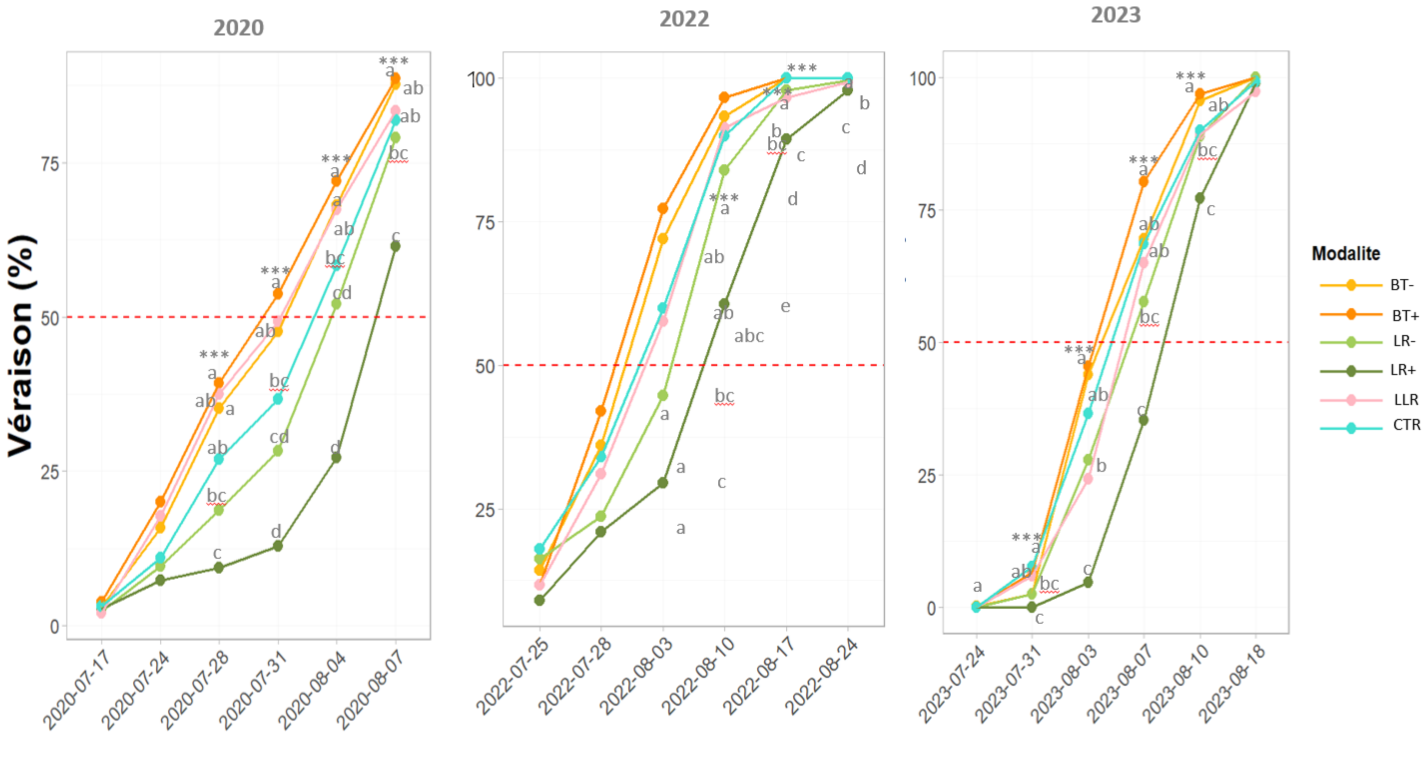


Figure 4.3 1: Veraison dynamics for the 2020, 2022 and 2023 vintages. The letters represent the results of post hoc tests Fischer-LSD after a non-parametric test Kruskal-Wallis.

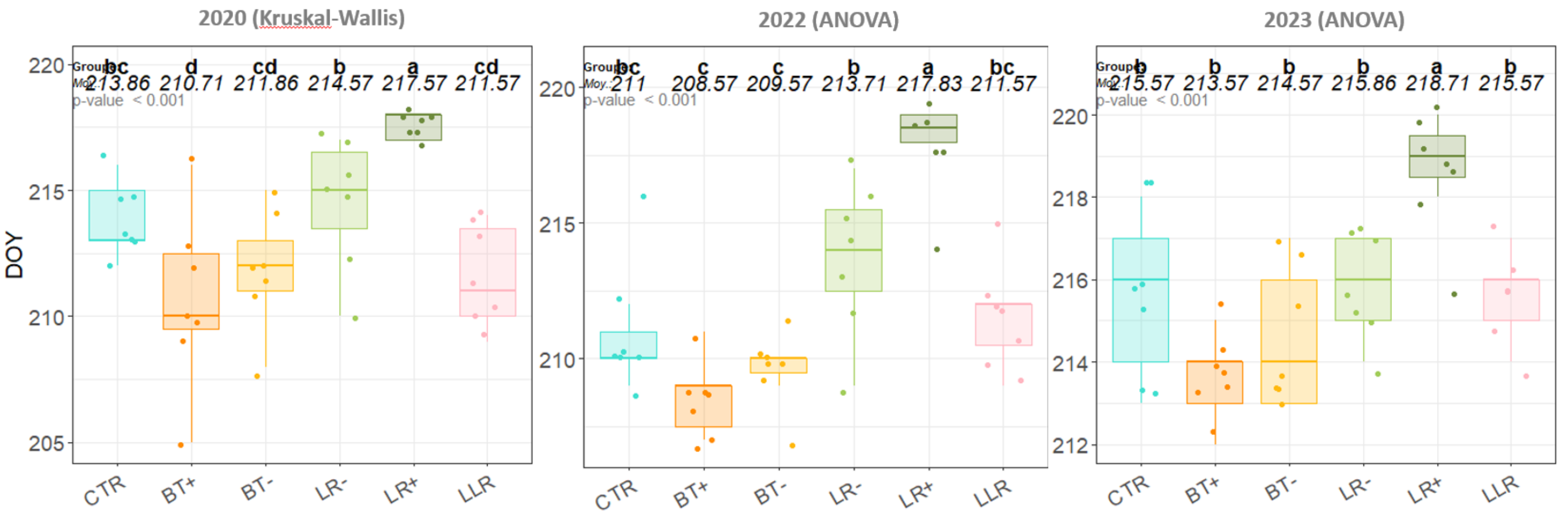


Figure 4.3 2Date of mid-veraison expressed in Day Of the Year (DOY)

4.4. Maturity dynamics

The evaluation of the ratio between sugar content and total acidity (S/AT) is an important parameter used by winemakers to assess maturity, especially for determining the harvest date. The higher this ratio, the more advanced the maturity. Therefore, part of the objective of this study is to limit this ratio by controlling sugar content in order to reduce the potential alcohol volume.

Our initial hypothesis is that the defoliated modalities would present a less advanced maturity level compared to the control, contrary to bunch thinning treatments, which would have a more advanced maturity level. Figure 4.4.1 shows the evolution kinetics of the S/AT ratio during the maturation period for the 2020, 2022, and 2023 vintages. Although there is no significant difference in the S/AT ratio among the modalities at the time of harvest during the last maturity check. A trend can be observed with a lower S/AT ratio for the defoliated modalities and a higher one for the BT+ and BT- modalities, and this is the case each year. Therefore, these outcomes coincide with the findings of Van Leeuwen et al. in 2017, that by reducing the LFR, a decrease in grape sugar without affecting grape acidity; as well as slowing the maturity of the berries. (Alberto Palliotti et al., 2013)

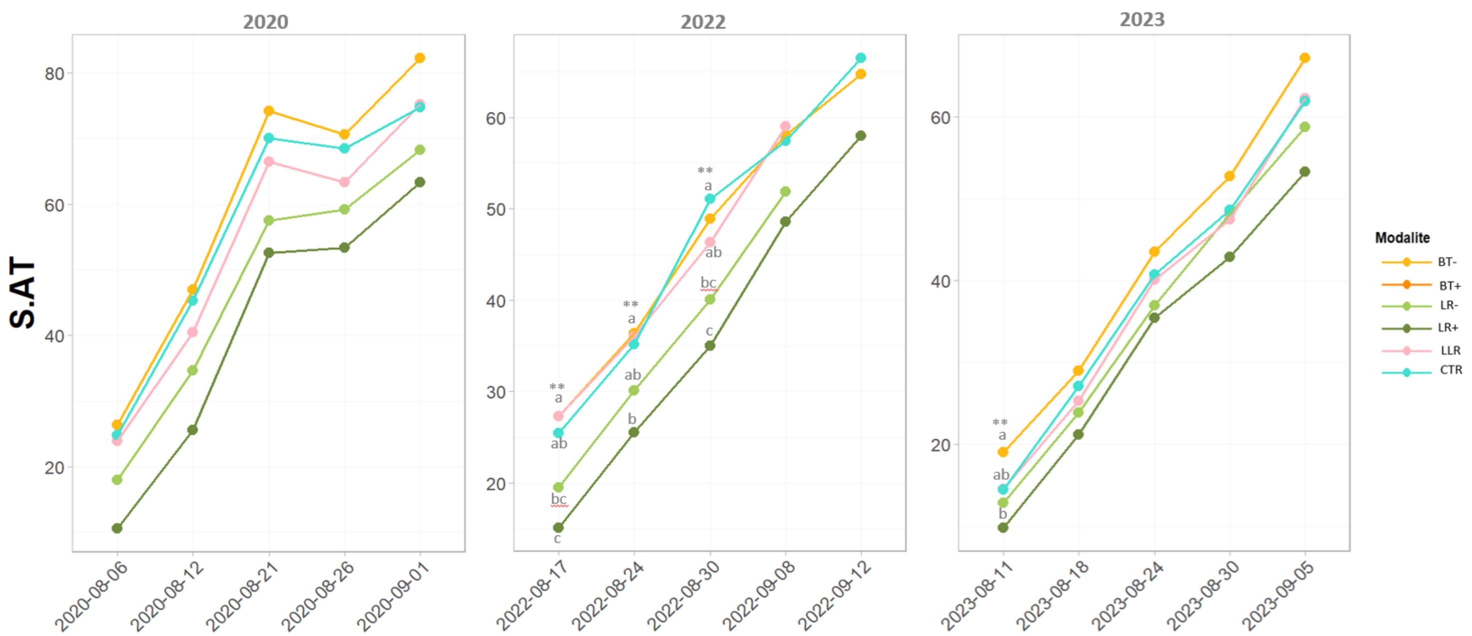


Figure 4.4 1: S/AT dynamics from mid-veraison until harvest.

4.5. Nitrogen status

We observe in Figure 4.5.1 that the chlorophyll index does not highlight any significant differences between the different modalities. However, it demonstrates a low to medium nitrogen status of the plot according to the scale presented in section 3.2.3., for the years 2020, 2022 and 2023. In 2021, the nitrogen status has a high nitrogen status. Hence, the method leaf removal apical then does not seem to affect the nitrogen status of the vine, which is positive.

The graphs in Figure 4.5.2 show the content of assimilable nitrogen at harvest of all the seasons. It is observed that this content is significantly different according to the treatment modalities each year. The bunch thinning modalities are not different from the control (CTR), while the defoliated modalities (LR-, LR+, and LLR) seem to have a lower content of assimilable nitrogen. Indeed, LR- and LR+ are significantly different from CTR in 2020 and 2022. The first three years show a difference between LLR and the CTR except in 2023. Therefore, the application of different severity of LFR seems to have an impact on the content of assimilable nitrogen, and the defoliated modalities lead to a reduction of it. This outcome is actually expected since it has been shown that a decrease in the LFR implies a reduction in the content of soluble solids in the berries (Zufferey et al., 2012). Moreover, the average level of assimilable nitrogen seems to have decreased between 2020 and 2023. In 2023, only the LLR and LR+ modalities present a content of assimilable nitrogen above the deficiency threshold of 140 mg/L. An assimilable nitrogen content below the threshold can lead to difficult alcoholic fermentation. (Ortiz-Julien et al., n.d.)

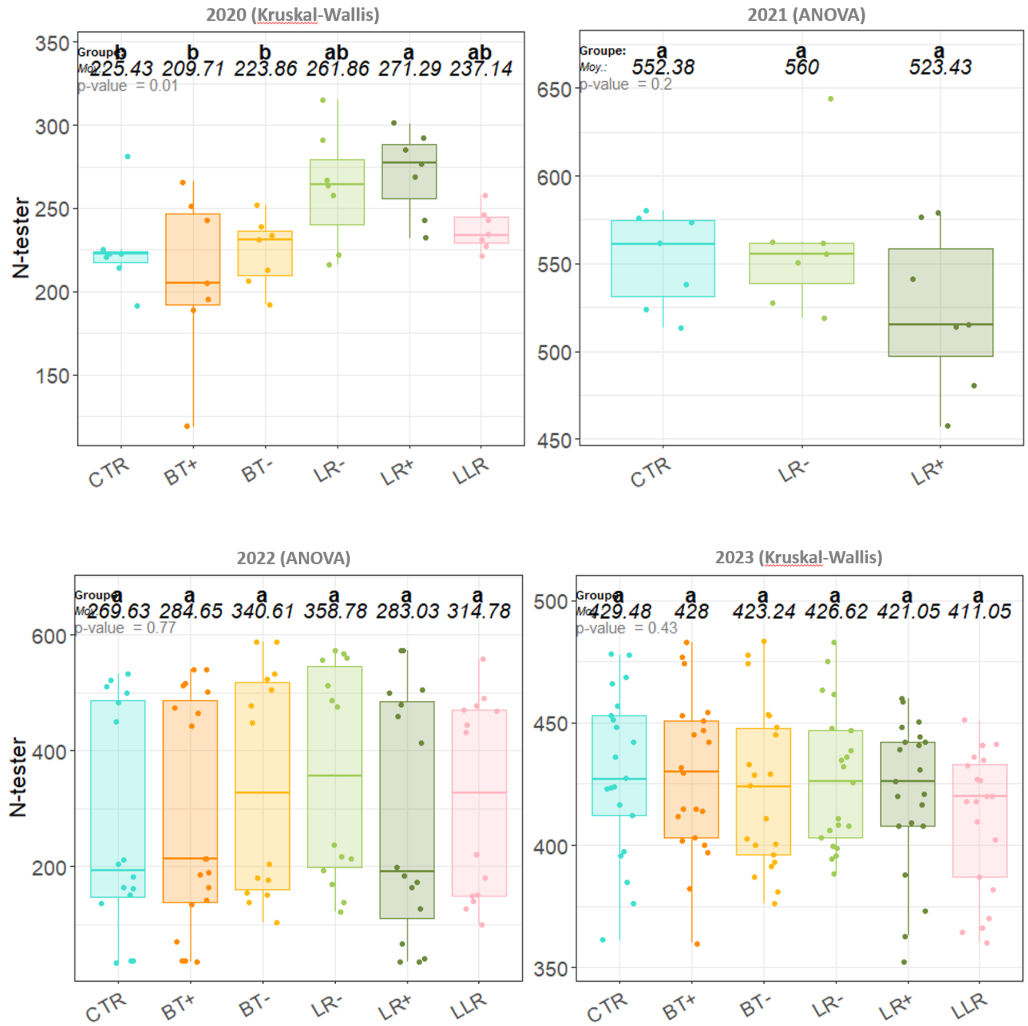


Figure 4.5 1: Chlorophyll index measured at mid-veraison for all four vintages

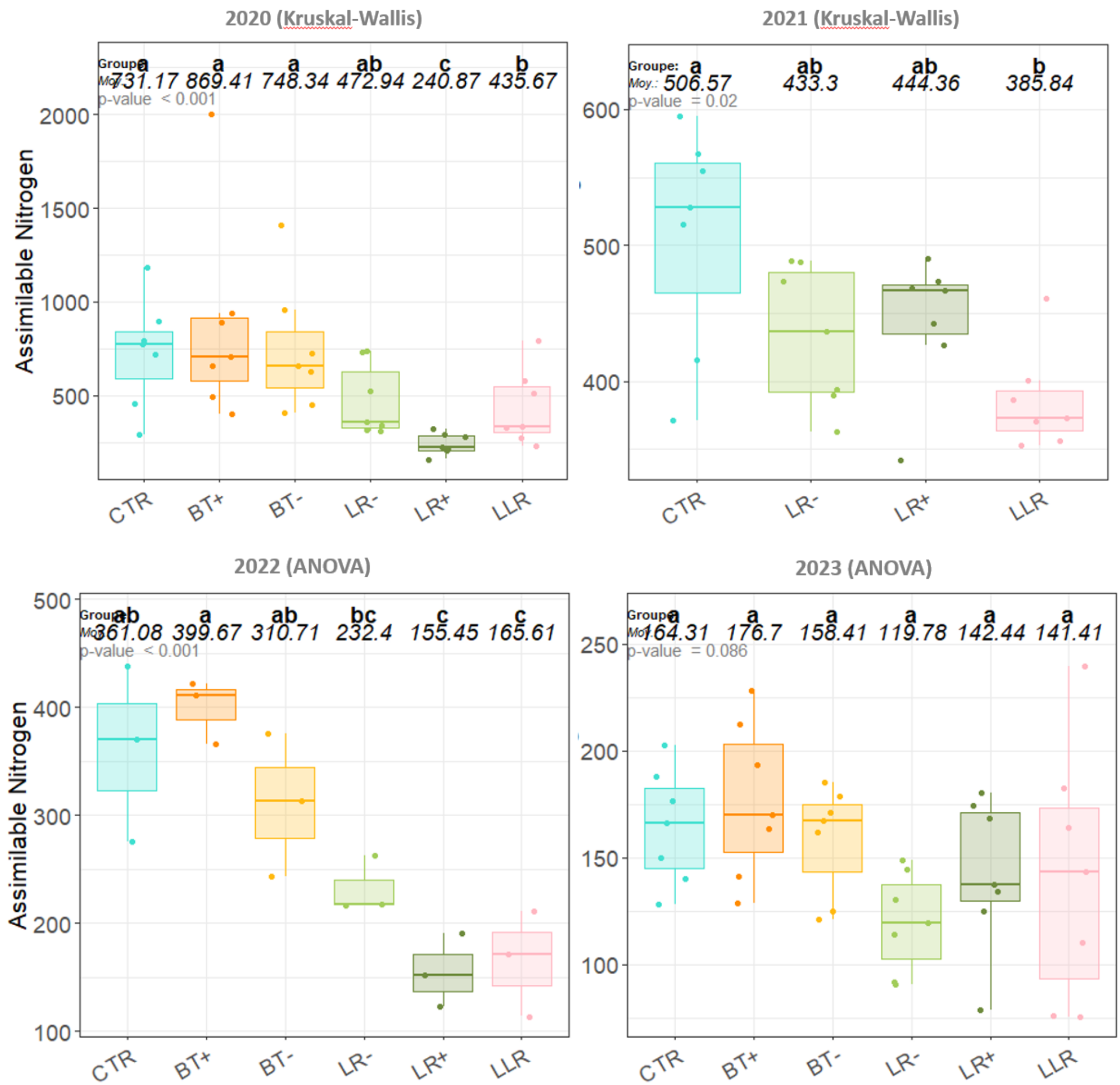


Figure 4.5 2: Yeast Assimilable Nitrogen at harvest for the years 2020, 2021, 2022 and 2023

4.6. Hydric state

Through this study, one of the aims is to reduce water stress by canopy management. It is assumed that by reducing the transpiring leaf area, the plant will save water and become more resistant to drought. It is observed that the BT+, BT- and TEM modalities are not significantly different, but they are significantly distinct from the vines where defoliation method was applied. Moreover, the δ13C is lower for LR+ and LR-, indicating less water stress. The LLR treatment also shows promising results in reducing hydric stress of the plant. (Bois et al., 2023)

This reduction in water stress might be due to a decrease in photosynthesis, the vine uses water more effectively when it has fewer leaves to lose water from.

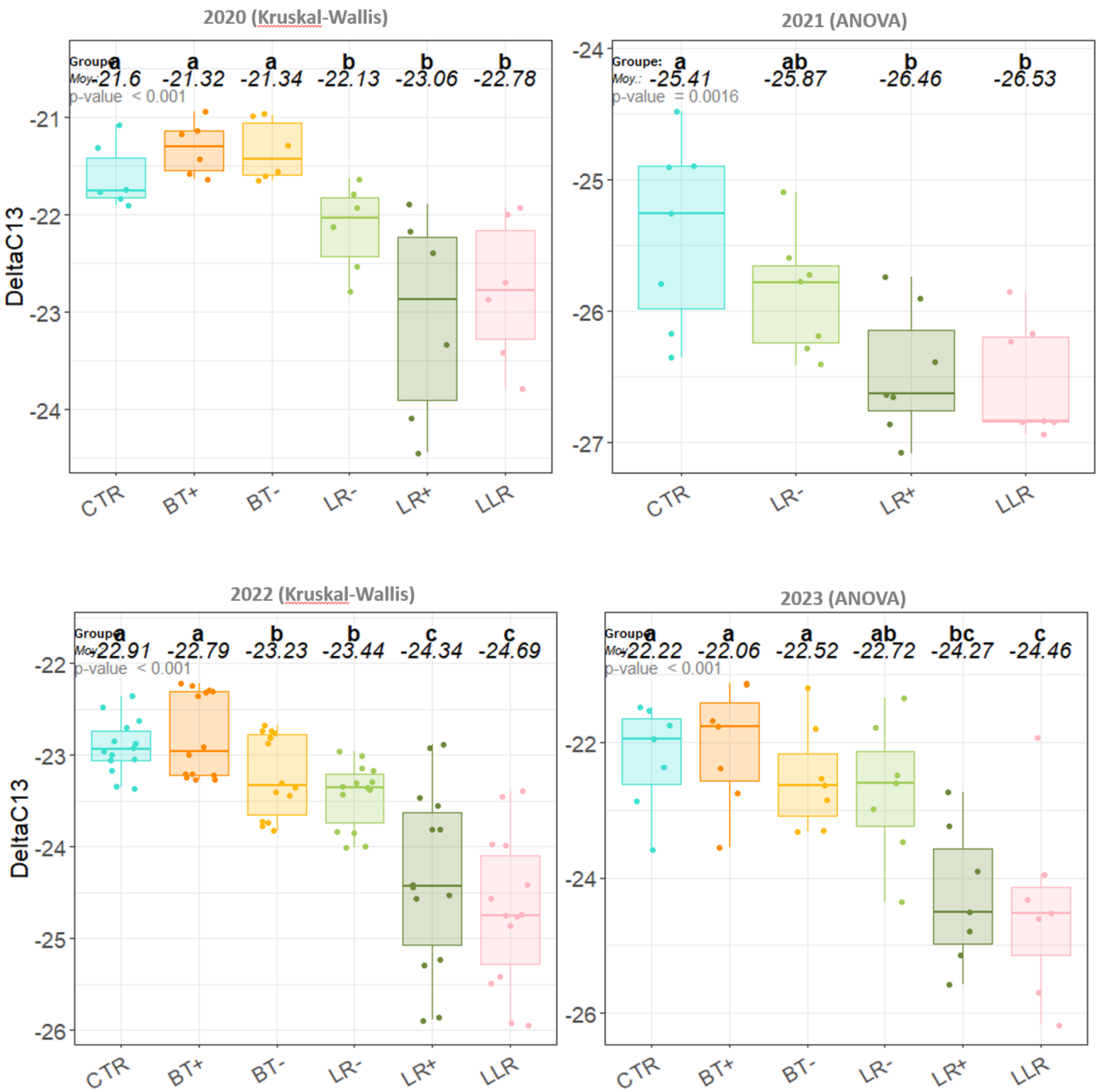


Figure 4.6: δ13C from 2020 to 2023

4.7. Sunburn Damage

As of the sunburn damage, Figure 4.7 shows that in 2020, the LR+ and LR- treatments significantly differ from the CTR and lowers the damages compared to the other modalities. This can positively affect the yield and quality of the harvest, and is consistent with the Δ13C results. In 2022, there is no difference among the treatments. However, in 2023, the LLR treatment shows a significant difference from the CTR, LR+ and LR- treatments, it resulted in higher scalding damages. This outcome coincide with the literature, as sunburn damage is higher when applied later in the season and lower when applied at an earlier stage (Gambetta, 2019). Indeed, this is the case of our treatments; LLR was applied 10 days after mid-veraison and the other modalities at bunch closure. Actually, berries become more vulnerable to sunburn as they soften, their reaction to high temperatures vary depending on their stage of maturity. (Hulands et al., 2014) Ultimately, by decreasing the leaf surface area, the vine experiences less damage and is better able to withstand heat waves, as this practice reduces the vine's hydric stress through water savings. This result is therefore promising for future prospects. Moreover, since the same person did the observation and counting, any potential "observer" bias is therefore limited.

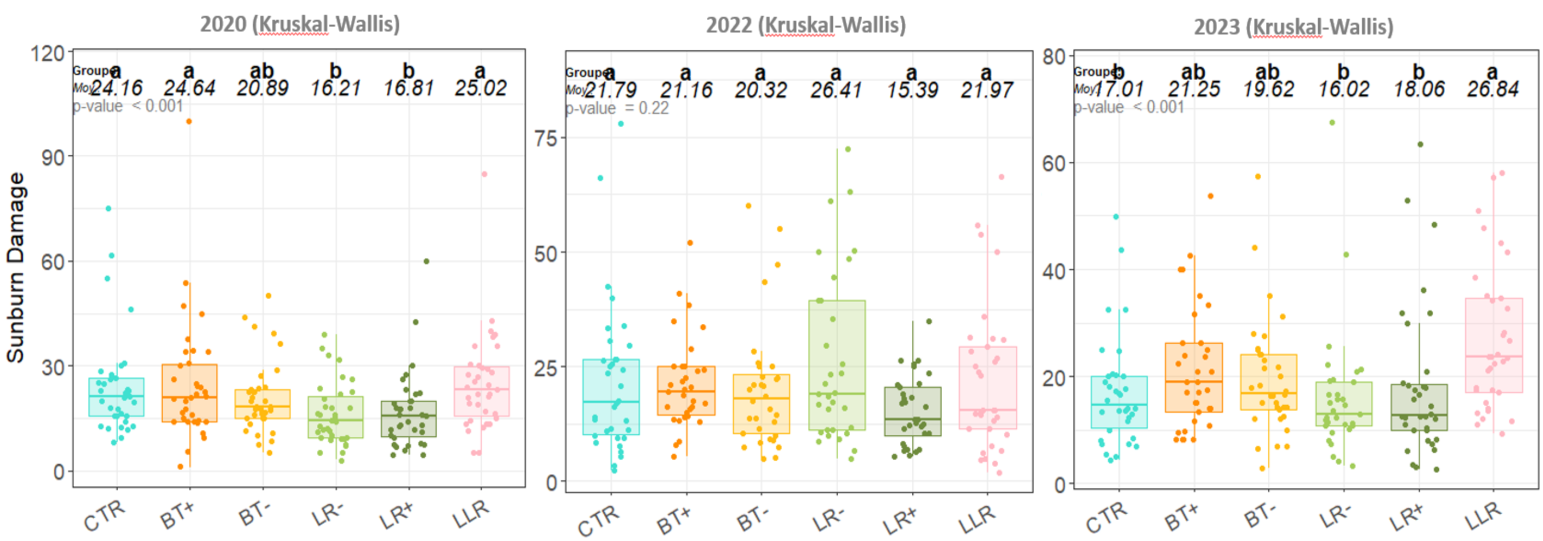


Figure 4.7: Scalding damage observed a day before harvest

4.8. Yield

Figure 4.8 shows the average harvest weight per vine (g) for the four vintages studied. The BT- and BT+ modalities show lower values since the vines were thinned: 6 and 4 clusters were left for each treatment, respectively. It is observed that there is no statistical difference between the defoliated modalities and the control in all of the four years. However, the overall yield is lower in 2020 and 2023 compared to the other two vintages.

Thus, applying leaf removal apical at different stages does not seem to affect the yield, but rather depends on the vintage. These results coincide with the scientific literature, as it has been shown that reducing RFF does not impact yield (Alberto Palliotti et al., 2013; Poni et al., 2018; Valentini et al., 2019).

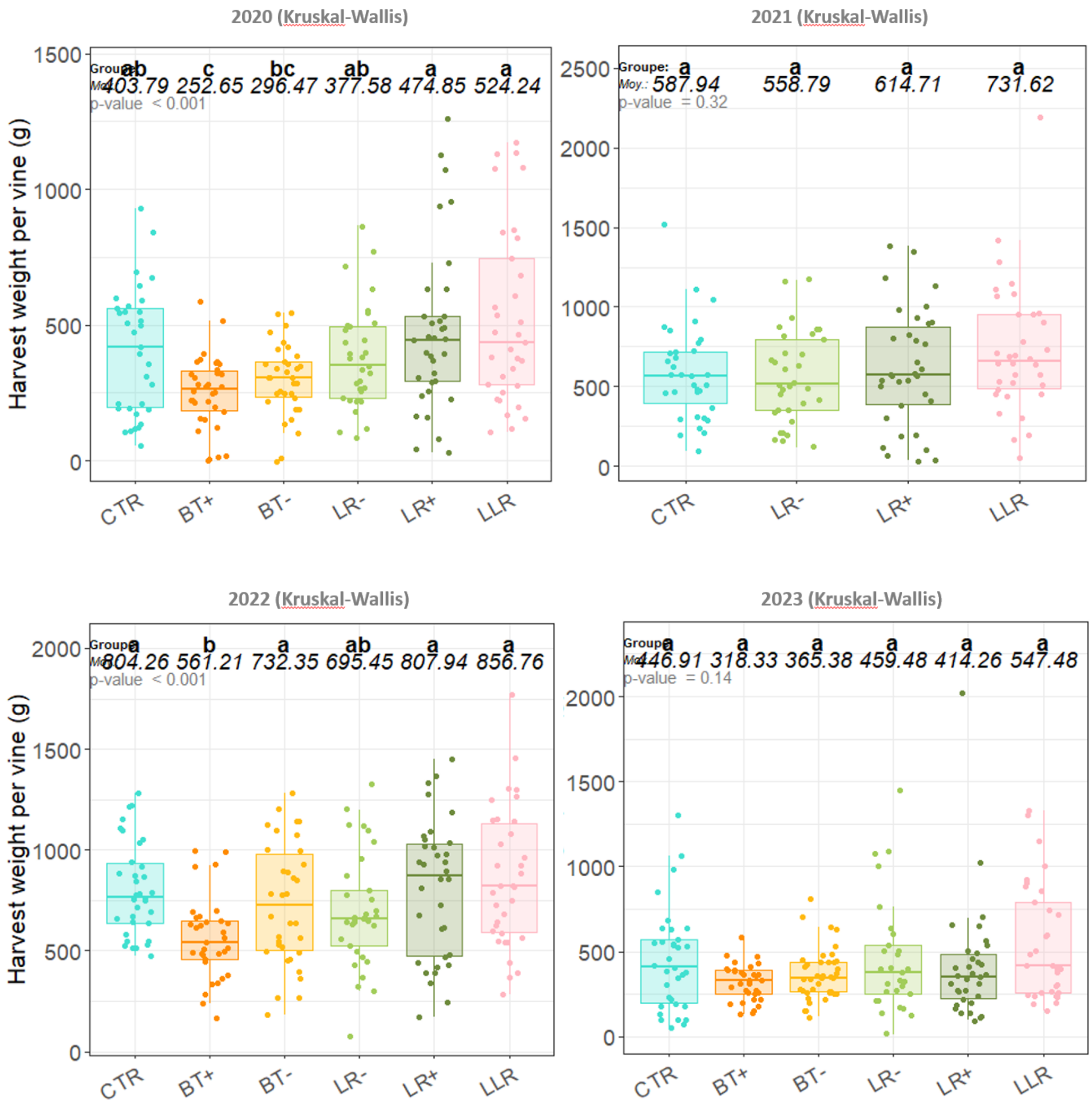
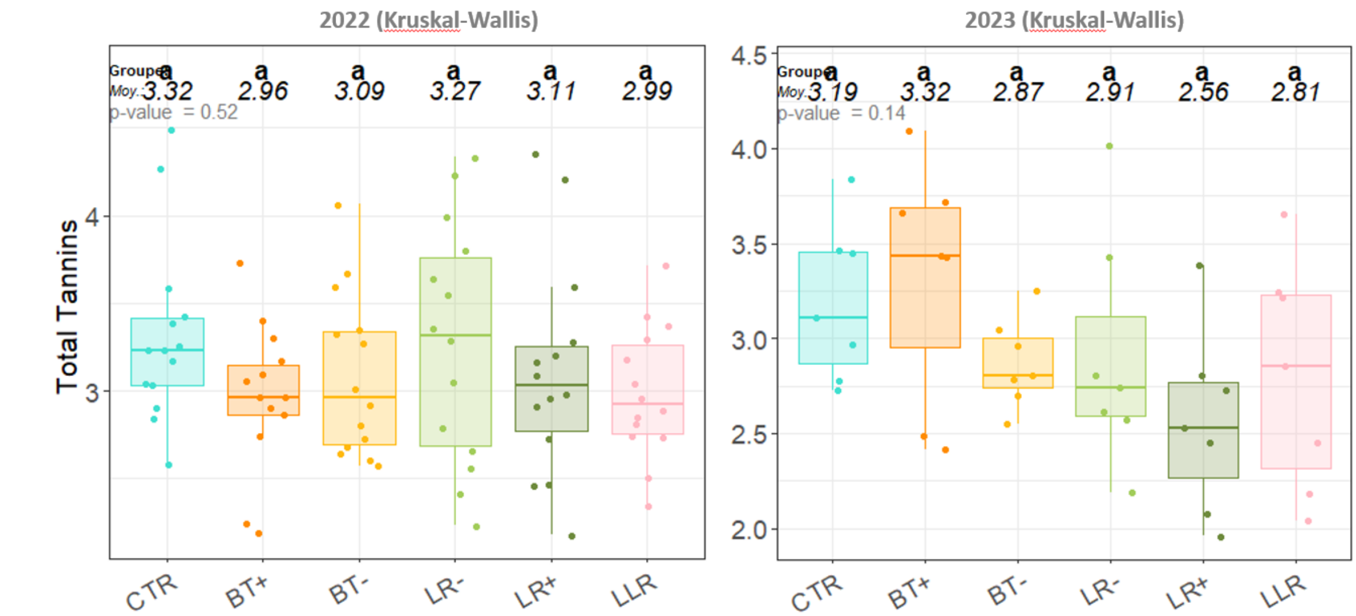


Figure 4.8: Yield per vine in (g) at harvest

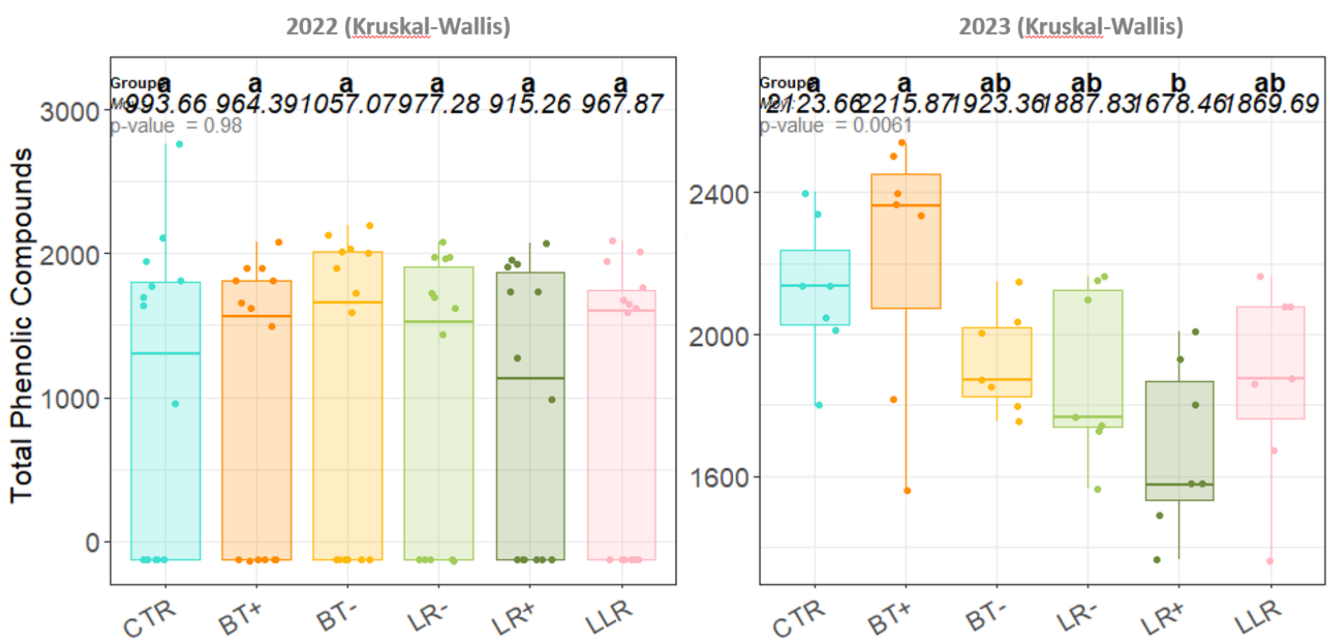
4.9. Phenolic compositions

The total phenolic compounds of 2022 and 2023 are presented in the following figure: Figure 4.9. If we look at the total tannins, the application of leaf removal method did not affect this composition of the berry skin and the seed. Nonetheless, The LR- and LR+ have lower content compared to the CTR. As of the total phenolic compounds, there is a significant difference between the CTR and the LF+ the defoliated vines have much lower concentration in 2023. However, in 2022, there is no difference, a laboratory error may have occurred, it might have been during the calibration range of the Gallic Acid Equivalent, which is the most crucial part of this experiment.

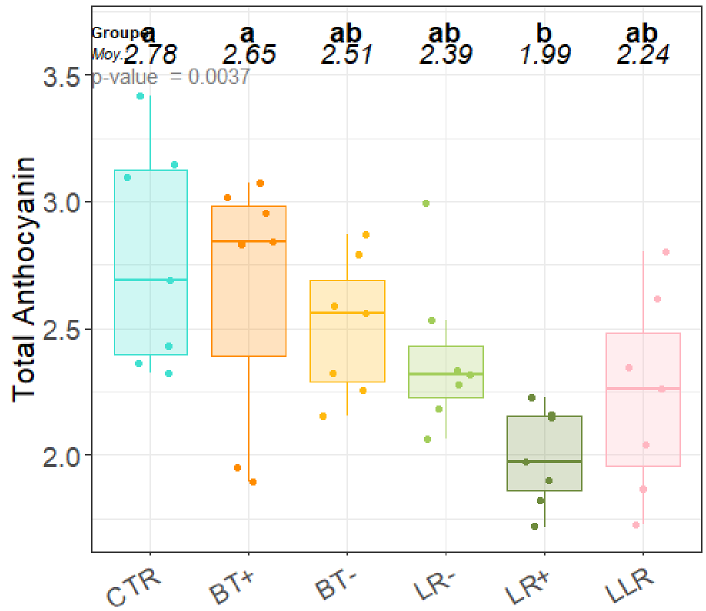
The same observation goes to the total anthocyanin in 2023 (2022 data is missing): the LR- and the LLR seem to have no significant difference with the other treatments. In fact, according to some authors, a reduction in LFR might possibly decrease grape phenolic compounds or an increase in herbaceous aromas in grapes and therefore in wines. (van Leeuwen and Destrac-Irvine, 2017) Hence, our results match with the literature.



(A)



(B)



(C)

Figure 4.9: (A) Total tannins (ANOVA), (B) Total phenolic compounds (ANOVA), (C) Total anthocyanin (Kruskal-Wallis)

However, in a study done on Trebbiano cultivar, when the treatment was applied in the post-fruit set, the phenolic composition was enhanced. Thus, according to the author, this method can be considered as an effective method in improving phenolic compounds of Trebbiano grapevines. (Quartacci et al., 2022)

CONCLUSION

The aim of this experiment was to assess the impact of managing the leaf-to-fruit ratio through apical defoliation on the agronomic behavior of the vine with the aim of reducing the sugar content and therefore alcohol content in wines. Our findings, corroborated by other studies, suggest that apical defoliation is a viable strategy for adaptating to climate change to control grape sugar content as well as grapevine water status. The results are actually promising; We observed that this practice can reduce the sugar content of the grape while not affecting the acidity and improve drought resistance. Moreover, leaf removal apical also lead to a later veraison compared to the control grapevines. However, we observed a marginal decline in yield. This strategy also resulted in a lower yeast assimilable nitrogen content probably leading to difficulties during alcoholic fermentation.

While apical removal proved efficient its manual application was time-consuming and labor-intensive, indicating a significant cost factor. Future studies should explore the mechanization of this strategy to save time and money for a profitable production, as already tested in Italy.(A. Palliotti et al., 2013) Furthermore, given that apical defoliation is still little known and not much studied in France, if not understudied, implementing this method in other regions will be beneficial to establish a solid database and refine this viticulture technique.

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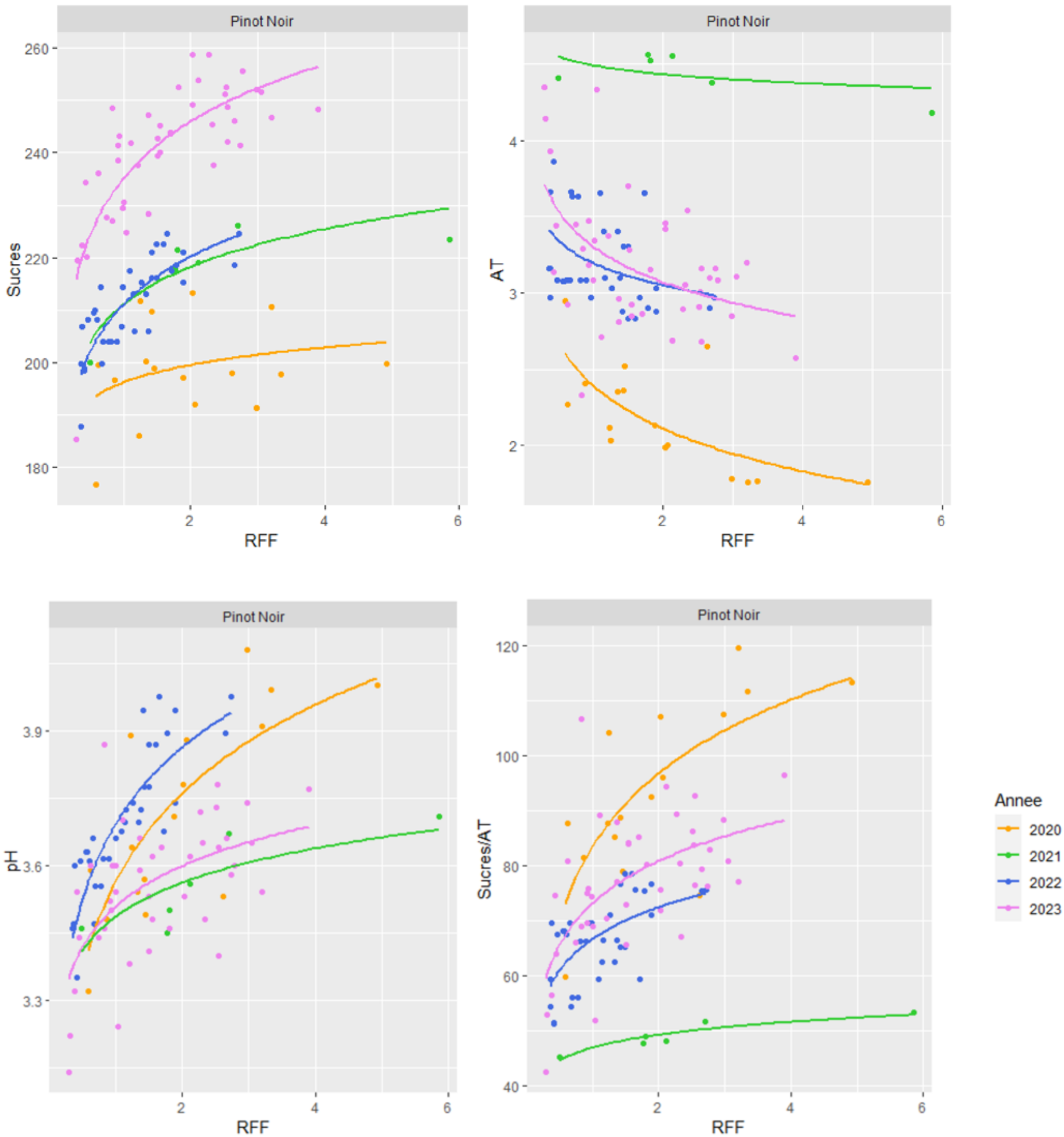
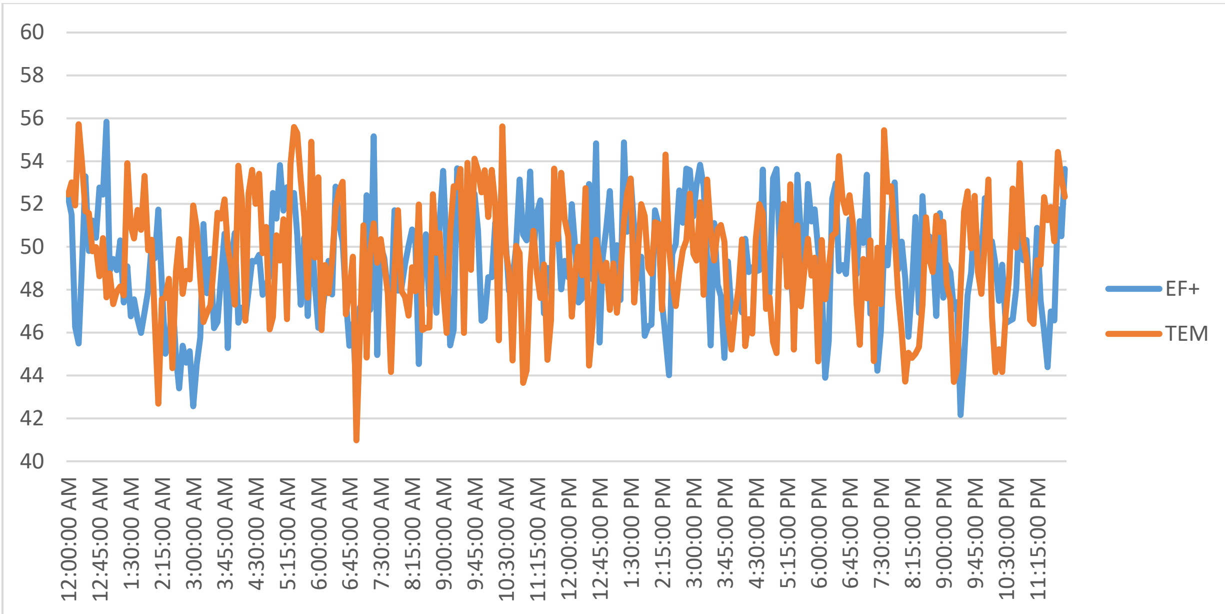
APPENDIX

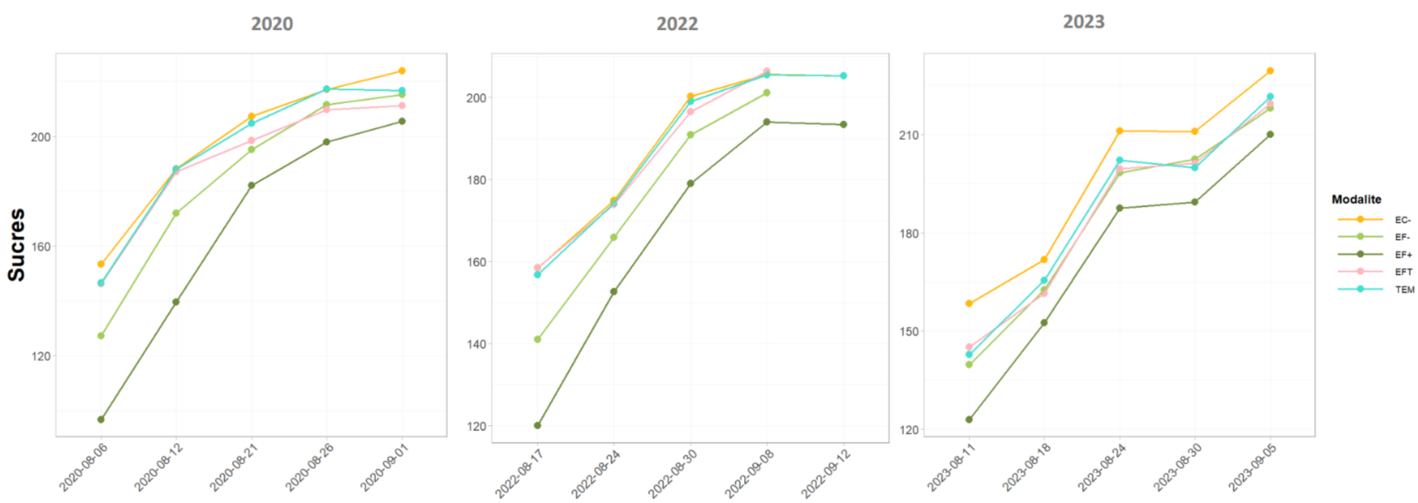
A.1 Correlation of the LFR and berry compositions: sugar, tartaric acid, pH and Sugar/AT



A.2 Humidity measurements



A.3 Sugar accumulation kinetics, defoliated modalities show a lower sugar accumulation



A.3 Comparison of hydric stress among blocs of the year 2023