

## Evaluation of site-specific management practices to reduce the heterogeneity in grapevine vigor, yield and grape composition

Cesar Gustavo Pereyra Alpuin

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## THÈSE POUR OBTENIR LE GRADE DE DOCTEUR **DE L'INSTITUT AGRO MONTPELLIER** ET DE L'UNIVERSITE DE MONTPELLIER

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**Evaluation of site-specific management practices** to reduce the heterogeneity in grapevine vigor, yield, and grape composition

## Présentée par Gustavo PEREYRA Le 5 septembre 2023

## Sous la direction de Milka FERRER et Anne PELLEGRINO

Devant le jury composé de

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UNIVERSIT

**DE MONTPELLIER** 









## Evaluation of site-specific management practices to reduce the heterogeneity in grapevine vigor, yield, and grape composition

Cesar Gustavo Pereyra Alpuin

Doctorado en Ciencias Agrarias

Setiembre 2023

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Tesis aprobada por el tribunal integrado por el Dr. Gustavo González Neves, Dra. Nathalie GAVEAU, Dr. Cornelis van LEEUWEN, Dr. Bruno TISSEYRE, Dr. Eduardo BOIDO y Dra. Mercedes FOURMENT, el 5 de setiembre de 2023. Autor: Gustavo PEREYRA. Directora: Dra. Milka FERRER. Codirectora: Dra. Anne PELLEGRINO

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"Prefiero equivocarme andando que detenerme en la marcha" Luis Batlle

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**Pereyra, G.,** Ferrer, M., Pellegrino, A., & Gaudin, R. (2022). *Montmorillonite content is an influential soil parameter of grapevine development and yield in South Uruguay*. *Agrociencia Uruguay*, *26*(2), 1124.

**Pereyra, G.,** Pellegrino, A., Ferrer, M., & Gaudin, R. (2023). **How soil and climate** variability within a vineyard can affect the heterogeneity of grapevine vigour and production . *OENO One*, 57(3), 297–313.

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2022. *"Soil management in vineyards".* Participation as speaker in a technical dissemination day INTA Concordia - Argentina. Virtual. 30/3/2022

2019. "*Traditional and innovative management techniques applied to the vineyard*". Brief description: it was an activity where the results of undergraduate and graduate theses of the Viticulture group were disseminated. Udelar, Montevideo, Uruguay.

2019. *Technical Conference day.* SOVICAR. Brief Description: Participation as speaker in a technical dissemination day in **Carmelo, Colonia, Uruguay**.

2019. *Vine Irrigation Conference*. Brief Description: An extension day was organized for winegrowers. **Udelar, Canelones, Uruguay** 

2019. *"Vineyard in trouble: difficulties when irrigating"* Brief description: Technical workshop for growers, technicians and students. **Udelar, Canelones, Uruguay**.

## LIST OF ABBREVIATIONS

A: Total anthocyanins ApH1: Total potential in anthocyanins **B/v:** Bunches per vine **BB** BL: budbreak to bloom (September to November) BL V: bloom to veraison (November to January) **BR:** Bunch rot Bw: Individual berry weight **Bz:** Bunch size **CC:** Volumetric moisture at field capacity **CEC:** Cation exchange capacity CI: % of clay **CP:** Cane Production **CT:** clav fractions **D:** Berry density **Db:** Bulk density Dw: Volumetric mass of water ELA: Exposed leaf area Reference Penman–Monteith ETo: evapotranspiration GD<sub>10</sub>: Cumulated thermal time **GIS:** Geographic information systems **GPS:** Global positioning system H-L: Leaf removal treatment in high vigour H-N: Nitrogen restriction treatment in high vigour **HV:** High Vigour treatment H-W: Water restriction treatment in high vigour Kc: Crop coefficient Kleaf: Leaf potassium L+N: Nitrogen supply treatment in low vigour L+W: Irrigation treatment in low vigour LA: Leaf area LAI: Leaf area index LV: Low Vigour treatment N stock: Nitrogen soil stock NBvn: Normalization of berry volume ND30: Temperatures above 30 °C from flowering to harvest (November to March) NDVI: Normalized difference vegetation index

Nleaf: Leaf nitrogen OIV: International Organisation of Vine and Wine **OM:** Organic matter **PA:** Precision agriculture PAR: Photosynthetically active radiation PARc: Photosynthetically active radiation in the canopy **PV:** Precision viticulture **PW:** Pruning weight **RH:** Relative air humidity **RHc:** Relative humidity in the canopy **RI:** Rooting index **RRff:** Cumulated rainfall from flowering to fruit set (November to December) **RRrip:** Cumulated rainfall during ripening (January to March). Sa: % of sand **SD:** soil depth Si: % of silt SI: Water stress integral **TAW:** Total Available Water (mm) TC: Total cations TD<sub>10</sub>: Trunk diameter TLA: Total Leaf Area TM: Maximum temperature at harvest **Tmax:** Maximum temperature **Tmc:** Mean temperature in the canopy **Tmin:** Minimum temperature TMv: Maximum temperature at Veraison (January) Tp: Total phenols **TPI:** Total phenol index Ts: Soil temperature **TSS:** Total sugar content Tx: Average temperature **V** H: Veraison to harvest (January-March). **VPD:** Vapor pressure deficit WA: Water availability WMO: World Meteorological Organization **XRD:** X-ray diffractometry Y: Yield **YAN:** Yeast available nitrogen **Ψp:** Water status at pre-dawn

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## SUMMARY

During eight consecutive seasons (2014-2021) the intra-plot heterogeneity of vigour in a cv. Tannat vineyard in Uruguay was studied. Such variability was assessed during three years (2015, 2016 and 2017) using the Normalized Difference Vegetation Index (NDVI). High-resolution (0.2 m) multispectral images were obtained over the ground to define contrasting vigour zones: high (HV) and low (LV). In winter 2020, trunk diameter was assessed to corroborate the interannual stability of vigour, and positive correlations were established between NDVI and vegetative growth parameters. A complete description of soil physical and chemical parameters was carried out. Climate data, vegetative growth, yield and grape composition were used. In addition, site-specific management techniques were employed according to vigour zone to influence yield and grape quality and reduce heterogeneity. For HV, treatments were aimed at reducing water and nitrogen inputs and improving microclimatic conditions in the cluster zone. In LV, on the other hand, treatments were aimed at increasing vegetative growth and yield with water and nitrogen supplementation. Although a 1 ha plot can be considered homogeneous from a topographic, edaphological and climatic point of view, this study demonstrated the existence of great variability in soil variables, production parameters and grape composition. The HV zone was associated with higher levels of leaf area, pruning weight, yield (higher berry and bunch weight) and bunch disease incidence than the LV zone. These HV zone characteristics were the result of a deeper and more structured soil, with higher organic matter content, nitrogen reserves and clay content, and abundance of montmorillonite-type clay. The LV zone, was distinguished by a shallower and more compact soil which negatively conditioned root growth. New information was provided on the interaction of the soil-plant-atmosphere system. In particular, the dominant role of water availability in the first place and soil nitrogen availability in the second place in establishing plant vigour. The gradient of vine vigour and yield between the two zones remained stable over the years, regardless of climatic conditions. This indicates that soil characteristics can mitigate or enhance the effects caused by climatic conditions. The determination (possible by remote sensing) of the vigour zones of a plot is a prerequisite for proposing soil and crop management practices that optimize the use of resources and ensure the economic and environmental sustainability of wine production.

Key words: heterogeneity, vigour, site-specific management, composition, Tannat

## RÉSUMÉ

Pendant huit saisons consécutives (2014-2021), l'hétérogénéité intra-parcellaire de la vigueur dans un vignoble cv. Tannat en Uruguay a été étudiée. Cette variabilité a été évaluée pendant trois années (2015, 2016 et 2017) à l'aide de l'indice de végétation par différence normalisée (NDVI). Des images multispectrales à haute résolution (0,2 m) ont été acquises au sol pour définir des zones de vigueur contrastée : haute (HV) et basse (LV). Durant l'hiver 2020, le diamètre des tiges a été évalué pour corroborer la stabilité interannuelle de la vigueur, et des corrélations positives ont été établies entre le NDVI et les paramètres de croissance végétative. Une description complète des paramètres physiques et chimiques du sol a été réalisée. Les données climatiques, la croissance végétative, le rendement et la composition du raisin ont été utilisés. En outre, des techniques de gestion spécifiques au site ont été utilisées en fonction de la zone de vigueur pour influencer le rendement, la qualité du raisin et réduire l'hétérogénéité. Pour la HV, les traitements visaient à réduire les apports d'eau et d'azote et à améliorer les conditions microclimatiques dans la zone des grappes. Dans le cas de LV, en revanche, les traitements visaient à augmenter la croissance végétative et le rendement avec une supplémentation en eau et en azote. Bien qu'une parcelle de 1 ha puisse être considérée comme homogène d'un point de vue topographique, édaphologique et climatique, cette étude a démontré l'existence d'une grande variabilité dans les variables du sol, les paramètres de production et la composition des raisins. La zone HV a été associée à des niveaux plus élevés de surface foliaire, de poids de taille, de rendement (poids plus élevé des baies et des grappes) et d'incidence des maladies des grappes que la zone LV. Ces caractéristiques de la zone HV étaient le résultat d'un sol plus profond et plus structuré, avec teneur en matière organique, des réserves d'azote et une teneur en argile plus élevées, et une abondance d'argile de type montmorillonite. La zone LV, s'est distinguée par un sol moins profond et plus compact qui a conditionné négativement la croissance des racines. De nouvelles informations ont été fournies sur le rôle dominant de la disponibilité de l'eau en premier lieu et de la disponibilité de l'azote du sol en second lieu dans l'établissement de la vigueur des plantes. Le gradient de vigueur et de rendement de la vigne entre les deux zones est resté stable au fil des ans, indépendamment des conditions climatiques. Cela indique que les caractéristiques du sol peuvent atténuer ou renforcer les effets causés par les conditions climatiques. La détermination (possible par télédétection) des zones de vigueur d'une parcelle est une condition préalable pour proposer des pratiques de gestion du sol et des cultures qui optimisent l'utilisation des ressources et assurent la durabilité économique et environnementale de la production viticole.

Mots clés: hétérogénéité, vigueur, gestion spécifique au site, composition, Tannat

## RESUMEN

Durante ocho temporadas consecutivas (2014-2021) la heterogeneidad intraparcelaria del vigour en un viñedo de cv. Tannat en Uruguay fue objeto de estudio. Dicha variabilidad fue evaluada durante tres años (2015, 2016 y 2017) mediante el índice de vegetación de diferencia normalizada (NDVI). Se obtuvieron imágenes multiespectrales de alta resolución (0,2 m) sobre el terreno para definir zonas de vigour contrastantes: alto (HV) y bajo (LV). En invierno de 2020, se evaluó el diámetro del tronco para corroborar la estabilidad interanual del vigour, y se establecieron correlaciones positivas entre el NDVI y los parámetros de crecimiento vegetativo. Se llevó a cabo una descripción completa de los parámetros físicos y químicos del suelo. Se utilizaron datos climáticos, de crecimiento vegetativo, de rendimiento y de composición de la uva. Además, se aplicó un enfoque de manejo sitio-específico a cada sitio de vigor para incidir sobre el rendimiento, la calidad de la uva y reducir la heterogeneidad. En el HV, los tratamientos tenían el objetivo de reducir los aportes de agua y nitrógeno, mejorando las condiciones microclimáticas de la zona de racimos. En LV, en cambio, los tratamientos buscaban aumentar el crecimiento vegetativo y el rendimiento mediante la suplementación de agua y nitrógeno. Si bien una parcela de 1 ha puede considerarse homogénea desde el punto de vista topográfico, edafológico y climático, este estudio demostró la existencia una gran variabilidad en las variables del suelo, los parámetros de producción y la composición de la uva. La zona HV se asoció con niveles más altos de área foliar, peso de poda, rendimiento (mayor peso de la baya y del racimo) e incidencia de enfermedades que la zona LV. Estas características de la zona HV eran el resultado de un suelo más profundo y estructurado, con mayor contenido de materia orgánica, reservas de nitrógeno y contenido de arcilla (tipo montmorillonita). La zona LV, presentó un suelo menos profundo y más compacto lo que condicionaba negativamente el crecimiento de las raíces. Se aportó nueva información sobre el rol dominante del agua en primer lugar y de la disponibilidad de nitrógeno del suelo en segundo lugar a la hora de establecer el vigor de las plantas. El gradiente de vigor de la vid y de rendimiento entre las dos zonas se mantuvo estable a lo largo de los años, independientemente de las condiciones climáticas. Esto indica que las características del suelo pueden mitigar o potenciar los efectos causados por las condiciones climáticas. La determinación (posible mediante teledetección) de las zonas de vigor de una parcela es un requisito previo para proponer prácticas de gestión del suelo y del cultivo que optimicen el uso de los recursos y garanticen la sostenibilidad económica y medioambiental de la producción vitivinícola.

Palabras clave: heterogeneidad, vigor, manejo sitio específico, composición, Tannat

# CHAPTER I

# DETERMINATION OF VINE VIGOUR AND INFLUENCE ON GRAPE PRODUCTION



### 1. GENERAL INTRODUCTION

The grapevine (*Vitis vinifera* L.) is cultivated in six continents, covering an estimated 7.3 million hectares<sup>1</sup>. In this area, there is a diversity of climatic environments (oceanic, temperate, Mediterranean, subtropical, continental, arid) and edaphic variability (depth, fertility, water reserve capacity, color, texture, structure, topography), which interact with cultural practices to determine the expression of vine vigour.

Vine vigour is a term that refers to vegetative growth and productivity (Smart & Robinson, 1991). This growth is not always spatially homogeneous, and variability analysis can be approached at three scales: macro, meso and micro. Macro-level variability refers to the variation found in a given region or geographical area. The meso level refers to the differences within each vineyard and can be assessed through vigour indicators. Finally, a micro approach focuses on the variability in shoot and bunch sizes within a given plant. This variability of growth at those three levels generates differences in production, grape maturity, sanitary status and wine attributes. Therefore, achieving balanced plants (in terms of leaf:fruit ratio) according to the specific climate and soil characteristics of the productive area is one of the most critical challenges faced by the winegrower in order to achieve stable yields and berry composition.

Several studies detail the relationship between variations in soil properties and plant development in perennial and annual crops. At the vineyard or plot level, variations in vegetative growth, root system, yield, grape ripening and berry health, among others, were shown to rely on variations in the soil characteristics. Indeed, the soil's physical, chemical and biological properties, such as a temperature, depth, texture, structure and microorganism activity, determine the volume of soil explored by the roots, the level of available nutrients and the soil's capacity to accumulate water. Thus, Bramley (2011) showed that Sauvignon blanc yields were higher in areas with deeper soils and greater water availability. Moreover, the climate in interaction with the soil is decisive for the development of the vineyard, limiting or modifying biological processes. Soil-climate parameters influence vine growth, vigour, physiology and grape ripening. Climatic variability of critical parameters such as temperature and water availability determine differences in production and grape quality from year to year.

In addition to soil and climate, vineyard management practices impact vine vigour. Several cultivation techniques can modify soil properties, water and nutrient availability and plant microclimate, ultimately impacting leaf area, yield and grape composition. It is well known that vine vegetative growth is dependent on water availability. Increased water and nitrogen availability may result in excessive vegetative growth (increased vigour) with negative implications for plant microclimate (lower temperature and light exposure, higher relative humidity) and physiological parameters such as photosynthetic activity, stomatal conductance and transpiration. On the other side, in situations of water deficit, lower photosynthetic activity and leaf fall can dramatically impair clusters development due to insufficient availability of assimilate and excessive exposure. In addition to water, nutrients, especially nitrogen, should be carefully controlled. Indeed, while an excess of nitrogen leads to increased vigour with reduced fruit set, insufficient nitrogen availability may result in poor vegetative growth and alterations in the secondary metabolism of the berry (anthocyanins and aromatic compounds). In situation of excessive vine vigour due to high water and/or nitrogen availabilities, leaf removal can be implemented in the short term as a corrective management practice. When applied in the bunch zone, leaf removal permits to increase the bunch exposure and favor grape metabolism (Smart et al., 1990). In the long term, other practices, such as the training system, row orientation, planting density, variety and/or rootstock combination, can permanently affect the expression of vigour. For example, an increase in planting distance provides more space for vine growth which, if not corrected by an increased number of buds at pruning, will result in an excess of vigour (Jackson & Lombard, 1993).

Regardless of the cause of the variation of vigour, the winegrowers face the choice of enduring or exploiting the heterogeneity of its vineyard or, in contrast, seeking for uniformity. In a traditional management approach, a parcel is managed uniformly without considering the natural within-plot variability in terms of resource availability. Thus, the same amount of resources (water and nitrogen) is applied at the plot level regardless of the likely unequal access to resources between the plants across the plot. A uniform irrigation applied in a heterogeneous plot (block of variable vigour), in addition to generating an inefficient use of water, enhances the heterogeneity of the plot. The same conclusion could be drawn with a uniform management of canopy or fruit load

throughout a plot displaying heterogeneity of leaf/fruit ratio. The characterization of the within-plot variability may be useful to identify areas with different ripening dates, sugar/acidity ratios and/or secondary metabolites profiles and generate different wine products. Another approach is to try reducing the within plot heterogeneity by adapting locally the inputs and soil/plant management practices to the variability observed in the vineyard. This zonal management approach or site-specific management maximizes the use of resources by applying what is needed in each zone. It seeks to reduce the existing variability in the vineyard, allowing more homogeneous products to be obtained.

The concept of variability is not new, and winegrowers are aware of the existence of variability at the plot level, but the difficulty to characterize this variability leads most of the time to the choice to manage it uniformly. With the development of new technologies, vine variability can be easily accessed through precision agriculture tools applied to viticulture. Remote sensors (thermal cameras, multispectral images), GPS (global positioning system), data analysis and information interpretation systems, Variable Rate Application (VRA) equipment, yield monitoring systems, real time data, among others, allow a rapid monitoring of variability and adjustment of cultural practices. Among the remote sensors, aerial or satellite images processed from red and infrared reflectance to generate vegetative indices such as NDVI (Normalized Difference Vegetative Index) have been widely used to assess regional and field vigour heterogeneity both in annual and perennial crops.

Also, sensors based on infrared thermography detect differences in water status that condition vine vigour (Stoll & Jones, 2007). Relationships between these remote sensor-based index and parameters of vine growth, vigour and production (yield and berry composition) have been parameterized (Tisseyre et al., 2008). In addition, it has been established that the variability observed is usually stable over time as it is associated with the physical and chemical characteristics of the soil. However, the magnitude of this heterogeneity can vary depending on the climatic conditions of the year and management practices. In any case, this stability in variability makes it possible to generate management strategies within delimited zones in order to buffer the heterogeneity of production from year to year.

In Uruguay, the vine growing activity began with the arrival of the first Spanish and Portuguese immigrants during the colonial era. Currently, the production area extends over approximately 7,000 ha and generates about 30,000 direct and indirect jobs<sup>2</sup>. Tannat is the emblematic Uruguayan variety due to its good performance in the soil and climatic conditions of the country. It represents 23% of the total vineyard area and 46% of the red varieties. Tannat wines are characterized by a high enological potential, with a high concentration of tannins, anthocyanins and acidity, which generates their particularities.

Six Uruguayan wine climate regions have been determined by Ferrer et al. (2007) using the Multicriterial Classification System of Tonietto & Carbonneau (2004). The most productive wine-growing region corresponds to the south (80 % of wine production). The climate in this region is temperate (Heliothermic index 1800-2100), with cool nights (12-4 °C), moderate drought (Ferrer et al., 2007). This region has an average annual rainfall of 1100 mm, but with high inter-annual variability. In addition, the distribution of monthly rainfall is not homogenous over the years (0 mm to 300 mm per month) thus generating periods of water deficit or excess during the grape-ripening period (from September to March in the southern hemisphere). Also, the soil types in Uruguay are very heterogeneous in the different regions, due to great diversity of the geological materials that have generated the soils. In general, they are moderately deep (50 to 100 cm), clayey and with B horizon of illuvial character. The predominant soils are classified as fine, smectitic, thermic Vertic argiudoll. In addition, they are characterized by a high content of organic matter over all horizons (Duran et al., 2005) and dominance of 2:1 clays (Silva et al., 2018). This heterogeneity of weather, together with the non-uniform soil characteristics, generates high spatial and temporal heterogeneity at the region and even at the vineyard level.

This work was entitled: *Evaluation of site-specific management practices to reduce the heterogeneity in grapevine vigour, yield, and grape composition*. The general objective was to evaluate in a Tannat vineyard in Uruguay the effects of site-specific

<sup>&</sup>lt;sup>2</sup> INAVI, 2020. Instituto Nacional de Vitivinicultura (<u>https://www.inavi.com.uy/</u>)

techniques aimed at controlling plant vigour (water and nitrogen supply, and leaf removal) on the overall plant physiology (growth, storage) and the heterogeneity in grape production (yield, quality) at different levels (field, plant, bunch). The selected vineyard is characterized by a high variability of vigour evaluated by NDVI during three consecutive vintages. The feasibility of site-specific management techniques applied to plant and soil to reduce the heterogeneity within-vineyard were analyzed.

The specific objectives of the project were to:

(i) Characterize the sources of heterogeneity at the plot level. Evaluate the role of climate-soil interaction in the generation of vigour heterogeneity.

(ii) Dissect the overall relationships between soil/plant water and nitrogen status, plant functioning (phenology, growth and carbohydrate/nitrogen storage) and yield and quality elaboration.

(iii) Quantify the heterogeneity in grape production (yield, quality) at different scales (field, plot, plant and bunch) and its response to management techniques. The dynamics of quality components (sugars and berry volume) will be determined for that purpose.

This document is built on the basis of six chapters. The first chapter reviews the general scientific context of this work and presents the methodology and experimental site selected to meet the objectives. The second chapter presents the methodology and the experimental device selected to meet the objectives. The third chapter identifies the soil and climatic factors that determine the expression of vigour in grapevines. The fourth chapter deals with site-specific management to modify vigour expression and plot variability. The fifth chapter focuses on berry development as a function of climate, vigour and management practices. Finally, a general discussion and perspectives of this work are addressed. Both chapters III and IV are published scientific articles.

#### 2. SCIENTIFIC CONTEXT

#### 2.1 DEFINITION OF VIGOUR AND PLANT BALANCE

Grapevine (Vitis vinifera L.) year after year productivity highly varies depending on soil, climatic conditions, the level of biotic or abiotic stress and the cultural practices used (Dobrowski et al., 2003). Maintaining a stable and balanced production is one of the challenges for the winegrower. Concerning the concept of a balanced plant, Winkler et al. (1974) defined the term "vigour" and "capacity" to refer to vine growth. These authors defined vigour as the growth rate of the different organs of the vine, while the concept of capacity refers to total production (i.e., biomass). Champagnol (1984) considered that vigour corresponds to the intensity of annual metabolic activity during which shoots, leaves and roots grow and accumulate carbohydrates. The reserves' levels thus determine the plant's vigour for the following season. Smart & Robinson (1991) group the two terms planted by Winkler et al. (1974) in the concept of "vine vigour" when referring to vegetative and productive growth. Rives (2000) also points out that vigour can be evaluated through shoot growth or shoot diameter. Carbonneau et al. (2020) defined vigour as the rate and intensity of shoot growth. Although there are nuances in the definition of vigour, it is generally accepted that vigour should be adapted to the crop load to reach a balanced vine functioning.

Winkler (1957) and Ravaz (1911) defined the concept of balanced vine as a plant with the minimum leaf area necessary to ripen (accumulate sugar) the fruits. Ravaz (1911) thus proposed an index called the Ravaz Index (RI; Y/CP), which makes it possible to determine when a plant is balanced according to the following equation (Eq. 1):

$$RI = \frac{Yield (kg)}{cane \ production (kg)}$$
 Eq. (1)

In this equation, grape production and cane production (CP) are considered in the same growing season. The optimal index value varies according to environmental conditions, variety, management techniques and production objectives (Figure 1.1). For example, according to Smart & Robinson (1991), plants are balanced when they reach a

Ravaz index between 5 and 10, while other authors indicated that balance is reached when the index is 5-7 (Vasconcelos & Castagnoli, 2000) or with values below 4 (Reynolds, 2005). For Kliewer & Dokoozlian (2005), a range of 3 to 6 may either be optimal for small cluster varieties such as Pinot Noir. Under Uruguayan pedo-climatic conditions and for the cultivar Tannat, Ferrer et al. (1997) determined that the optimum range of this index is reached with values between 6 and 8. Champagnol (1984) added in the definition of balanced vine the starch reserves accumulated during the season, which conditions the following year's production. Vine equilibrium was assumed to be reached when leaf area was sufficient to mature the grapes in other studies (Smart & Robinson 1991, Dry & Loveys 1998, Fredes et al. 2010). Ultimately, another index used is the relationship between leaf area and yield (leaf area/load; LA/Y) through the following equation (Eq. 2):

$$LA/Y = \frac{Total \ Leaf \ area \ (m^2)}{Yield \ (kg)}$$
 Eq. (2)

This index indicates the amount of leaf area needed to support a unit of fruit weight. More recent studies (Martínez-Lüscher & Kurtural, 2021) question this relationship, since this index does not consider the accumulation of reserves in the trunk and roots. In any case, the LA/Y ratio and RI vary according to variety, climatic region, management practice and production objective (Kliewer & Dokoozlian, 2005) as shown in Figure 1.1. For example, Vance (2012) pointed out that to achieve moderate vigour in a system with Guyot pruning in vertical shoot position system (VSP) it is necessary to have 0.8 to 1.2 m<sup>2</sup> kg<sup>-1</sup>, while other authors point out an area of 1-1.5 m<sup>2</sup> to complete the ripening of 1 kg of fruit (Kliewer & Dokoozlian, 2005). Howell (2001) indicated that a balanced production is achieved with a ratio of 0.7-1.4 m<sup>2</sup> kg<sup>-1</sup>. Other indicators of balance were proposed, including pruning weight per linear meter of row (Kliewer & Dokoozlian, 2005) and canopy exposed area (m<sup>2</sup>)/total leaf area (Carbonneau, 1983). However, the Ravaz Index and the leaf:fruit ratio are the most widely used indicators of equilibrium in the literature.





Regardless of the definition used, a balanced vine is assumed to permit stabilizing yields (Smart & Robinson, 1991) and achieving adequate grape ripening across years (Dry 2000, Archer & Hunter 2004, Terry & Kurtural 2011). Indeed, unbalanced growth generates variability in vegetative growth that results in heterogeneity of yield and berry composition (Bramley & Hamilton 2004, Reynolds et al. 2007, Cortell et al. 2008). It should be clarified that a situation of high or low vigour is not bad *per se*, but excess or lack of vigour can generate suboptimal plant functioning in the short term during the season or in longer terms over years (Dry & Loveys, 1998).

## 2.2 FACTORS THAT DETERMINE VIGOUR

Excessive vigour (Figure 1.2, A; Figure 1.3) is manifested by exacerbated vegetative growth, more shoots per plant, larger diameter shoots with longer internodes and increased lateral shoot growth (Vance, 2012). Associated with this excess vegetative growth, long-term productivity problems are also reported due to excessive shading that decreases floral induction the year before harvest (Buttrose, 1969).



FIGURE 1.2. Vineyard with vigour difference.

Vineyard of *Vitis vinifera* cv. Tannat, in southern Uruguay grafted on SO4 trained on trellises. A: High vigour; B: Low vigour

Increased susceptibility to fungal diseases such as bunch rot has been reported, particularly in situations of humid climates or rainfall during ripening (English et al., 1989; Ferrer et al., 2020(a)) (Figure 1.2, A). Finally, Loveys et al. (2012) reported that vigorous varieties have higher respiration:photosynthesis ratio than low vigour varieties, thus lowering the whole plant carbon balance. Berry size (Cortell et al., 2005) and yield (Bramley et al., 2011) are higher in high vigour. Inadequate planting density (Intrieri & Filipetti, 2000), excess nutrient availability (particularly N), high water availability, or low yields imposed by the grower (Edson et al., 1993) are conditions that may favor excess vigour. In contrast, plants with low vigour (Figure 1.2, B; Figure 1.3) show little vegetative growth, with few shoots, short shoots and a higher fruit/leaf ratio (Vance, 2012). Low vigour was generally associated with small berries, low yields and more exposed clusters. Low vigour can be a natural consequence of soil-climatic conditions or generated through various crop situations over several seasonal cycles. Excessive crop load (Poni et al., 1994), inadequate fertilization (Spayd et al., 1993), lack of water in the soil, or the use of competitive cover crops (Tesic et al., 2007), among others, can induce situations of very low vigour.



FIGURE 1.3. Main parameters for assessing vigour and determinants in two contrasting

#### vigour situations.

Plants of *Vitis vinifera* cv. Tannat, in southern Uruguay (34° 36 S, 56° 14 W) grafted on SO4 trained on trellises. Commercial vineyard with high spatial variability defined by Ferrer et al. (2020) using NDVI. Uniformly managed vineyard. Evaluations carried out in 2019. A: High vigour; B: Low vigour. Blue lines indicate non-modifiable factors. Green dotted line indicates modifiable factors. Vigour indicator variables: ELA: Exposed Leaf Area (m<sup>2</sup> vine<sup>-1</sup>; according to Carbonneau, 1995); TLA: Total Leaf Area (m<sup>2</sup> vine<sup>-1</sup>); CP: Cane production (kg vine<sup>-1</sup>); Y: Yiled (kg vine<sup>-1</sup>); Y/CP: Ravaz Index; LA/Y: Leaf Area:Yield ratio; %BR: Bunch rot; RN: Root number, N: soil nitrogen (kg ha<sup>-1</sup>). Climate parameter: Light, rain, wind, air temperature, relative humidity. Soil parameter: TAW: Total Available Water (mm); PE: penetration resistance; Ts: Soil Temperature; EC: soil electrical conductivity; OM: Organic matter; N stock: Nitrogen soil stock; CEC: cation exchange capacity and TC: total cations. Ruler represents soil depth in meters for each vigour condition.

After the definition of the concepts of "vigour" or "capacity" or "vine balance", the impact of environmental and crop management factors on vigour are detailed. As mentioned above, the vigour and different index of vine balance such as Y/CP and AF/Y vary according to cultivar, variety/rootstock combination, canopy management, production objective and soil-climate conditions (Figure 1.2; data based on a production situation and a particular year). Management plays a key role in the sustainability of perennial crops because decisions in one season affect subsequent seasons (Howell, 2001). Over time, the definition of a sustainable system involves several productive,

ecological, environmental, social, cultural and economic factors (Sarandón & Flores, 2009). Within these factors, we can distinguish the ones that are not modifiable, related to climate and soil, from the ones that are modifiable, such as plant material and cultivation practices. The edaphic-climatic factors are the most influential in the generation of vigour and will ultimately condition cultural practices.

<u>Climate:</u> Climate variations have a short-term and long-term effect on the variability in vigour and production within a vineyard (Hunter & Bonnardot, 2011). The impact of climate on vigour can be assessed at three scales. A first scale is the macroclimatic scale, which refers to the region (hundreds of kilometers) and is influenced by latitude (Bonnardot et al. 2004, Quixley 2007). A second scale is the meso-climatic scale, which describes the climate in a smaller area and alludes to topographic changes, terrain elevation or proximity to water (Dry & Smart, 1988). Finally, a plant or micro-climatic scale is limited to the area close to the plant or the organs (leaves, berries) and is determined by the planting frame, plant vigour and canopy architecture (Bonnardot et al. 2004, Hunter et al. 2004).

Among the climatic variables, photosynthetically active radiation (PAR), temperature, wind speed, relative humidity, rainfall and evapotranspiration all influence plant microclimate (Dry, 2000) and affect vegetative development and growth, yield components and grape composition.

<u>Soil</u>: Geology, topography and soil-generating material determine plant vigour (Steyn et al., 2016). The physical properties of soil including its texture, structure, drainage, depth, color and temperature can be mentioned as major influential factors (Bekker, 2011) conditioning grapevine vigour and quality (Reynolds et al., 2007). Within the chemical properties pH, electrical conductivity and nutrient availability are also important factors for vine functioning. Variations in these physical and chemical soil parameters at the site level can be related to the spatial variation of vigour (Zerihun et al. 2010, Priori et al. 2019, Gatti et al. 2022). Indeed, vigour is generally higher for deeper soils (Bramley & Hamilton 2004, Bramley et al. 2011, Tardáguila et al. 2013), cation exchange

capacity (Tardáguila et al. 2011), % clay (Bramley & Hamilton 2004, Tardáguila et al. 2011, Willwerth & Reynolds 2020), water availability (Bramley et al. 2011, van Leeuwen et al. 2018), soil drainage (Olivier & Conradie, 2008) and plant nitrogen availability (Balachandra et al. 2009, van Leeuwen et al. 2018, Gatti et al. 2020).

The greater availability of water for plants is associated to the soil's capacity to store water (Schmitz & Sourell, 2000). However, there are differences in access to stored water due to differences in root colonization in the different soil layers. Other aspects of cultural management (planting density, pruning intensity, rootstock) affect plant vigour in synergy with soil characteristics. The choice of rootstocks can contribute to the management of vine vigour, as rootstocks can confer low, medium or high vigour (Keller et al. 2012, Marguerit et al. 2012). However, the vertical and horizontal distribution of the root system relies on the interaction between the genotype and soil characteristics (Hunter et al. 1995, Mortlat & Jacquet 2003). Indeed, the volume of soil to be explored by roots is conditioned by the physical and chemical properties of the soil. Nutrient availability (especially nitrogen) directly impacts plant vigour (Tesic et al. 2007, Celette et al. 2008). At this point, it is essential to note that an adequate development of fine roots determines a greater water uptake and soil nutrients (Archer & Strauss, 1990).

<u>Cultural practices</u>: Cultural management also impacts the expression of vigour by modifying aspects of climate and soil. Cultural management modifies plant ecophysiology with changes in growth and balance. Decisions such as location and planting design (row orientation and training system) permanently influence plant growth and determine vigour (Carol, 2007). Other cultural practices allow managing the expression of vigour, such as pruning, bunch thinning, leaf removal, weeding, green pruning, fertilization, irrigation and others (Dry et al. 2005, Reynolds et al. 2005). As detailed above, rootstock/variety combination is also a structural factor determining plant vigour.

#### 2.3 PLANT VIGOUR AND BERRY DEVELOPMENT

The previous sections defined vigour, the concept of a balanced plant, the determinants of vigour and how it can be determined or characterized. This section will deal with aspects related to berry development in relation to vigour and climatic characteristics. These aspects will be further discussed in chapter V of this work.

The berry is a non-climacteric fruit (Coombe & Hale, 1973) consisting of the exocarp or skin, the mesocarp or pulp and the endocarp, which is the tissue surrounding the seed (Hardie et al., 1996). A double sigmoid curve represents the growth of the berry with three distinct phases, two growth phases separated by a latent growth phase (Conde et al., 2007). Berry growth is associated with the flow of water and solutes from the plant to the fruit through the xylem and phloem. Phase I of fruit development is characterized by rapid fruit growth due to cell division and expansion (Ojeda et al. 1999, Schlosser et al. 2008). During phase II or latency, berry size varies little, while the main changes are in seed size and fresh weight (Ristic & Iland, 2005). During phase III of fruit development, significant changes occur in berry physiology (Dai et al., 2010). These changes include berry softening, decreased acidity, increased sugar concentration and color change in red varieties.

Transpiration is one process that provides the driving force that moves water and solutes into the berry (Dreier et al., 2000). Sugars arrive via the phloem to the berries and can be transported via symplastic and/or apoplastic transport from the phloem cells to the mesocarp cells, where they will be stored in the vacuole (Conde et al., 2007). Apoplastic transport involves the movement of solutes through the extracellular space and is the dominant transport after veraison (Afoufa-Bastien et al., 2010). In contrast, symplastic transport is a movement of solutes across cells, facilitated by plasmodesmata that allow cytoplasmic continuity (Lalonde et al., 2003). Sugar transport processes involve passive diffusion, mass flow (turgor-driven) or active transport facilitated by sugar transport proteins (Hayes et al., 2007). Sucrose is the main sugar transported by phloem sap and is hydrolyzed to glucose and fructose by invertase enzymes after unloading by the phloem (Dai et al., 2010).


**FIGURE 1.4.** Diagram of fruit development. The most critical stages and periods of compound accumulation and water flow into the berry are shown. Source: Kennedy, 2014.

Berry composition can change dynamically during berry development and is affected by many environmental and cultural factors (Coombe, 1992). The impact of vigour on grape composition has been reported in many papers with variable results. It has been reported that vineyards classified as "high vigour" would show incomplete or delayed ripening (Hall et al., 2011; Arnó et al., 2012). Sugar concentration is lower and pH higher (Santibáñez 2004, Kliewer & Dokoozlian 2005, Cortell 2006, Bramley et al. 2011, Vance, 2012) probably due to lower bunch exposure to sunlight. A negative correlation between vigour and anthocyanins was found in bigger berries (Lamb et al., 2004). As for secondary composition, they showed lower anthocyanin content (Lamb et al. 2004, Bramley et al. 2011). A negative correlation between higher vigour and flavonoid accumulation in berries was reported in several studies (Baluja et al. 2013, Yu et al. 2016). While "low vigour" plots or areas could present full ripening with better wine quality (Hall et al. 2011, Arnó et al. 2012).

Vine vigour indirectly influences grape composition through the modifications generated in the canopy that determine the microclimate near the cluster zone. Microclimate refers to the climatic conditions inside the canopy and the underlying environment (Smart & Robinson, 1991). Microclimate influences different factors of agronomic importance (Deloire et al., 2005), such as yield (Samrt & Robinson 1991, Sánchez & Dokoozlian 2005), grape composition (Smart, 1985) and grape health suitability at harvest (English et al. 1989, Molitor et al. 2011, Arrillaga et al. 2021). Among the microclimatic variables, temperature, relative humidity and luminosity are the most affected by canopy structure. Denser canopies present an excessive amount of leaf layers with an increase in relative humidity in the cluster zone with consequences on grape health (Zoecklein et al. 1992, Ferrer et al. 2020a, Arrillaga et al. 2021). In addition, with a greater number of leaf layers, the efficiency of phytosanitary treatments is reduced, increasing the risk of infection by pathogens (Valdés-Gomez et al., 2008). In contrast, less dense or more porous canopies have a greater airflow allowing lower relative humidity. Increased bunch exposure represents changes in fruit ripening (Downey et al. 2004, Tarara et al. 2008). The temperature of the berry can increase between 2° and 10 °C compared to air temperature, depending on the exposure of the clusters (Kliewer & Lider, 1986). Temperatures exceeding 35 °C have a negative impact on anthocyanin and phenol accumulation (Spayd et al. 2002, Mori et al. 2007, Tarara et al. 2008) by favoring the degradation of these metabolites. In vines with a good leaf: fruit balance, adequate exposure of the clusters stimulates the biosynthesis of phenols (Price et al. 1995), anthocyanins (Reynolds et al. 1995, Mori et al. 2007) and aromatic compounds (Bureau et al. 2000, Skinkis et al. 2010).

#### 2.4 METHODS USED TO QUANTIFY PLANT VIGOUR

Because vine vigour highly relies on the interactive effects of year to year climate fluctuations, soil variability or season-specific management, the characterization of vineyard's vigour at plot level is necessary to better orientate the production strategy

(i.e., the strategy based on exploiting heterogeneity or reducing variability at the plot level). The literature identifies several ways of quantifying vigour in a vineyard or plot with various advantages and disadvantages. Among the ways to determine vigour are mentioned: leaf area measurements (Myburgh 2005, Cloete et al. 2006), cane production (Ravaz 1911, Smart et al. 1990, Kliewer & Dokoozlian 2005, Demestihas et al. 2018), trunk diameter (Dobrowski et al. 2003, Strever, 2007; Trought et al., (2008), shoot length (Constanza et al., 2004), yield maps (Tisseyre et al., 2007), remote sensing (Johnson 2003, Bramley et al. 2011, Ferrer et al. 2020, Sams et al. 2022) and mobile applications (de Bei et al., 2014). All these techniques are valid for determining vigour, but some of them imply a great use of manpower, error in the determinations and a more limited spatial scope (Strever, 2007). Other techniques employ technologies that allow spatial quantifications of vigour on a larger scales and in real time. Some of the most common forms of vigour determination are detailed below.

#### 2.4.1 Trunk diameter

Trunk circumference is an indicator of vine vigour and, unlike other forms of assessment, it expresses vigour as a cumulative result over the life of the plant (Dobrowski et al., 2003). Indeed, pruning weight or vegetative growth are vigour indicators over a shorter period (Bramley, 2022). Differences in soil's physical and chemical properties are associated with spatial variations in trunk circumference (Trought et al., 2008). Bramley et al. (2011) reported that vines with larger trunk circumference had lower soluble solids content and higher total acidity, associated with excess vigour.

#### 2.4.2 Leaf area

As mentioned above, there is a direct relationship between leaf area and various productive aspects such as berry sugar accumulation and reserve accumulation. In addition, it, directly and indirectly, affects the accumulation of primary and secondary metabolites by modifying the clusters' microclimate. Leaf area establishment depends mainly on water availability and nitrogen fertilization (Lebon et al., 2006). To characterize

leaf area, the leaf area index (LAI) has been defined as the total area of leaf tissue per unit of soil surface area (Watson, 1947). The monitoring of leaf area (LAI) and canopy characteristics has been used as a way to estimate the crop coefficient (Kc) and thus adjust irrigation rates (Williams & Ayars, 2005).

Given the importance of this parameter, a great deal of work has focused on developing methods to measure leaf area. These methods can be classified into destructive vs. non-destructive and direct vs. indirect. Direct methods generally measure leaf area directly. These methods are accurate but time-consuming, labor-intensive, and, therefore, impractical. Indirect methods, on the other hand, are based on estimations of leaf area from allometric relations, imagery or simulations (Sepúlveda & Kliewer 1983, Barbagallo et al. 1996, Constanza et al. 2004).

An example is the use of the correlation between vine midrib length and leaf area (Carbonneau et al. 1978). Other methods use equipment to estimate LAI through diffuse radiation inside the canopy using sensors or photographs with hemispherical cameras (Weiss, 2002). However, with the technological advance and the accessibility of the smartphone to a georeferencing system, high-definition digital cameras, information registration, etc., new indirect estimations of leaf area have been developed through imagery (Fuentes et al., 2014). In particular, the smartphone application called VitiCanopy has been reported as an alternative for leaf area determination, as a quick tool for determining LAI (Leaf Area Index) and other parameters of canopy architecture (De Bei et al., 2014).

#### 2.4.5 Multispectral imagery

Remote sensing refers to acquiring information about the vine plant without direct contact. The technologies available for remote sensing have been used to estimate crops' vegetative development/vigour. Multispectral imaging quantifies vegetation reflectance at different wavelengths (green, red and near-infrared) through radiometric sensors (Hall et al., 2001). This technology is based on the interaction of solar energy (wavelengths) and leaf characteristics. Chlorophyll and other plant pigments are related

to the visible spectrum (400 to 700 nm) (Gitelson et al., 2003) for use in the photosynthesis process. These pigments absorb red and blue light while green light is reflected. In addition, infrared light is not altered by the chlorophylls in the leaf, but it does change its path through the leaf (half goes through the leaf and half is reflected). Healthy plants reflect more near-infrared light than damaged plants (which reflect more visible light), which is explained by the lower chlorophyll content in unhealthy plants (Boshoff, 2010).

This relationship between red (R) and near-infrared (NIR) wavelengths has been used to establish various vegetation indices. A vegetation index can be defined as an indicator of relative plant growth and/or vigour (Huete et al., 1994). Many remote sensing works (Hall et al. 2001 and 2002, Dobrowski et al. 2003, Johnson et al. 2003, Lamb et al. 2004) have confirmed that vine vigour difference can be characterized through multispectral image processing using vegetation indices such as the normalized difference vegetation index (NDVI, Eq. 3, Rouse et al., 1973). The mathematical calculation of this index is:

$$NDVI = \frac{NIR - R}{NIR + R} \quad Eq. (3)$$

where NIR is the reflectance value in the near infrared wavelength (700 to 900 nm) and R is the reflectance value in the red wavelength (600 to 700 nm). This index has been correlated with LAI (Johonson 2003, Hall et al. 2008), pruning weight (Dobrowski et al., 2003), leaf area (Ferrer et al., 2020a) and even with grape composition parameters (Lamb et al., 2004). Multispectral images can be acquired by satellite, aircraft, dorn, or tractormounted sensors. In all cases, the key to the successful use of this technology is to be able to achieve an understanding between the images generated and the characteristics of the grapevine.

#### 2.5 PRECISION VITICULTURE

Precision agriculture (PA) emerged in the late 1980s in the United States and focused on fertilization management in extensive crops such as cereals (Stanfford, 2000). Precision agriculture can be defined as the "process of adopting differentiated cropping practices based on spatial and temporal variability" (Moran et al. 1997, Cook and Bramley 1998). Precision viticulture (PV) is an example of precision agriculture.

In PV, combining technologies and methodologies is aimed at improving and optimizing productive, economic and environmental issues (Tisseyre et al., 2008). Indeed, according to Bonilla (2015), precision viticulture has three main objectives: (i) to maximize productive yield and/or harvest quality by adjusting management techniques, (ii) to maximize harvest quality by separating grapes according to different qualities and (iii) to minimize environmental impact by reducing the use of pesticides and fertilizers and improving the efficient use of water. In this approach, management is based on the fact that each homogeneous crop unit must be treated differentially, thus achieving optimized management of resources according to the vineyard's variability and the plant's interaction with the environment (Hall et al., 2002). The first work on precision agriculture applied to grapevines was carried out in Australia (Bramley & Proffit, 1999). Then, its application has spread to many viticultural regions: the United States (Johnson et al. 2003, Taylor et al. 2013), France (Bobillet et al. 2005, Tisseyre et al. 2008), Italy (Filippetti et al. 2013, Gatti et al. 2021), Spain (Arnó et al. 2005, Arnó et al. 2012, Santesteban et al. 2013), Portugal (Cunha et al., 2010), New Zealand (Praat et al., 2004), South Africa (Strever, 2007), Argentina (Bragachini, 2002), Chile (Ortega-Farias et al. 2003, Acevedo-Opazo et al. 2008), Brazil (Oldoni et al., 2020) and Uruguay (Ferrer et al., 2020a).

Bramley (2022) proposes to follow three distinct steps for a correct PV (Figure 1.5). The first step is observation and data collection through various sensors that allow the collection of geo-referenced information. In the second step, the information collected is interpreted and analyzed. And finally, in the third step, a management plan is implemented based on the information collected and processed. These three stages are

developed cyclically due to the perennial nature of the vine. The adjustment of this management can be oriented towards seeking homogeneity in the plot (with the differential application according to input needs) or taking advantage of this natural variability by determining management zones. An example of a strategy using PV technologies is optimizing fertilizer application or improving water use efficiency by adjusting irrigation according to canopy characteristics (Tisseyre et al., 2008).



FIGURE 1.5. Precision viticulture phases

Successful of PV relies on essential tools at each step, some of which will be detailed below. Georeferencing is one of the critical points in precision viticulture and usually requires an accuracy of about 1 m (Tisseryre et al., 2007). Through the use of global positioning system (GPS), the spatial information of the plants can be known in a format of geographical coordinates (longitude, latitude and altitude). The development of geographic information systems (GIS) has made it possible to use the georeferenced database (generated in step 1) to apply geostatistical analysis, thus allowing for easy visualization of the information (Bonilla, 2015). Advances in remote sensing are also a key tool for the development of PV that allows obtaining information from the use of multispectral sensors or thermal cameras (Stoll & Jones, 2005). As mentioned above the development of lateral proxy detection sensors accompanied by GPS can be coupled to

drones and tractors to collect geo-referenced information. Soil property monitoring is also widespread to establish associations between soil heterogeneity (variability in physical and chemical properties) and plant growth (Taylor et al., 2019). Among the sensors used for soil evaluation, those that determine soil electromagnetic properties (ECa) stand out. Soil electrical conductivity can be related to soil moisture parameters, presence of salts, clay content, among others (Corwin & Lesch, 2005). Among ECa sensors, electrical resistivity (ER) and electromagnetic induction (EMI) sensors stand out (Tisseyre et al., 2008). Another important point is the use of VRD (Variable Rate Dosing) technologies that allow achieving a dosage of inputs based on information generated instantaneously or previously (Tisseyre et al., 2008). The main uses of this technology are in phytosanitary applications (Raynal, 2004).

# CHAPTER II GENERAL MATERIALS AND METHODS



#### 1. MATERIALS AND METHODS

#### **1.1. EXPERIMENTAL SITE**

This work was carried out in a 1.1 ha commercial vineyard planted in 1998 with *Vitis vinifera* L. cv. Tannat, grafted on SO4 rootstock. This vineyard is located in Canelones, Uruguay (34° 36 S, 56° 14 W), 56 km from Montevideo (Figure 2.1).





The continental and regional location are presented in A and B. In C location of the experimental plot (red box) ( $34^{\circ} 36 S$ ,  $56^{\circ} 14 W$ ). Arrow indicates north

The vineyard has a gradual slope of 1-3% (north/south). Vine spacing is 2.5 m x 1.2 m (3333 vines ha<sup>-1</sup>). The rows are oriented north/south. The vines are pruned using a double guyot system and the shoots have been formed in VSP (vertical shoot position). The winegrower managed the vineyard in the same way throughout the plot. The vineyard is not irrigated. In addition, it receives standard fertilization with urea (46% N), distributed half at pre-flowering and half at post-harvest, with a total dose of 140 kg of fertilizer per hectare. Weed management was controlled in the row with herbicides. The inter-row consisted of a mixture of *gramineae* and *asteraceae*, with oats sown in winter. Vegetation growth was controlled in the inter-row by periodic mowing (six times a year).

This vineyard was selected due to its high east-west variability in vine vigour. For this plot, the Ravaz index averaged around 14, indicating that plants vigour was outside the optimum range established by Ferrer et al. (1997) for this variety (6-10). In addition, the coefficient of variation of yield at the plot level was 47%.

#### CHAPTER II | MATERIALS AND METHODS

Ferrer et al. (2020a) assessed the vigour of this plot using precision viticulture tools (Figure 2.2). Crop vigour was assessed at veraison (January in the southern hemisphere) during three years (2015, 2016 and 2017) from Normalized Difference Vegetation Index (NDVI), calculated using aerial imagery (620 m altitude and 50 m/s speed). High-resolution multispectral images were obtained on the ground (0.2 m) that allowed establishing or defining three vigour zones, high (HV), medium (MV) and low (LV). As each NDVI class (high, medium and low) was systematically located in the same parts of the vineyard each year (Ferrer et al., 2020a), a synthesis map could be established and is presented in Figure 2.2. In this map the NDVI ranges consisted of high vigour (NDVI 0.57 to 0.61), medium vigour (NDVI 0.55 to 0.57) and low vigouur (NDVI 0.55 to 0.48). In winter 2020, trunk diameter was evaluated to corroborate vigour stability. A grid evaluation was performed with 84 points throughout the plot and the diameter was measured at 10 cm above the graft with a digital caliper (Neiko 01407  $\pm$  0.2 mm). Vines were georeferenced with a GPS (Thales Navigation Inc., San Dimas, CA, USA). Trunk diameter correlated positively with NDVI (r = 0.60, p-value <0.05).



FIGURE 2.2. NDVI map resulting from the average NDVI during 2015-2017.

#### **1.2. EXPERIMENTAL STRATEGY**

The experimental strategy to meet the objectives of this project is summarized in Figure 2.3. The description of the particular methodology is described in each chapter.

The first step, presented in chapter III of this document, was to determine the causes of the variation in vigour at the plot level. For this purpose, an exhaustive description of the physical and chemical parameters of the soil was made. In addition, an 8-year database (2014-2021) was used, which included climatic data, vegetative growth, yield and grape composition. The aim was to establish the relationships between soil or climate variables and plant response and how these relations were affected in the two situations of contrasting vigour, i.e. High Vigour (HV) vs Low Vigour (LV).

The next step relied on the evaluation of different vigour management strategies (chapter or part IV). For this purpose, treatments were adjusted according to vigour zone to improve yield, berry quality and reduce vigour heterogeneity at the plot level. Only the most contrasting vigour zones of the vineyard (HV vs. LV) were selected. In the preestablished zones and for three seasons (2019, 2020 and 2021), treatments (Table 2.1) were arranged in a randomized block design with three replicates and 21 vines per replicate (63 plants per treatment). For HV, treatments aimed to reduce water and nitrogen supply and improve micro-climatic conditions in the cluster zone. Water restriction (H-W) was applied from veraison to harvest by covering the soil with polyethylene (white on both sides, 220 micrometers thick, with ultraviolet protection). No nitrogen was applied (0 N units in the cycle) in the nitrogen restriction treatment (H-N). For two cycles (2019 and 2020) the grower stopped fertilizing with urea in one subplot (rows 5 to 15, where this treatment was randomly installed). A leaf removal treatment (H-L) was imposed prior to flowering, at a rate of 60% of leaves removed. In contrast, for LV, treatments aimed to increase water and nitrogen supply.

Irrigation treatment (L+W) was supplied compared to the control (LV) to achieve 100% of climatic demand (ETo) from bud to flowering and from harvest to leaf drop, and 70% of ETo from flowering to harvest. For the supplemental nitrogen (L+N) treatment, 210 kg urea per hectare were supplied (70 kg urea per hectare was added to the 140 kg mentioned above before flowering). In addition, a treatment combining water and nitrogen supplementation (L+WN) was carried out.

### VIGOR DETERMINATION SOIL PARAMETERS CLIMATE VARIABLES



# CHAPTER

IV

CHAPTER

Ш

YEARS:

2014 to 2021

**TREATMENTS:** 

HV

LV

YEARS: 2019 to 2021

TREATMENTS: HV, H-N, H-W, H-L LV, L+N, L+W

### VIGOR MANAGEMENT

VIGOR AND BERRY

DEVELOPMENT

MICRO-CLIMATE VARIABLES NITROGEN AND WATER STATUS VEGETATIVE GROWTH YIELD COMPONENTS BERRY COMPOSITION INTRA-PLOT HETEROGENEITY





# CHAPTER

YEARS 2014 to 2021

TREATMENTS: HV, H-N, H-W, H-L LV, L+N, L+W





FIGURE 2.3. Diagram of the general methodology proposed to meet the objectives of this study.

#### CHAPTER II | MATERIALS AND METHODS

Finally, part V discusses the role of vigour, environmental conditions and cultivation techniques on berry development, with emphasis on water and sugar accumulation. In this chapter, information is addressed at two levels. A first level characterizes berry growth dynamics through the analysis of historical data (2014-2021) for the two contrasting vigour situations (HV vs. LV) according to climatic conditions. And a second level analyzes the impact of water availability, nitrogen and leaf:fruit ratio (all treatments, table 3.1) on berry growth dynamics in two contrasting years (2019 vs. 2020).

Table 2.1.	Description of water,	nitrogen and leaf r	emoval treatment	s in each vigour z	one
and years.					

Vigour	Treatment	Nitrogen (Units)	Irrigation	Rainfed	Cover crop	Season
HIGH	Control (HV)	64	NO	YES	YES	2014 to 2021
	Water restriction (H-W)	64	NO	NO*	NO*	2019 to 2020
	Nitrogen restriction (H-N)	0	NO	YES	YES	2019 to 2020
	Leaf removal (H-L)	64	NO	YES	YES	2019 to 2021
ΓΟΜ	Control (LV)	64	NO	YES	YES	2014 to 2021
	Irrigation (L+W)	64	YES (All season)	YES	YES	2020 and 2021
	Nitrogen supply (L+N)	96	NO	YES	YES	2019 to 2021
	Irrigation + nitrogen supply (L+WN)	96	YES	YES	YES	2021

\*From veraison to harvest

# CHAPTER III

# SOIL AND CLIMATIC FACTORS DETERMINING THE HETEROGENEITY OF VIGOUR WITHIN A VINEYARD



#### 1. INTRODUCTION CHAPTER III

The spatial variability of the physical environment such as soil, topography and climate conditions crop functioning. Soil properties such as texture, structure, depth, nutrition, water reserve, among others, and their interaction with agronomic management and climate, were shown to have major impact on the variability of grapevine vigour and production (Runge & Hons 1999, Machado et al. 2002, Arnó et al. 2012). Knowing the specific causes that generate such spatial variability is important for winegrowers to optimize production and reduce production costs. But assessing variability is not a simple task and is time-consuming.

Precision agriculture (PA) technologies applied to viticulture (PV) allow a comprehensive approach of spatial variability of soil and plant variables. Spatial characterization of grapevine vigour can be beneficial to improve the vineyard management and increase the productivity (Bramley et al. 2011, Gatti et al. 2021). Deeper soils, with higher organic matter, higher available water have been reported to favor high yields (Bramley et al. 2011, Tardáguila et al., 2011). Spatial differences in yield and vigour are rather consistent over time (Priori et al., 2019). In contrast, grape quality which highly relies on microclimate (notably temperature and light), does not follow a stable the spatial distribution pattern (Tisseyre et al., 2008).

Elevated temperatures lower sugar accumulation toward berries by decreasing the photosynthesis and sugar flow in the phloem (Lecourieux et al., 2017). Anthocyanins are also affected by elevated temperatures, due to an increase in anthocyanin degradation or inhibition of anthocyanin biosynthesis (Mori et al., 2007). In addition, temperature significantly affects hydraulic and soluble solute transport relationships by affecting transpiration (Rebucci et al. 1997, Pascual et al. 2022). Photosynthesis is optimum within a temperature range of 25 to 30 °C (Kriedemann, 1968). Temperatures above 35 °C usually inhibit photosynthesis, impacting in the fruit ripening processes (Greer & Weedon, 2012). Also, severe water deficit (predawn leaf potential < -0.5 MPa) negatively impacts physiological processes such as photosynthesis and sugar accumulation in the berry (Carbonneau, 1995).

This chapter focuses on determining the environmental factors that determine the expression of vigour. It is structured in two parts. The first part deals with how soil heterogeneity, combined with annual climatic variability, determine vine growth at the plot level during 8 years of evaluation. The second part presents the relationship between clay type and plant vigour.

## 2. HOW SOIL AND CLIMATE VARIABILITY WITHIN A VINEYARD CAN AFFECT THE HETEROGENEITY OF GRAPEVINE VIGOUR AND PRODUCTION

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#### 2.1. ABSTRACT

#### ENGLISH

This study aimed to determine how within-plot soil heterogeneity combined with yearly climate variability can promote the heterogeneity of vine growth at plot level, and which soil-climate parameters influence final yield and berry composition the most. An 8-year experiment was conducted on grapevine in two zones of a vineyard (1 ha) differentiated according to grapevine vigour as determined by NDVI: high vigour (HV) and low vigour (LV). The heterogeneity of the soil properties (depth, texture and composition), plant growth (shoots and roots) and plant production (yield components and berry composition) were determined at plot level. Compared to the LV zone, the HV zone was associated with deeper soils, higher soil water and nitrogen availability, CEC and montmorillonite/illite ratio. More extended root systems, higher vegetative growth and higher yield were observed in the HV zone compared to the LV zone. Drier and warmer vintages increased the difference in heterogeneity of vine growth and yield between the two zones. Berry composition (primary and secondary metabolites) also differed between HV and LV zones but seemed unconnected to vigour and mainly depended on soilclimate-plant interactions over the years. The heterogeneity of plant vigour within the vineyard mainly resulted from differences in root exploration, soil profile and composition (notably montmorillonite/illite ratio). The present study identified soil and crop factors that, depending on weather conditions, can be drivers for reducing the heterogeneity of plant development and improving productivity at vineyard level.

**Keywords:** Within-field heterogeneity . Precision viticulture . Root growth . Berry composition . Soil water . *Vitis vinifera* L. . Tannat

#### FRENCH

Cette étude vise à déterminer comment l'hétérogénéité du sol à l'intérieur d'une parcelle, combinée à la variabilité annuelle du climat, peut favoriser l'hétérogénéité de la croissance de la vigne au niveau de la parcelle, et quels sont les paramètres pédoclimatiques qui influencent le plus le rendement final et la composition des baies. Une expérience de 8 ans a été menée sur la vigne dans deux zones d'un vignoble (1 ha) différenciées en fonction de la vigueur de la vigne déterminée par NDVI : haute vigueur (HV) et faible vigueur (LV). L'hétérogénéité des propriétés du sol (profondeur, texture et composition), la croissance des plantes (pousses et racines) et la production végétale (composantes du rendement et composition des baies) ont été déterminées au niveau de la parcelle. Par rapport à la zone LV, la zone HV était associée à des sols plus profonds, à une plus grande disponibilité de l'eau et de l'azote dans le sol, à une CEC et à un rapport montmorillonite/illite plus élevé. Des systèmes racinaires plus étendus, une croissance végétative plus importante et un rendement plus élevé ont été observés dans la zone HV par rapport à la zone LV. Les millésimes plus secs et plus chauds ont augmenté la différence d'hétérogénéité de la croissance de la vigne et du rendement entre les deux zones. La composition des baies (métabolites primaires et secondaires) différait également entre les zones HV et LV, mais ne semblait pas liée à la vigueur et dépendait principalement des interactions sol-climat-plante au fil des ans. L'hétérogénéité de la vigueur des plantes au sein du vignoble résulte principalement de différences dans l'exploration des racines, le profil et la composition du sol (notamment le rapport montmorillonite/illite). La présente étude a permis d'identifier des facteurs liés au sol et à la culture qui, en fonction des conditions météorologiques, peuvent être des facteurs de réduction de l'hétérogénéité du développement des plantes et d'amélioration de la productivité au niveau du vignoble.

**Mots-clés :** Hétérogénéité intra-champ . Viticulture de précision . Croissance des racines . Composition des baies . Eau du sol . *Vitis vinifera* L. . Tannat

#### SPANISH

El objetivo de este estudio fue determinar cómo la heterogeneidad del suelo dentro de la parcela, combinada con la variabilidad climática anual, puede promover la heterogeneidad del crecimiento de la vid a nivel de parcela, y qué parámetros edafoclimáticos influyen más en el rendimiento final y en la composición de las bayas. Se realizó un experimento de 8 años con vides en dos zonas de un viñedo (1 ha) diferenciadas según el vigor de la vid determinado por NDVI: alto vigor (HV) y bajo vigor (LV). Se determinó a nivel de parcela la heterogeneidad de las propiedades del suelo (profundidad, textura y composición), el crecimiento de la planta (brotes y raíces) y la producción vegetal (componentes del rendimiento y composición de las bayas). En comparación con la zona LV, la zona HV se asoció con suelos más profundos, mayor disponibilidad de agua y nitrógeno en el suelo, CEC y relación montmorillonita/illita. Se observaron sistemas radiculares más extendidos, mayor crecimiento vegetativo y mayor rendimiento en la zona HV en comparación con la zona LV. Las añadas más secas y cálidas aumentaron la diferencia en la heterogeneidad del crecimiento de la vid y el rendimiento entre las dos zonas. La composición de las bayas (metabolitos primarios y secundarios) también difería entre las zonas HV y LV, pero no parecía estar relacionada con el vigor y dependía principalmente de las interacciones suelo-clima-planta a lo largo de los años. La heterogeneidad del vigor de la planta dentro del viñedo se debió principalmente a diferencias en la exploración de las raíces, el perfil y la composición del suelo (en particular, la relación montmorillonita/illita). El presente estudio identificó los factores del suelo y del cultivo que, dependiendo de las condiciones climáticas, pueden ser impulsores para reducir la heterogeneidad del desarrollo de la planta y mejorar la productividad a nivel del viñedo.

**Palabras clave:** Heterogeneidad . Viticultura de precisión . Crecimiento radicular . Composición de las bayas . Agua del suelo . *Vitis vinifera* L. . Tannat

#### 2.2. INTRODUCTION

One of the main issues that farmers face is the within-plot variability of production. Spatial (within-plot) yield variability is associated with stable seasonal physical features (soil and topography) that interact with seasonal abiotic and biotic conditions (climate, water and nitrogen availability and presence of disease) and agronomic management strategy (Machado et al., 2002; Tisseyre et al., 2008; Jasse et al., 2021). The availability of water and nutrients (mainly nitrogen) are well known factors that condition plant development, growth and yield. Water is one of the most critical factors determining the vegetative development of grapevine (Pellegrino et al., 2005). Severe water deficit can result in limited aerial and root growth due to decreased cell turgor and increased root penetration resistance in dry soil (Bengough et al., 2011). In addition, water availability determines nutrient uptake (Keller 2015), stomatal conductance, photosynthesis (Flexas et al., 1998) and berry growth. Several studies have reported a reduction in stomatal conductance without impact on photosynthesis during a water deficit, thus increasing water use efficiency.  $\delta$ 13C in berry must can be used as an integrative indicator of water status and water use efficiency during the ripening period (Brillante et al., 2018; Yu et al., 2021). Another factor that impacts plant functioning is soil temperature, which depends on soil characteristics and water availability. Notably, soil temperature influences shoot and root growth in grapevine through its impacts on carbon and nitrogen allocations from the pool of reserves (Clarke et al., 2015; Field et al., 2020). Ultimately, the physical characteristics of soil, including texture, structure and depth, are important factors to consider because of their effects on root temperature and on water and mineral supply to the plant (Schmitz and Sourell, 2000; Brillante et al., 2016). The above physical characteristics (soil/climate) of the vineyard are part of the "Terroir" concept. Indeed, "Terroir" refers to the combination of geographical (soil) and climatic characteristics of a region that are influenced by human practices, and which in turn enables the production of a product with unique characteristics (Vaudour 2003; van Leeuwen et al., 2006; OIV, 2010).

Precision viticulture (PV) comprises a set of tools that allows the viticulturist to characterise the spatial variability of terroir components at the vineyard scale to make well-informed decisions. It enables resource management to be optimised or selective

harvesting based on yield or quality parameters to be conducted (Bramley and Hamilton 2004). The use of a vegetation index like the NDVI (normalised difference vegetation index, defined by Rouse et al., (1973)) provides a source of information for potential delimitation of zones with contrasting plant growth (Bramley et al., 2011; Ferrer et al., 2020 (a); Sams et al., 2022). The NDVI is often used in viticulture to estimate vine vigour (Tisseyre et al., 2007). The concept of vine vigour refers to the vine's growth capacity; i.e., vegetative and productive growth (Winkler et al., 1974; Smart and Robinson 1991). Thus, 'vigour' is a term that encompasses both the plant growth rate and its production potential (total dry matter produced). The heterogeneity of weather, together with nonuniform topography and soil characteristics, generate plant vigour or an NDVI with high spatial and temporal heterogeneity within a single vineyard (Bramley and Hamilton 2004; Jasse et al., 2021). Many studies have reported the impact of grapevine vigour on yield and grape composition. In general, vines with high vigour are associated with high yields and big berries (Bramley et al., 2011; van Leeuwen et al., 2018), but they are prone to greater sensitivity to Botrytis cinerea (Filippetti et al., 2013; Ferrer et al., 2020(a); Gatti et al., 2022) and to a delay in ripening (van Leeuwen et al., 2018) compared to low vigour vines. By contrast, vines with low vigour, which often result from lower water and nitrogen availability at the plot level, generate lower yields (Arno et al., 2012) and can result in excessive exposure of the bunches to sunlight (Tardaguila et al., 2011; McClymont et al., 2012; Ferrer et al., 2020 (b)). Vigour has been shown to affect berry composition (sugars, acids and anthocyanins) in different ways, depending on the climatic conditions in a given year (Tisseyre et al., 2008; McClymont et al., 2012; Gatti et al., 2022).

While the physical characteristics of soil generate consistent heterogeneous zones of productivity over the years, specific weather conditions and/or crop management during the cropping cycle can alternatively lower or exacerbate the withinplot variability of production (Tisseyre et al., 2007; Gatti et al., 2022). Thus, understanding the factors underlying soil heterogeneity at the plot level and how weather conditions can enhance their effects on plant development and productivity is essential for optimal and more sustainable crop management.

The present study was conducted over eight consecutive growing seasons in a representative vineyard in the south of Uruguay characterised by a temperate climate,

with the aim of mapping soil heterogeneity (texture, depth, organic matter, nitrogen stock and temperature) within the vineyard. The influence of soil factors combined with weather conditions on the expression of plant vigour, yield and berry quality components were then assessed. Whether precise vineyard management could help to reduce the effects of heterogeneity on plant growth and production is discussed.

#### 2.3. MATERIALS AND METHODS

#### 2.3.1. <u>Study site</u>

The experiment was carried out over eight consecutive years (2014-2021) in a commercial vineyard of 1.1 ha planted in 1998 with Vitis vinifera L. cv. Tannat grafted on SO4 rootstock. This vineyard was located in Canelones, Uruguay (geographic coordinates: 34° 36 S, 56° 14 W), 56 km from Montevideo. The vineyard was on a gradual slope of 1-2% (north /south). The rows were orientated north-south. The vine spacing was 2.5 m x 1.2 m (3333 vines /ha). The vines were pruned using a double guyot system (12 buds per plant) and the shoots trained to a VSP (vertical shoot position). The vineyard was not irrigated. Standard post-harvest fertilisation was carried out using urea, at a dose of 64 units of N per ha, half of which was distributed at pre-flowering and half at post-harvest.

The vineyard has high variability from east to west in terms of vigour and yield/pruning ratio (Ravaz Index), which ranged between 5 and 20. Crop vigour was assessed at veraison (January in the southern hemisphere) in 2015, 2016 and 2017 using the Normalized Difference Vegetation Index (NDVI), which was calculated using aerial imagery (altitude 620 m and speed 50 m/s), as described by Ferrer et al., 2020 (a). High resolution (0.2 m) multispectral aerial images were obtained to define three vigour zones: high (NDVI 0.57 to 0.61), medium (NDVI 0.55 to 0.57) and low (NDVI 0.55 to 0.48) (Figure 1A). The reflectance generated by the cover crop in the intermediate rows was eliminated using the algorithm described by Primicerio et al., (2015). Further details on sensor type and image processing are available in Ferrer et al., 2020(a). The high and low NDVI zones were located in the same part of the plot in all three years (2015, 2016 and 2017), indicating perennial stability in terms of the spatial heterogeneity of the vegetative growth. Two random blocks with three replications were then defined in each the zones of high vigour (HV) and low vigour (LV). Each replication comprised twenty-one vines distributed within two rows. The vines were geo-referenced using a GPS (Thales

Navigation Inc., San Dimas, CA, USA). In 2020, the variability of trunk diameter (TD10) in the different vigour zones was determined. Eighty-four points were measured in the plot following a grid design (Figure 1B) and a trunk diameter variation map was produced. TD10 was evaluated at 10 cm above the graft using a digital caliper (Neiko 01407  $\pm$  0.2 mm). The value obtained was the average of the transverse and longitudinal diameters with reference to the direction of the row.

Both zones (HV/LV) were managed by the winegrower in the same way: the weeds were controlled under the row using herbicides, mixed grass comprising oats and Asteraceae was grown in the inter-row, and growth was systematically controlled in the middle row by periodic mowing (six times a year).

#### 2.3.2. Climate characterisation

The climate in this region is temperate, with warm nights (14 to 18°C) and moderate drought. Uruguay has an average annual rainfall of 1100 mm. However, the inter-annual variability of rain is high, and the distribution of monthly rainfall is not homogenous over the years (0 mm to 300 mm per month). During the growing season (September to March, in the southern hemisphere) the average rainfall is 600 mm.

The mesoclimatic data were collected from a meteorological station (geographic coordinates: 34° 40' S, 56° 20' W), which is less than 10 km from the study plot and managed according to WMO (World Meteorological Organization) standards. The weather of the area was characterised based on the following variables: maximum temperature (Tmax), minimum temperature (Tmin), average temperature (Tx), reference evapotranspiration (ETo) and rainfall. From these data, the following indicators (Ferrer et al., (2020 (b)) were determined: cumulated rainfall from budbreak to flowering (September to November; RRbb), cumulated rainfall from flowering to fruit set (November to December; RRff) and cumulated rainfall during ripening (January to March; RRrip).

Plant and soil microclimates were measured using Ibutton thermochron sensors (USA, DS-1921g,  $\pm$  0.5°C increment). Three sensors were randomly distributed in the canopy within each zone (HV/LV) during the 8 years of experimentation (2014-2021). Using the temperature data, the following indicators (Ferrer et al., 2020 (b)) were

determined: number of days with temperatures above 30°C from flowering to harvest (November to March; ND30), maximum temperature at Veraison (January, TMv) and maximum temperature at harvest (February, TMh). Soil temperature (Ts) was also determined from bud-break to leaf fall in 2019 and 2020. For this purpose, three sensors were randomly installed in each vigour zone under the row at depths of 20 and 40 cm. Using a base temperature of 10°C GD10 (Lebon et al., 2004), the growing degree days were calculated using the data obtained from these sensors (soil and canopy).



FIGURE 3.1. Experimental site location and vigour maps.

A: Map of average NDVI (aerial images) values at veraison (2015 to 2017) depicting the three vigour zones (white: low; sky-blue: medium; dark blue: high) for an experimental site (34° 36 S, 56° 14 W). The distribution of replicates in rectangles (yellow: high vigour, green: low vigour). B: Trunk diameter distribution map. Trunk diameter in mm. It was evaluated at bud-break in 2020 (September). Yellow points in B correspond to the soil sampling location (n=84). C: Combination map of NDVI and trunk diameter. Triangles indicate locations of representative plants for each vigour zone (yellow: high vigour; green: low vigour), selected for root profile diggings determination. Stars indicate auger-based soil inspection points.

#### 2.3.3. Soil measurements

The soil types in Uruguay are very heterogeneous, but the predominant soils in the study region have been classified as Fine Smectitic Thermic Vertic Argiudoll (Duran et al., 2005). The soil was characterised in the field according to FAO (2006) and classified following the USDA Soil Taxonomy (Soil Survey Staff 1999). Two soil diggings and twelve

soil profile inspection points, using a soil auger, were made in the middle of the row (see location in Figure 3.1 C) to determine the chemical and physical properties of the soils. A characteristic soil profile for each vigour zone is shown in Figure 4. In the winter of 2015, 252 soil samples were taken within the vineyard at three sampling depths (0-20, 20-40 and 40-60 cm) within a grid area design (10.8 m x 12.5 m) (Figure 1B, yellow points) applying the methodology proposed by Alliaume et al., (2017). Extractable phosphorus (Bray method), pH, exchangeable bases (calcium, magnesium, potassium and sodium), organic matter (OM), nitrates, cation exchange capacity (CEC), % of sand (Sa), clay (CI) and silt (Si) were determined from samples taken at 0-20 and 20-40 cm. From the 40-60 cm samples, only OM and pH were determined. Furthermore, six soil inspections were performed with an auger in each vigour zone. The horizons, depth, texture, consistency, structure, bulk density and percentage of organic matter were determined in each inspection. According to its physical and chemical characteristics, in particular its CEC (14.5 – 33.7 cmol+/kg) and total cations (12.6 – 33.5 cmol+/kg) the soil belongs to the Vertic Argiudoll unit of the regional map (Silva et al., 2018).

In addition, organic matter, nitrate and ammonium contents at three depths (0-20, 20-40 and 40-60 cm) were determined in the winter of 2018, 2019 and 2020 in both vigour zones. The N stock was calculated as the sum of NO3- and NH4+ contents and a yearly O.M. mineralisation rate. The O.M. mineralisation rate was set to 0.02 g/g/year, which is slightly less than the rate observed by Salvo et al., (2014) for a no till crop system (with rotation) in similar soils of Uruguay. Nitrogen leaching was not taken into account.

To estimate the distribution of water and nitrogen in the root zone, a 0.1 m x 0.1 m grid was used in the soil profile at dormancy in 2020 (see supplement 4C). Two samples of 30 g of soil were taken at every 0.2 m depth and every horizontal distance separated by 0.2 m. A total of 50 samples were collected from each vigour zone. Soil moisture and N stock were determined in these samples. Soil moisture was measured using the gravimetric method.

In each vigour zone (HV/LV) a mixed soil sample was taken at a depth of 20-30 cm in order to quantify and identify the different clay mineralogy (CM). Clay mineralogy was determined by X-ray diffractometry (XRD), as described by Beaux et al., (2019), in the laboratory of the Technological Development Department of CURE

(http://www.cure.edu.uy/). The methodology for sample preparation and clay analysis was adapted from Carroll (1970). Organic matter, carbonates and gypsum were removed from the samples, then the samples were deflocculated and the clay fraction was separated. Three oriented samples were prepared from this fraction: one was measured naturally (without processing), another was placed in a glycol chamber (for ethylene glycol saturation) for 24 hours and then measured, and another was calcined at 550oC for 2 hr and then measured.

The Total Available Water (TAW) (Allen et al., 1998) was calculated from the soil and root analyses as described below.

The TAW over the soil depth (SD) was calculated from Eq. 1:

TAW (mm) = (FC - PWP) \*soil depth /10 cm (1)

with FC (volumetric moisture at field capacity) and PWP (volumetric moisture at permanent wilting point) both in cubic centimetres of water per cubic centimetre of dry soil, and BD as bulk density. FC and PWP in the soil profile were estimated using Eqs. (2) and (3) developed by Fernández (1979) and Silva et al., (1988) for Uruguayan soils:

FC (% vol.) =[21.977-0.168\*(Sa, %)+2.601\*(OM, %) +0.127\*(Cl, %)]\*BD/Dw (2)

PWP (%vol.) = [-58.1313 + 0.3718 (OM, %) + 0.5682 (Sa ;%) + 0.6414 (Si ;%) + 0.9755 (Cl ; %)]\*BD/Dw (3)

In Eqs. 2 and 3, BD (bulk density, g/cc-1) is divided by Dw (volumetric mass of water, 1 g/cc-1) in order to keep RHS dimensionless.

The soil depth applied in Eq. (1) was the maximum depth of the root system observed from the soil profile. Following Fernández (1979) and Silva et al. (1988), BD/Dw was determined using Eq. (4):

BD/Dw = 3.6725 - 0.0531\*(OM, %) - 0.0210\*(Sa, %) - 0.0228\*(Si, %) - 0.0221 (Cl, %) (4)

#### 2.3.4. Plant growth and yield components

#### Root growth

One representative plant per zone (HV/LV) was selected based on average trunk diameter in each vigour zone. Root exploration (up to the maximum root depth) of each selected plant was assessed at dormancy (2020) 5 and 30 cm from the trunk. A 0.1 m x 0.1 m grid was placed on the root profile after the surrounding soil was removed according to Böhm (1979). the roots were grouped according to their diameter as follows (Van Zyl, 1988): less than 0.5 mm (fine), between 0.5 and 2 mm (thin), between 2 and 5 mm (medium), more than 5 mm (thick). The location and number of the roots per category in each grid were recorded using a digital caliper. The rooting index (RI) was calculated from the number of roots in each class (Eq. 5, Callejas-Rodriguez et al., 2012):

RI= [(fine roots number + thin roots number) / (medium roots number + thick roots number)] (5)

#### Aboveground growth and nutrient status

Canopy variables were measured over 8 consecutive years (2014-2021) in each vigour zone. Exposed Leaf Area (ELA, in m2/ha) was assessed at veraison on 9 vines per zone (HV/LV) according to Carbonneau (1995). Leaf nitrogen (%NI) and potassium (%KI) were measured on 20 healthy and exposed leaf blade at veraison. Yield per vine (Y, kg/vine), number of bunches per plant (B/v) and bunch size (Bz) were determined at harvest on 63 plants per vigour zone. Pruning Weight (PW, kg/vine) was measured during winter on the same 63 plants that were used for yield assessment. The Ravaz index was calculated using this information (Y.PW-1).

#### Berry weight and composition

Two samples of 250 berries were collected at harvest for each replicate (21 plants), with three replicates for each zone (HV/LV), using the method proposed by Carbonneau et al. (1991). One sample was used for classical grape analysis and another for phenolic analysis.

For the basic berry analysis, the weight of the berries (Bw, g) was determined with an Ohaus Scout scale (Ohaus Corp., USA). The juice was obtained from manual crushing

of the berries and the crushing of the pulp with a juice extractor, Phillips HR2290 (Phillips, Netherlands). Berry analyses (sugar content, total acidity and pH) were carried following the OIV protocol (OIV 2014) using an Atago N1 refractometer (Atago, Japan) for Brix, Hanna HI8521 pH meter (Hanna Instruments, Italy) and burette for acidity (gH2SO4/L).

The other berry samples were assayed for total potential in anthocyanins (ApH1, g/l) and total phenols index (Tp), according to Glories and Augustin (1995) and González-Neves et al. (2004). The measurements were carried out by duplication with a Shimadzu UV-1240 Mini (Shimadzu, Japan) spectrophotometer, using glass (for the anthocyanin analyses, absorbance at 520 nm) and quartz cells (for the analyses of phenols, absorbance at 280 nm) with 1 cm path length.

#### 2.3.5. Statistical analyses

We used the QGIS (Geographic Information System; 2021) programme to create the vineyard maps of available soil water, CEC, % clay, total cations (TC) and trunk diameter using IDW (Inverse Distance Weighting) interpolation. The maps of soil moisture, nitrogen concentration and root density in the area of influence of the vine were made using OriginPro 9.1 software.

Statistical analyses were conducted with the statistical package InfoStat Version 2011. Analyses of variance, followed by the Fisher test for means comparison, were conducted to determine the effect of vigour on soil microclimate and plant responses. Correlations between all plant and soil variables were determined though a correspondence factor analysis (ACF). Multiple linear regression models were used to quantify the effects of edaphoclimatic variables (13) on the most important plant variables during the period 2014 to 2021. Although the characterisation of soil variables (TAW, Cl, CEC, CM) had been done in 2015, it was considered unlikely for these parameters to vary throughout the duration of this trial. Taking this into account, it was feasible to perform the interannual analysis on the variables analysed. Parameters such as O.M., Stock N and Ts were adjusted according to the values obtained in 2018, 2019 and 2020. The best explanatory models were selected through step-wise selection by setting limits of the model parameters at 0.10 p-value.

#### 2.4. RESULTS

#### 2.4.1. Temporal and spatial variabilities in weather and soil at the plot level

The average air temperatures (mean, minimum and maximum) over the cropping season were similar for all years (2014 to 2021) (Supplementary data 3.1). The mean temperature was 20°C, with a minimum of 13.5°C and a maximum of 25°C for the average temperatures. The cumulated thermal time (GD10) was higher than 2100°Cd in all years, and even higher than 2200°Cd in 2016, 2020 and 2021. Water supply could be characterised according to three groups of years (Figure 3.2 A): i) cumulated rainfall of over 600 mm during the plant cycle (wet years) in 2014, 2015 and 2019, ii) rainfall of less than 500 mm (dry years) in 2016, 2018 and 2020, and iii) rainfall of between 500 and 600 mm (intermediate) in 2017 and 2021 . For the wettest years, 60% of rainfall occurred during the ripening months. The atmospheric evaporative demand (ETo) was higher than the cumulated rainfall for all years, reaching 870 mm over the cropping season on average (Figure 3.2 B). The dynamics of soil temperature (Supplement 3.3) over two contrasting wet/dry seasons (2019 and 2020 respectively) did not show any significant differences between vigour zones. The average temperature was 20°C, regardless of soil depth (0-20 cm; 20-40 cm).





#### (2014-2021).

A- Cumulated rainfall. Black line indicates the mean rainfall over the growing cycle (601 mm). B- Cumulated reference evapotranspiration (ETo). Black line indicates the mean ETo over the growing cycle (877 mm). RRbb: cumulated rainfall/ETo from budbreak to flowering (September to November); RRff: cumulated rainfall/ETo from flowering to fruit set (November to December); RRrip: cumulated rainfall/ETo during ripening (January to March).



FIGURE 3.3. Maps representing soil variability for different soil parameters.

A- Clay (%) at 0-20 cm. B- Organic matter (OM, %) at 0-20 cm. C- Total Available Water (mm) over the soil depth explored by roots (0-90 cm for High Vigour; 0-60 cm for Low Vigour; see Fig.6). D- Total cations (cmol+.kg-1) at 0-20 cm. E- Cation exchange capacity (CEC, cmol+.kg-1) at 0-20 cm. The maps were built from the soil physico-chemical measurements carried out in August 2015 from the soil sampling points presented in Fig. 1 B.

The classical physical and chemical characteristics of the soil showed a strong spatial variability, mainly in percentage of clay, cation exchange capacity (CEC), total cations and total available water (TAW) (Figure 3.3). Clay content ranged from 26-45% in the HV zone (west side of the vineyard) to 27-35% in the LV zone (east side), with a CV for the whole plot of 20% (Figure 3.3 A). The mean CEC decreased from the HV to the LV

zone, ranging from 24-35 cmol+ /kg in the HV zone and 14-23 cmol+ /kg in the LV zone (Figure 3.3 E). The CV of CEC for the plot was 54%. The total cations ranged between 14 and 37 cmol+/kg, and the overall CV for total cations was 44% (Figure 3.3 D). Among the different cations, calcium showed the most significant within-plot variation of the two zones (CV 29%), followed by potassium (CV 25%). The TAW estimated from soil texture and root depth (Figure. 3.3 C, 3.4 and 3.6 A see below) was more than 180 mm in the HV zone, and less than 140 mm in the LV zone, with a CV of 15% for the plot. Soil pH (data not shown) was close to neutral (6.3) and slightly varied within the plot (CV: 6.4%). Organic matter (O.M.) tended to be slightly higher (0.4%) in the HV zone than in the LV zone (Figure 3B). However, O.M. was high in both zones, reaching 0.7 to 1% in the deepest soil layers (50 cm).

#### 2.4.2. Variability of soil and plant mineral status in the vigour zones

In the HV zone, the main soil horizons were: Ap (0- 0.10 m), Bss (0.15-0.40 m) and C (> 0.60 m), with transitional horizons (Figure 3.4). The texture was mainly clay loam. Slicken-sides (=pressure faces) were present from 0.20 m down to the deepest horizon. Carbonates increased from the surface to reach up to 20% in the C horizon.

No physical limitations were observed for root exploration and grapevine roots were detected in horizon C.

The soil was shallower in the LV zone than in the HV zone (0.20 m), and the variability of soil depth (SD) at the plot level was 19%. The profile was characterised as: horizon Ap(0-0.15 m); Bss (0.15 to 0.40 m) and C (> 40 m). No transitional horizon was present. In addition, the Bss horizon had an extremely firm consistency. The presence of carbonates was also lower than in the HV zone. No roots were observed at a depth greater than 60 cm (Figure 3.6 C) in the LV zone.



FIGURE 3.4. Soil profiles for the High and Low vigour zones.

The main soil parameters evaluated were: soil depth, texture, consistency, structure, bulk density (BD) and organic matter. The vigour zones were defined from the NDVI and trunk diameter values. High vigour: NDVI 0.57-0.61 and trunk diameter: 58.0 mm. Low vigour: NDVI 0.48-0.57 and trunk diameter 36.0 mm. Images and description collected in winter 2019.



#### FIGURE 3.5. Changes in the N stock (absolute values and percentages) from 2018 to

2020 in the High and Low vigour zones.

A- High Vigour; B- Low Vigour. Black: NO3- (kg ha-1); Red: NH4+(kg ha-1); Blue: N potentially mineralized from organic matter (kg ha-1) calculated with the same mineralization rate (2%) in the two vigour zones. Asterisks indicate significant differences according to Fisher (p-value < 0.05) between vigour conditions for each year evaluated. The vigour zones were defined from the NDVI and trunk diameter values. High vigour: NDVI 0.57-0.61 and trunk diameter: 58.0 mm. Low vigour: NDVI 0.48-0.57 and trunk diameter 36.0 mm

	Ν	1	КІ		
Year/Vigour	(% of dry	' matter)	(% of dry matter)		
	High vigour	Low Vigour	H. Vigour	L. Vigour	
2015	$1.64 \pm 0.07$	1.53 ± 0.07	0.56 ± 0.03*	0.36 ± 0.02*	
2016	1.70 ± 0.04 *	1.39 ± 0.09 *	0.75 ± 0.06	0.67 ± 0.05	
2017	1.66 ± 0.09 *	0.69 ± 0.07 *	n.d.	n.d.	
2018	n.d	n.d.	n.d.	n.d.	
2019	1.58 ± 0.15	$1.42 \pm 0.18$	n.d.	n.d.	
2020	1.79 ± 0.08 *	1.28 ± 0.11 *	n.d.	n.d.	

TABLE 3.1. Leaf concentration of N (%) and K (%) according to year and vigour condition

Mean and standard deviation. Values expressed as percentage of dry matter. Leaf nitrogen (%NI) and potassium (%KI) evaluated from a sample composed of 20 healthy and exposed leaf blade collected at veraison of each season. \* Asterisks indicate significant differences according to the Fisher test (p-value< 0.05). n.d. No data available. n= 3

The mineralogical analysis of the clay showed differences in the abundance of 2:1 clay types between the vigour zones (Supplement 3.4). The abundance of the clays in the

HV zone was 79% montmorillonite and 20% illite, th contrast to 34% montmorillonite and 60% illite in the LV zone. Both soil zones had a low content of kaolinite, the 1:1 clay type, (between 1% for HV zone to 6% for the LV zone).

Differences were observed in the absolute amounts of nitrogen stock between the vigour zones (Figure 5). A higher availability of N was observed in the HV zone than in the LV zone in all three study years. These differences were due to a higher presence of mineral nitrogen (NO3- and NH4+) and to a higher potential mineralisation of the O.M (greater soil depth, slightly higher O.M. content) in the HV zone (p-value<0.05).

Leaf N (NI) and K (KI) contents were globally higher in the HV zone than in the LV zones in most years (p-value<0.05) (Table 3.1). Over the period 2015 to 2020, %NI varied between 0.63 and 1.87 % in the plot. %NI was significantly higher in the HV zone in 2016, 2017 and 2020. K values ranged from 0.34 to 0.76 % over the period 2015 to 2016 and they were also higher in the HV zone in 2015.

#### 2.4.3. Water, nitrogen and root distribution for the representative vigour plants

The trunk diameter (TD10) of the plants chosen for root and soil exploration was nearly the same as the mean TD10 for HV (58 mm) and LV (35 mm). Trunk diameter correlated with NDVI and other soil (TAW, CEC) and plant variables (ELA, Y) (Supplement 2). At dormancy (in 2020), the distribution of soil moisture along the 1m deep soil profile (0.5m from both sides of the vine) differed depending on the vigour zone (Figure 3.6 A). In the HV zone, higher % moisture values were observed (38% to 68%) throughout the soil profile and with a more uniform distribution than in the LV zone (25% to 55%).

Soil N concentration in winter (in 2020) was higher in the HV zone than in the LV zone (Figure 6B): up to 50 mg/kg in the first 30 cm of soil and greater than 20 mg/kg until 80 cm depth. By contrast, in the LV zone, concentrations of N higher than 20 mg/kg were only observed in soil above a depth of 0.50 m and did not exceed 40 mg/kg.


FIGURE 3.6. Soil moisture, nitrogen and root distribution in soil profile according to the vigour condition.

Maps in the area of influence of the vine (1.0 m depth, 0.5 m both sides of the vine). The plants chosen were representative for each vigour zone in terms of trunk diameter. The evaluations were carried out in winter 2020. Maps above represent the high vigour zone. Maps below represent the low vigour zone. A- Volumetric soil moisture (%) contour lines. B- Nitrogen concentration contour lines (mg kg-1). C- Root density maps (number of roots 0.01 m-2). The observations were made 5 cm apart from the plant. Maps on the left represented the total density of roots, and maps on the right represented the roots with a diameter lower than 2 mm.

The root exploration maps showed differences at dormancy (2020) depending on the vigour zone (Figure 3.6 C). HV plants had greater root density than LV plants, and the roots reached a greater depth (roots were detected down to 0.9 m) than LV plants (absence of roots below 0.6 m). Moreover, the roots were spread over a wider area in the HV zone than in the LV zone (Supplement 3.5), thus allowing them to colonise a greater volume of soil. The number and distribution of roots thinner than 2 mm differed between the HV and LV zones (Figure 3.6 C). In the HV zone, these were more numerous than in the LV zone, occupying a larger area and volume above the depth of 0.7 m, compared to just a depth of 0.4 m in the LV zone. Root growth in the inter-row (0.3 m from the plant) was also evaluated. A greater root density (of all the classes, including those thinner than 2 mm) was found in the HV zone (Supplement 3.5).

### 2.4.4. Temporal and spatial variation of plant vigour and yield

Plant variables such as pruning weight, exposed leaf area, yield and concentration of berry anthocyanins (PW, ELA, Y and ApH1) varied highly over time depending on the vigour zone (Figure 7, Supplement 6 and 7). The HV zone systematically showed higher pruning weight (0.4 to 0.7 kg/vine), exposed leaf area (1.2 to 2.3 m2/vine) and yield 5.1 to 8.0 kg/vine) than the LV zone (pruning weight: 0.1 to 0.4 kg/vine; exposed leaf area: 0.8 to 2.0 m2 /vine; yield: 3.9 to 6.8 kg/vine) (p-value<0.05), except in 2014 when the yield was affected by sanitary conditions. The higher bunch size (Bz) and Bw values explain the higher yields obtained in HV compared to LV. In both zones (Supplement 3.6), PW and ELA were the highest in years 2014, 2015, 2017 and 2019. The years 2015, 2016, 2017 and 2018 were characterised by the highest yields in both vigour zones. The lowest yields were observed in 2014 and 2019 in the HV zone, and in 2014 and 2021 in the LV zone. Total anthocyanin concentrations also varied at plot level (ranging from 773 to 2345 mg/l); however, this variable was not clearly associated with vigour. Total anthocyanin concentration was alternatively higher in the HV or LV zone, depending on the year.





anthocyanins content according to the vigour level during each studied year.

The vigour zones were defined from the NDVI and trunk diameter values. High vigour: NDVI 0.57-0.61 and trunk diameter: 58.0 mm. Low vigour: NDVI 0.48-0.57 and trunk diameter 36.0 mm. The bars represent the average value, and the error bars represent the standard deviation. Green rectangle indicates optimum Ravaz Index values for Tannat (6-8). Pruning weight (PW) in kg vine-1 (n=63); Yield (Y) in kg vine-1 (n=63) and Anthocyanins in g/l (n=3). The asterisks indicate significant differences according to the Fisher test (p-value < 0.05).





### (from 2014 to 2021).

HV: High Vigour; LV: Low Vigour. Soil parameters: CEC: Cation exchange capacity; Stock N: Stock nitrogen (kg ha-1); OM: organic matter (%); Cl: Clay (%); TAW: Totally Available Water (mm); TC: total cations and Ts: soil temperature (°C). Climate variables: RRff: cumulated rainfall from flowering to fruit set; RRrip: cumulated rainfall during ripening; TMV: maximum temperature at veraison: TMh: maximum temperature at harvest; ND30: numbers of days with temperatures above 30° C from flowering to harvest. Plant parameters: RI: Rooting index; Y: Yield (kg vine-1); BW: Berry weight (g); B/v: bunch/vine; ELA: Exposed leaf surface (m2 ha-1); PW: Pruning weight (kg vine-1); %NI: Leaf nitrogen (%); Brix (°); Acidity (g H2SO4/L); ApH1: Total anthocyanins (g/l) and Tp: Total phenols index.

An exploratory analysis (Correspondance Factor Analysis, ACF) on all plant, soil and weather variables and climate variables was conducted for all the years (Figure 3.8). The first two axes on the ACF explain 77.3% of total inertia (Axis 1: 48.0%; Axis 2: 29.3%). The two vigour zones (HV/LV) are on opposing ends of axis 1. The HV zone can be seen to be highly associated with soil variables Total Available Water (TAW), stock of nitrogen (N stock) and clay content (%), and, to a lower extent, to cation exchange capacity (CEC) and total cations (TC). By contrast, the LV zone is opposite these soil variables. Plant variables related to vegetative development (RI, ELA and PW), leaf nitrogen content (%NI), and the production variables (Y and Bw) were associated with the HV zone. However, vine fertility (B/v) was more closely associated with the LV zone than with the HV zone. The LV zone was also associated with higher total phenol (Tp) concentrations. Micro-climatic variables such as maximum temperature in the canopy at veraison (TMv) and soil temperature (Ts) were found to be associated with the HV zone, while the number of days with temperature above 30°C (ND30) in the canopy were found to be

more closely associated with the LV zone. On the second axis, the years are distributed on both sides of the diagram, with the most extreme and opposing years being 2014 and 2018. Rainfall variables (RRff and RRrip) were positively correlated with the wet years (2014, 2015 and 2019) and negatively correlated with the dry years (2016, 2018 and 2020). The intermediate years (2017 and 2021) are close to the centre of this axis. The rainy years were associated with high berry Acidity, pH, Bw and %NI. Dry years were associated with high canopy temperature (TMv, TMh and ND30), and high sugar and total anthocyanins (Brix, ApH1).

Lastly, a multiple regression was performed on a set of 12 variables related to plant vigour, yield components and berry composition to determine which soil variables had the most influence on spatial variations at plot level. Significant regressions were obtained for only 8 of the 12 variables: NDVI, Leaf area, Pruning Weight, Yield, Berry weight, Brix, Total anthocyanin and Total Phenol concentrations (Table 3.2). In general, total available water (TAW), soil temperature (Ts), organic matter (OM) and stock N were the soil parameters that were the most closely correlated with plant response. The climatic variables that were the most closely associated with the plant variables were maximum temperature at veraison (TMv), cumulative rainfall from flowering to fruit set (RRff) and cumulative rainfall during ripening (RRrip). Vegetative variables (NDVI, ELA and PW) were positively correlated with edaphic parameters, such as TAW, Stock N and O.M. and with climate variables such as RRff and RRrip. Similarly, yield (Y) and berry weight (Bw) were positively correlated with TAW, Stock N, RRff, RRrip and TMv. Yield was also positively correlated with soil temperature (Ts). Primary and secondary berry metabolisms (brix, ApH1, Tp) were more closely correlated with climatic variables. The edaphoclimatic variables that were the most closely associated with grape composition parameters were TMv, TMh, RRrip, RRff, O.M. and Ts (Table 3.2).

Response variable	Model	R <sup>2</sup>	p- value
NDVI	-1.69 + 0.02 (Cl) + 2.8 x 10 <sup>-3</sup> (TAW) + 1.3 x 10 <sup>-3</sup> (RRrip) + 8.5 (Ts)	0.74	< 0.01
Exposed leaf area (ELA; m² ha-1)	-5.58 + 0.01 (Stock N) + 0.40 (OM) + 0.01 (TAW) + 9.2 x 10 <sup>-4</sup> (RRff) + 0.13 (TMv)	0.64	< 0.05
Cane Production (CP; kg vine <sup>-1</sup> )	-0.93 + 0.01 (Stock N) + 3.7 x 10 <sup>-4</sup> (RRff) + 0.01 (TAW) + 1.1 x 10 <sup>-3</sup> (CEC)	0.77	< 0.01
Yield (Y; kg vine <sup>-1</sup> )	6.27 + 0.03 (TAW) + 0.50 (Stock N) + 1.6 x 10 <sup>-3</sup> (Ts) + 0.34 (TMv)	0.63	< 0.10
Berry weight (Bw, g)	2.50+ 0.11 (TAW) + 1.2 x10 <sup>-3</sup> (RRff) + 4.9 x 10 <sup>-4</sup> (RRrip) + 0.01 (Stock N) - 0.07 (TMv)	0.78	< 0.05
Brix (°)	53.88 – 2.09 (TMv) + 0.98 (TMh) - 0.01 (RRrip) + 1.07 (OM) + 0.04 (TAW)	0.67	< 0.01
Total anthocyanins (ApH1; g/l)	3046 + 0.57 (Ts) - 564 (OM) + 240 (TMh) – 104 (TMv)	0.61	< 0.10
Total Phenols (Tp; %)	103 - 0.01 (Ts) + 1.76 (TC) + 0.66 (ND30) + 0.01 (RRff) +2.47 (TMv)	0.55	< 0.01

**Table 3.2.** Multiple regression analysis for the vegetative, yield and berry composition variables as a function of soil parameters in 2015 and climate condition (2014-2021).

Abbreviations: TAW: Total Available Water; OM: Organic matter; Cl: clay %; Ts: soil temperature; CEC: cation exchange capacity; TC: total cations; RRff: cumulated rainfall from flowering to fruit set; RRrip: cumulated rainfall during ripening; TMv: maximum temperature at veraison.

### 2.5. DISCUSSION

### 2.5.1. The variability of soil and root density between high and low vigour zones

The HV zone has a high natural fertility (CEC >20 cmol+/kg), while the LV zone has medium natural fertility (CEC 10-20 cmol+/kg) (Silva et al., 2018). This difference in CEC between zones is related to contrasting percentages of clay and of the type of clay (Pereyra et al., 2022(a)). When applying a decomposition rule, the clay types are 28% montmorillonite and 7% illite in the HV zone, and 10.2% montmorillonite, 18% illite and 1.8% kaolinite in the LV zone. Assuming typical values of CEC for montmorillonite (90 cmol+/kg), illite (30 cmol+/kg) and kaolinite (10 cmol+/kg), the CEC (clay) are 27.3 and 14.8 cmol+/kg in the HV and LV zones respectively. These values are superior to the OM contribution to CEC, particularly in the HV zone. With a CEC for OM of around 250 cmol+/kg and 2.7% OM, we find a contribution of OM to CEC that is less than 7 cmol+/kg. The differing amounts and types of clay depending on the vigour zone may explain the soil water retention in the HV zone as compared to the LV zone (van Leeuwen and Seguin 2006; Tardaguila et al., 2011). Soils containing a higher amount of montmorillonite have

a greater expansion capacity (Brady and Weyl 2008), which may be responsible for the lower bulk density (BD) calculated for the HV zone. The swelling properties of montmorillonite is linked to changes in the hydration of calcium (Sun et al., 2015) when the soil becomes wet. The clay-humic association is then subject to strong geometric constraints, which could explain why the humus of mollisol is called soft humus (Brady and Weyl, 2008). In the LV zone, the higher density at deeper soil layers is probably a result of some internal processes linked to soil evolution. As the soil contains less calcium, it is possible that acidification in the root zone progressively reduced the positive effect of calcium carbonate, leading to soil degradation.

The differences in root development in the vigour zones were associated with the physical and chemical properties of the soil. Total available water, aeration, soil depth, penetration resistance, bulk density and cultivation practices are known to affect root development (Morlat and Jacquet, 1993; Callejas-Rodriguez et al., 2012; Gatti et al., 2020). Deeper soil, greater water reserve capacity, higher cation exchange and lower bulk density (Figure 5.3 and 5.4), as found in the HV zone, lead to greater root development (Figure 5.6) with a higher abundance of fine roots (higher RI). The presence of calcium carbonates in the HV zone may have been favourable for the formation of bigger textural aggregates (Ubade et al., 2011), facilitating root development in deep layers. Moreover, because of the (medium) tolerance of rootstock SO4 to carbonates, no iron deficiency symptoms were observed during this trial. Due to the greater soil compaction (higher bulk density and extremely firm consistency) in LV zone, 70% of the roots thinner than 2 mm were located in the top 0.30 m-deep layer of soil, and no roots were observed below 0.5 m (Figure 6C). In addition to soil water availability, soil temperature influences the development of fine roots that have an important role in water and nutrient uptake (Mahmud et al., 2019). Soil temperature directly affects the root system by impacting root metabolism, root growth, nutrient and water uptake (Clarke et al., 2015; de Souza et al., 2022; Mezzatesta et al., 2023) and indirectly affects it by conditioning N mineralisation rates (Zogg et al., 1996; Verdenal et al., 2021). These factors, especially Ts and TAW, could explain the heterogeneity in soil nitrogen availability and root development between HV and LV zones (Figures 5 and 6). Indeed, the areas with higher fine root density in both the HV and LV zones were associated with soil zones containing

higher soil moisture content and available N (Figure 6). Insufficient resources (water and N) in the zone of influence of the LV plants (Figure 6-A, 6-B) could explain the observed low root density. Under similar conditions, plants have been found to apply a nitrogen/carbohydrate "saving" strategy by reducing the synthesis of new roots and extending the lifespan of existing roots (de Souza et al., 2022). This situation would lead to negative feedback in the long term due to a reduction in water and nutrient uptake capacity (Centinari et al., 2016) as root age increases.

### 2.5.2. Canopy and berry development responses to soil and climate

Zones of differing vigour within a vineyard tend to be stable over time (Bramley and Hamilton 2004; Gatti et al., 2022) - even up to 6 years (Tisseyre et al., 2008). In this study, the NDVI maps from 2015 to 2017 were corroborated in 2020 by measuring trunk diameter (Figure 1 D; Supplement Data 2). The leaf area (ELA, NDVI), pruning weight (PW) and yield (Y, Bw and Bz) were higher in the HV zone than in the LV zone, regardless of the climatic conditions of the years (Figure 7 and 8). The stability of the vigour zones over time was mostly associated with edaphic factors, as reported in other studies (Priori et al., 2019, Gatti et al., 2022;). In turn, soil factors such as Cl, O.M, SD, conditioned soil water availability (TAW) and were strongly associated with the abovementioned productive factors (ELA, NDVI, PW, Y; Table 3.2). This indicates that soil characteristics play a dominant role in vigour establishment and can mitigate or enhance the effects of weather. The root system has the ability to adapt to different edaphic situations, thus impacting vegetative growth, production and grape quality (Tomasi et al., 2015). Higher TAW, Ts in the HV zone resulted in higher soil N concentration and higher %NI (Figure 3.6; Table 3.1). The limitation of water content in the LV zone may have decreased plant N availability by negatively impacting microbial activity, and N mobility and uptake. In addition, a high soil bulk density also limits root elongation and decreases N uptake. The lower %NI values reflected such a limitation in the LV zone, which was more marked in the drier years (2016, 2017 and 2020) (Table 3.1, Figure 3.2), thus making it possible to conclude that %NI may be an indicator of vine vigour (Balachandra et al., 2009; Gatti et al., 2022).

The ability of HV and LV vines to provide a constant supply of water and nutrients for vegetative development, yield and grape quality was determined by the differing root

distribution and density depending on the physical and chemical properties of the soil (Morlat and Jacquet, 1993; van Leeuwen et al., 2009). In addition, climatic variables, such as water supply (RRff, RRrip) and temperature (Tmv), also impacted those plant variables (Table 3.2). Rainy and intermediate years (high RRff and RRrip) were favourable for vegetative growth in both zones (Figure 2 and 7). Yield tended to be lower when rainfall during the month prior to harvest (RRrip) increased in the HV zone (e.g., in 2014) because of higher susceptibility to bunch rot (Figure 3.7 and 3.8); this has also been observed in other studies (Filippetti et al., 2013; Ferrer et al., 2020(b)). The higher soil water content (during rainfall) in the HV zone than in the LV zone can be attributed to the swelling properties of montmorillonite and higher humus levels. Such characteristics buffered the effects of water deficit (in dryer years), with less impact on plant growth and yield in the HV zone than in the LV zone, as was also reported by Tomasi et al., (2015). The plants in the HV zone achieved a better production/vegetative balance (Figure 3.7) than those in the LV zone, as expressed using the Ravaz index (11.5 vs. 14.2). In the rainy years, the plants reached the optimum Ravaz index value (6-8) reported for Tannat. Bud fertility was higher in the LV zone than in the HV zone, and the canopy microclimate (ND30) tended to be warmer (Figure 8). The lower ELA in the LV zone may have allowed greater exposure of the buds to light which explains the higher number of clusters per plant (Sanchez and Dookozlian, 2005).

In this trial, the harvest date was set according to the evolution of pH, Brix and Bw in each year. These parameters showed a coefficient of variation of less than 10% (data not shown) between the vigour zones. Our results indicate that the inter-annual variability in pH and Brix were poorly linked to soil properties (Figure 3.8; Table 3.2; Supplement 3.7). The concentrations of variables linked to berry composition (sugar and acidity) mostly depended on air and canopy temperature in both zones and on precipitation in the LV zone (due to its low water reserve capacity). Tisseyre et al. (2008) and Gatti et al. (2022) reported less intra-annual variation in Brix and pH than in berry anthocyanin content. Although the secondary metabolism variables (ApH1 and Tp) showed significant variations between the HV and LV zones, the differences were not consistent from one year to another.

Anthocyanin and phenol concentrations in berries are known to depend highly on soil properties and climatic characteristics of the year (van Leeuwen et al., 2004). Our results show that the concentrations of these variables depended highly on the climatic and microclimatic conditions of a given year, tending to be higher in the dry years (Figure 3.7 and 3.8, Table 3.2). The more open canopies of the LV zone increased light interception in the cluster zone, thus increasing anthocyanin content when temperature was not excessive (< 35°C) (Haselgrove et al., 2000; Mori et al., 2007). Indeed, the concentrations of berry secondary metabolites (ApH1 and TP) were shown to be associated with temperature parameters (Ts, TMV, TMh and ND30, Table 3.2). These results are in line with Ryu et al. (2020), who reported that high berry temperatures around the onset of ripening (veraison) inhibited anthocyanin synthesis. In addition, high spring rainfall (2016, 2019 and 2021), which was an important factor influencing vine vigour in the LV zone, had a positive impact on ApH1 in the same zone (Figure 3.7, Supplement 7). Sams et al. (2022) reported that the spatial variability of phenolic compounds was fairly consistent with that predicted by remote sensing data, such as NDVI and the canopy temperature index. Canopy temperature relies on energy balance and plant water status and also affect grape composition (Brillante et al., 2016; Sams et al., 2022).

### 2.5.3. Site-specific management

This study showed that the variation in yield and vigour at the plot level was mainly determined by the heterogeneity of the soil and exacerbated by weather conditions. As mentioned previously, the stability over time of plant vigour and yield gradients related to soil heterogeneity will allow differential management zones to be established within the same plot (Bramley and Hamilton 2004). A strategy that could be applied is the division of the vineyard into homogeneous zones to take advantage of this variability and generate wines of different qualities (Bramley and Lamb 2003; Gatti et al., 2022).

Alternatively, the site-specific management of the vineyard comprising different uses of inputs, could help to lower heterogeneity in plant vigour (Tisseyre et al., 2008; McClymont et al., 2012). Since the distribution of vigour zones was consistent in this trial, it would be possible to establish two differentiated site-specific management zones. We

recently reported the impact of site-specific management following this approach (Pereyra et al., 2022(b)). In the HV zone, a reduction in nitrogen fertilisation could be an option to reduce vine vigour (Pereyra et al., 2022(b)), but the accumulation of reserves in the medium- and long-term must be considered. In addition, favouring the growth of cover crops in the inter-row would decrease grapevine growth (Celette et al., 2009; Coniberti et al., 2018). However, cover crops must be carefully managed, for example the variability of rainfall in Uruguay (enhanced under climate change) could lead to excessive competition, even in the HV zone, during dry years. Lastly, the improvement of the microclimate in the bunch zone via leaf removal could be necessary to increase berry secondary metabolism and reduce pest pressure in the HV zone (Filippetti et al., 2013). Pre-flowering leaf removal at high vigour in the vineyard of this study improved grape quality (sugars and anthocyanins) and reduced the incidence of bunch rot without any sunburn damage (Pereyra et al., 2022(b)).

In the LV zone, a different irrigation strategy and/or supplementary fertiliation would help overcome water and nitrogen deficits resulting from certain soil characteristics (TAW, depth and nutrients) and increase vine vigour. Working on Shiraz, McClymont et al. (2012) managed to increase yield and water use efficiency by implementing differential irrigation according to vigour conditions. Supplementary irrigation during the flowering and fruit set periods is also a possibility (Table 2). In the LV zone, the cover crop should be controlled. More exhaustive maintenance through periodic mowing and the use of less competitive species would improve water availability for the vine.

Other management levers could be proposed in the future when planting in new vineyards to reduce vine heterogeneity. Our results suggest that the vines can support more shoots and produce a higher yield than was the case in the HV zone. Therefore, increasing bud number per plant and adopting open canopy trellis/training systems may be a way of reducing vine vigour while improving the microclimate in the cluster zone (Gladstone and Dokoozlian, 2003); however, this would require vine density to be decreased accordingly. Furthermore, it may be necessary to evaluate the adaptation of rootstocks to specific soil characteristics (Ollat et al., 2015). Field phenotyping approaches still need to be improved to characterise and select grapevine rootstocks and

varieties better adapted to heterogeneous soil conditions (Carvalho et al., 2021). Finally, based on the results, the soil water reserve could be used as a criterion for plot delimitation in future plantations. Plots of similar TAW would result in the homogenisation of vigour.

### 2.6. CONCLUSIONS

This work has provided new information on soil-plant-environment interactions. The variability in soil characteristics (clay type and content, TAW, O.M, SD and Stock N) at the vineyard level conditioned plant response, generating two well-defined zones according to vigour (HV, LV). Compared with the LV zone, the HV zone was characterised by greater soil water and nitrogen availability and better vine root exploration, which enabled higher wood production and yield. The predominance of montmorillonite over illite was an important factor contributing to soil fertility in the HV zone, which is compatible with the use of cover crops between the vine rows. The gradient of vine vigour and yield in both zones was stable over the years, regardless of weather conditions. These results suggest that the differences found in the soil properties in the HV zone attenuated the effects of the climatic conditions, which did not occur in the LV zone. However, no consistent grape composition in either zone was observed. Secondary metabolite concentrations (anthocyanins and phenols) were mainly affected by the climatic and micro-climatic conditions each year, highlighting the complexity of the interactions of these compounds in the soil-plant-atmosphere system. Defining vigour zones (by remote sensing) within a plot would be required for more precise soil and crop management and for more sustainable vineyard management and berry production.

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# 3. MONTMORILLONITE CONTENT IS AN INFLUENTIAL SOIL PARAMETER OF GRAPEVINE DEVELOPMENT AND YIELD IN SOUTH URUGUAY

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## 3.1. ABSTRACT

## ENGLISH

Soil physical and chemical characteristics play a key role on vine growth and yield. The soils of South Uruguay display high content of montmorillonite or illite. The proportions of these minerals deserve special attention as they influence the soil structure and hydric properties. The present study was conducted in a 1.1 ha vineyard of this region (Canelones) characterized by a high heterogeneity of plant vigour. It was aimed to determine and map the physical and chemical properties of the soil and their relations with plant vigour and yield. The cation exchange capacity (CEC) and the clay and organic matter contents were measured in 84 locations within this vineyard to calculate the montmorillonite and illite contents of the soil. In addition, the type and abundance of clays was corroborated by X-ray diffractometry analysis. The CEC and montmorillonite contents were positively correlated with vine vigour, expressed by the Normalized Vegetation Index (NDVI), trunk diameter, pruning weight, leaf area, and with yield. Thus, the within vineyard distribution of the ratio montmorillonite/illite conditioned the heterogeneity of vine growth and yield at the field level. The impact of those minerals on water and mineral supply to the plant is discussed.

Keywords: clay type 2:1; CEC; vertic argiudol; soil heterogeneity

## FRENCH

Les caractéristiques physiques et chimiques du sol jouent un rôle clé sur la croissance et le rendement de la vigne. Les sols du sud de l'Uruguay présentent une teneur élevée en montmorillonite ou en illite. Les proportions de ces minéraux méritent une attention particulière car elles influencent la structure du sol et ses propriétés hydriques. La présente étude a été menée dans un vignoble de 1,1 ha de cette région (Canelones) caractérisée par une forte hétérogénéité de la vigueur des plantes. L'objectif était de déterminer et de cartographier les propriétés physiques et chimiques du sol et leurs relations avec la vigueur des plantes et le rendement. La capacité d'échange cationique (CEC) et les teneurs en argile et en matière organique ont été mesurées en 84 endroits de ce vignoble pour calculer les teneurs en montmorillonite et en illite du sol. En outre, le type et l'abondance des argiles ont été corroborés par une analyse par diffractométrie aux rayons X. Les teneurs en CEC et en montmorillonite ont été positivement corrélées avec la vigueur de la vigne, exprimée par l'indice de végétation normalisé (NDVI), le diamètre du tronc, le poids de la taille, la surface foliaire, et avec le rendement. Ainsi, la distribution intra-vignoble du ratio montmorillonite/illite conditionne l'hétérogénéité de la croissance et du rendement de la vigne au niveau du champ. L'impact de ces minéraux sur l'approvisionnement en eau et en minéraux de la plante est discuté.

Mots clés : type d'argile 2:1; CEC; argiudol vertical; hétérogénéité du sol.

### SPANISH

Las características físicas y químicas del suelo desempeñan un papel fundamental en el crecimiento y el rendimiento de la vid. Los suelos del sur de Uruguay presentan un alto contenido de montmorillonita o illita. Las proporciones de estos minerales merecen especial atención ya que influyen en la estructura del suelo y en sus propiedades hídricas. El presente estudio se realizó en un viñedo de 1,1 ha del sur de Uruguay (Canelones) caracterizado por una alta heterogeneidad de vigor de las plantas. El objetivo fue determinar y cartografiar las propiedades físicas y químicas del suelo y su relación con el vigor de las plantas y el rendimiento. La capacidad de intercambio catiónico (CEC) y los contenidos de arcilla y materia orgánica se midieron en 84 lugares de este viñedo para calcular los contenidos de montmorillonita e illita del suelo. Además, se corroboró el tipo y la abundancia de arcillas mediante análisis de difractometría de rayos X. Los contenidos de CEC y montmorillonita se correlacionaron positivamente con el vigor de la vid, expresado por el Índice de Vegetación Normalizado (NDVI), el diámetro del tronco, el peso de la poda, el área foliar y con el rendimiento. Así, la distribución dentro del viñedo de la relación montmorillonita/ilita condicionó la heterogeneidad del crecimiento de la vid y del rendimiento a nivel de campo. Se discute el impacto de estos minerales en el suministro de agua y minerales a la planta.

Palabras clave: arcilla tipo 2:1; CEC; argiudol vertical; heterogeneidad del suelo

### 3.2 INTRODUCTION

Uruguay's viticultural history began with the arrival of the first Spanish and Portuguese immigrants during the colonial period. Currently, production extends over approximately 7,000 ha and generates about 30,000 direct and indirect jobs. Tannat is the emblematic Uruguayan variety due to its good performance in the soil and climatic conditions of the country. The diversity of geological materials have generated very different soil types in Uruguay<sup>(1)</sup>. In South Uruguay, these soils have developed on sediments. They are moderately deep (50 to 100 cm), clayey, and have a B horizon of illuvial character<sup>(2)</sup>. The predominant soils are classified as fine, smectitic, thermic Vertic argiudoll. They have particular properties: high organic matter content and richness in expansive clays of type  $2/1^{(3)}$ . The temperate climate of Uruguay, with around 1100 mm rain per year, allows grape-vine cultivation with high yield objectives.

The soil physical properties (texture, structure, soil depth, colour, and temperature) and chemical properties (pH, electrical conductivity and nutrient availability) are determinant for plant functioning<sup>(4-5)</sup>, determining grape vigour and quality<sup>(5-6)</sup>. Thus, the soil variability can generate an heterogeneity of plant growth and functioning, as observed by vegetation imagery in some vineyards<sup>(7)</sup>. The non-uniformity of yield, berry composition and berry sanitary status can lead to technical, economic, and environmental issues<sup>(8-9-10-11)</sup>. It is then important to identify the soil factor(s) playing key role(s) in vine heterogeneity to propose corrective management levers. The presence of montmorillonite and illite is a typical signature of soils of South Uruguay<sup>(3)</sup>, but the proportion of these two clay types is unknown and seems variable throughout the region. Variation at the field level seems to have escaped any investigation.

The objective of this short communication was to evaluate the relationship between the field heterogeneity of grapevine in terms of plant variables (vigour and yield) and physical and chemical soil characteristics including in particular the content of illite and montmorillonite. For this purpose, a vineyard displaying a strong heterogeneity of plant vigour was used as a case study.

### **3.3 MATERIALS AND METHODS**

## 3.3.1 Study site

The field of the study was a commercial vineyard of 1.1 ha planted in 1998 with Vitis vinifera L. cv. Tannat, grafted on SO4 rootstock. This vineyard is located in Canelones, Uruguay (34° 36 S, 56° 14 W), 56 km away from Montevideo. The vine spacing was 2.5 m x 1.2 m (3333 vines ha-1). The vineyard was on a gradual slope of 1-2% (north /south). Vines were cultivated using a double guyot system and trained to a vertical shoot position. The vineyard was not irrigated. An intercrop occupied the inter-row and was regularly cut (mixture of Gramin-aceae spp. and Asteraceae spp.). The vineyard was characterized by high variability of yield and berry composition. Crop vigour was assessed at veraison by the Normalized Difference Vegetation Index (NDVI), calculated using aerial images, as described in Ferrer et al.<sup>(10)</sup>. The vines were geo-referenced using a GPS (Thales Navigation Inc., San Dimas, CA, USA).

## 3.3.2 Soil determinations and characteristics

The chemical and physical properties of the soil were determined. For this purpose, soil samples were taken within the vineyard following a grid layout (10.8 m x 12.5 m), obtaining 84 sampling points. The depths of sampling were 0-20 cm and 20-40 cm. During winter 2015, organic matter (OM), the percentage of sand (Sa), clay (Cl), and silt (Si) were determined for the two depths, while cation exchange capacity (CEC) was determined for the 0-20 cm samples.

To identify and quantify the different clay fractions (CT), a mixed soil sample was taken at a depth of 20-30 cm for each vigour zone (High Vigour: HV and Low Vigour: LV). The clay fractions were analysed by X-ray diffractometry (XRD), as described by Beaux et al.<sup>(12)</sup> at the laboratory of the Department of Technological Development of CURE (http://www.cure.edu.uy/). The methodology for sample preparation and clay analysis was adapted from Carroll<sup>(13)</sup>.

To determine the montmorillonite and illite contents in the soil, we have admitted that kaolinite was negligible<sup>(3)</sup> and we have considered that soil CEC is the sum of partial CEC (Eq. 9), and that the clay content is the sum of clay type contents (Eq. 10).

CEC of soil = CEC of OM + CEC of illite + CEC of montmorillonite (Eq. 9)

illite content + montmorillonite content = clay content (Eq. 10)

Considering 30 and 90 cmol+.kg<sup>-1</sup> for CEC of illite and montmorillonite (Brady and Weyl, 2008) and 250 cmol<sup>+</sup>.kg<sup>-1</sup> for OM, the equation (Eq. 9) becomes:

30 illite content + 90 montmorillonite content = soil CEC – 250 OM content (Eq.9 bis)

The set of equations (Eq. 9 bis) and (Eq. 10) was solved for every point corresponding to a soil sample. The QGIS (Geographic Information System; 2021) program was used to draw maps of the trunk diameter, CEC, montmorillonite, illite and OM using TIN interpolation.

## 3.3.3 Plant growth and yield components

In winter 2020, trunk diameter (TD) was evaluated, following the same grid scheme used for soil analysis (n=84), by a digital caliper (Neiko 01407  $\pm$  0.2 mm) at 10 cm above the grafting point following the methodology proposed by Santesteban et al.<sup>(16)</sup>. For each plant, two measurements were taken, one transverse and one longitudinal to the row. The average of both measures was used as the average trunk diameter for that plant.

The influence of soil properties on plant response was addressed within two randomized blocks characterized by high vigour (HV) and low vigour (LV) with three replications for each of them. Each replication included twenty-one vines distributed in two rows (63 plants per treatment). The plant parameters were the following: Exposed

leaf area (ELA, m2/vine) assessed at veraison by the meth-od proposed by Carbonneau et al.<sup>(17)</sup>, Yield (Y, kg/vine) measured at maturity and Pruning weight (PW) measured during winter. Pearson's linear correlation between plant parameter and soil parameters, or between soil parameters were calculated. Soil parameters included the illite content, ill-30 [for illite at 30 cmol+ kg-1] and montmorillonite content, mmt-90 [for montmorillonite at 90 cmol+ kg-1] in the 0-20 cm layer, clay (Cl 0-20, Cl 20-40) and organic matter (OM 0-20, OM 20-40) in the two layers and CEC of the 0-20 cm layer.

## **3.4 RESULTS**



FIGURE 3.9. Vigour and soil properties maps.

Vigour by NDVI (A), vigour by trunk diameter (φ in mm, B), CEC map (cmol+ kg-1, C), illite content (%, D), montmorillonite (%, E), organic matter (%, F). Soil maps in a depth of 0 to 20 cm. n=84

Three NDVI ranges were delimited within the vineyard, corresponding to high (NDVI 0.57 to 0.61) / medium (NDVI 0.55 to 0.57) / low (NDVI 0.55 to 0.48) (Fig. 1, A). The vineyard's west part reached NDVI corresponding to the high vigour class (HV), while the NDVI on east part corresponded to the low vigour class (LV). Those zones were corroborated by the range of trunk diameter values (Figure 3.9-B). The variation of the trunk diameter at plot level was 20% (CV) with a mean value of 49 mm (range: 30 to 66 mm).

The mean CEC decreased from HV to LV zones (Figure 3.9-C), ranging from 24-35 cmol+ kg-1 in the HV zone and 14-23 cmol<sup>+</sup> kg<sup>-1</sup> in the LV zone. The spatial variation of CEC was similar to the one of NDVI, with higher CEC in the HV zone and lower CEC in the LV zone. On average the content of illite (Figure 3.9-D) was 18.8% (range 4.6 to 28.8%), while montmorillonite (Figure 3.9-E) was 14.4% (range 3.7 to 26.7%). Organic matter was on average 2.9% at 0-20 cm depth. Some areas presented higher OM content (Figure 3.9-F) than others. These areas did not follow the patterns of NDVI map.

Mineralogical analysis of the clays showed differences in the abundance of 2:1 clays. The HV zone had 79% montmorillonite and 20% illite. In contrast, the LV zone reached 34% of montmorillonite and 60% of illite. Both soil zones had a low content of the 1:1 clay type kaolinite (between 1% for the HV zone and 6% for the LV zone).

The high vigour (HV) zone was characterized by higher yield and vegetative growth compared to the LV zone (Table 3.3). The most significant heterogeneity (CV) was found in low vigour for the four variables studied. ELA and PW were the variables with the greatest dispersion in the plot. As well, for these variables, the CV is particularly higher in low vigour zone compared to high vigour. Trunk diameter was correlated with the following plant variables: NDVI ( $r^2$ : 0.58; p-value: 0.05), ELA ( $r^2$ : 0.60; p-value: 0.05); Y ( $r^2$ : 0.69; p-value: 0.001) and PW ( $r^2$ : 0.63; p-value: 0.05).

Vigour	High	vigour	Low Vigour		
variables	Mean	CV (%)	Mean	CV (%)	
Y	6.96*	17	5.11*	23	
ELA	1.76*	19	1.2*	30	
PW	0.57*	20	0.35*	27.8	
TD <sub>10</sub>	58*	17	36*	21	

**TABLE 3.3**. Plant variables mean values and variability according to the vigour zone.

Y: Yield (kg/vine) ELA: Exposed Leaf Area ( $m^2$ /vine); PW: Pruning weight (kg/vine); TD<sub>10</sub>: Trunk Diameter (mm). The asterisks indicate significant differences according to the Fisher test (p-value < 0.05). n=63 (Y, ELA and PW) n=84 (TD).

Para	ameters	ill-30	mmt-90	Cl 0-20	CI 20-40	OM 0-20	OM 20-40	CEC
Plant	NDVI	-0.34 *	0.49 ***	0.29 *	-0.07	-0.20	0.12	0.52 ***
	Yield	-0.46 **	0.50 ***	0.49 ***	0.38 *	-0.24	-0.15	0.59 ***
	ELA	-0.41 **	0.43 **	0.17	0.39 *	-0.26	-0.01	0.49 ***
	TD <sub>10</sub>	-0.57 ***	0.78 ***	0.25	0.22	-0.44 **	0.06	0.68 ***
	СР	-0.52 ***	0.65 ***	0.30 *	0.41 **	-0.21	-0.05	0.68 ***
Soil	ill-30	1	-0.75 ***	-0.21	-0.52 ***	0.44 **	0.06	-0.66 ***
	mmt-90		1	0.45 **	0.36 *	-0.41 **	0.01	0.91 ***
	Cl <sub>0-20</sub>			1	0.24	-0.15	-0.14	0.65 ***
	Cl 20-40				1	-0.16	-0.07	0.37 *
	OM 0-20					1	-0.05	-0.46 **
	OM 20-40						1	0.10
	CEC							1

TABLE 3.4. Coefficient of Pearson

Plant variables: NDVI: Normalized Difference Vegetation Index; ELA: Exposed Leaf Area; TD: Trunk Diameter; PW: Pruning weight. Soil parameters: ill-30: illite, mmt-90: montmorillonite, Cl<sub>0-20</sub>: Clay at 0-20cm, Cl<sub>20-40</sub>: Clay at 20-40cm, OM<sub>0-20</sub>, OM<sub>20-40</sub>: Organic matter at 0-20cm and 20-40cm; CEC: Cation Exchange Capacity. (\*: p<0.05; \*\*: p<0.01; \*\*\*: p<0.001).

Correlations between plant and soil variables, or between soil variables, were analysed. NDVI correlated negatively with illite content and positively with montmorillonite content. The illite and montmorillonite contents also correlated with the trunk diameter, exposed leaf area, cane production and yield (Table 3.4). The correlations were negative for illite and positive for montmorillonite, with higher level of significance for montmorillonite. All the plant parameters also correlated positively with CEC. However, no correlation of plant parameters with organic matter was observed (except a negative one for trunk diameter).

The soil parameters showed little correlation except for the clay types. The organic matter in 0-20 cm correlated positively with illite but negatively with

montmorillonite. This is concordant with the negative correlation of CEC and organic matter in 0-20 cm because montmorillonite has more weight in soil CEC than organic matter. Illite content (in 0-20 cm) correlated negatively with the clay content in 20-40 cm while the opposite was true for montmorillonite. The montmorillonite content (in 0-20 cm) correlated positively with the clay content in 0-20 cm) correlated positively with the clay content in 0-20 cm. Higher clay levels thus reflected higher montmorillonite levels in the two horizons.

### 3.5 DISCUSSION

Based on the soil chemistry analyses and CEC the soil in the site study is a Vertic Argiudoll<sup>(2)</sup>. Differences in clay content and clay type between the two vigour zones can explain the differences in water retention in each vigour zone and differences in plant growth and yield<sup>(4-5)</sup>. Indeed, soil water retention and nutrient availability (mainly nitrogen) impact the overall plant functioning<sup>(18)</sup>. Montmorillonite is a swelling clay<sup>(19)</sup>. The positive correlation of the soil's montmorillonite content with the vine's vegetative development is plausibly due to the weakening of the clay-humus association as the soil moisture varies<sup>(14)</sup>. The tips of the roots are thus facing aggregates with poor stability ("soft humus"). In places where illite is dominant over montmorillonite, the clay-humus association is in contrast more stable favouring a harder soil structure. Another factor is the high pluviometry in the region. Drought is relatively rare. The montmorillonite keeps its hydrated state so that the bulk density of the soil has lower values in areas where this type of clay predominates over illite.

Higher montmorillonite content or higher montmorillonite/illite ratio in the 0-20 cm horizon of the soil has thus favoured grapevine growth as reflected by the NDVI, leaf exposed area, pruning weight and trunk diameter and ultimately has also favoured high yields.

The negative correlation between organic matter (in 0-20 cm) and montmorillonite could reflect the weaker association of this clay type with humus. As grapevine is a perennial crop, the roots could have experienced an improved growth in

these areas because the root tips have encountered a weaker resistance to penetration. With CEC higher in these zones (higher CEC of montmorillonite), the mineral nutrition should not have been a limiting factor compared to the zones where illite is the dominant type of clay. The negative correlation between illite content in 0-20 cm and clays in 20-40 cm is more difficult to explain. Clay type migrations during soil history could be one of the explanations.

High vigour has been associated with deeper soils<sup>(5-20)</sup>, higher organic matter content<sup>(4)</sup>, higher cation exchange capacity<sup>(5-11)</sup>, % clay<sup>(5-21)</sup>, higher water availability<sup>(15-20)</sup> and plant nitrogen availability<sup>(11-22)</sup>. These associations between soil and plant vigour are confirmed by our results (Table 1, Figure 1). The trunk diameter is an indicator of vine vigour but, unlike the other plant variables ad-dressed in the present study it expresses vigour as a cumulative result over the plant's life<sup>(23)</sup>. The correlation between TD and other vigour variables (PW and Y) makes the trunk diameter an appropriate (and simple) indicator to characterize the heterogeneity of vigour. Also, differences in soil's physical and chemical properties were shown to be linked to spatial variations in trunk circumference as in other studies<sup>(24)</sup>. Similar to our results, Bramley et al.<sup>(20)</sup> observed greater trunk circumference in grapevine with excessive vigour over years. Other plant variables such as pruning weight, vegetative growth and yield were also positively correlated with soil variables such as CEC and montmorillonite. These plant variables are used as vigour indicators over a short period (one year)<sup>(20)</sup>. We recently reported that those annual variables related to vigour also correlated with berry primary metabolism<sup>(25)</sup>.

## **3.6 CONCLUSIONS**

From our knowledge, no study of the within-field variation of CEC, illite, and montmorillonite has been reported. The main reason is plausibly that research has rarely investigated soil CEC at decametric granularity. Corroboration with mineralogical analysis of clays seems to support our assumptions. Although the number of samples for this

analysis was very low (one for each vigour zone), the authors consider that it provides a good approximation of the relationship between plant vigour and clay types.

This study was carried out in a single vineyard and has a series of methodological limitations that prevent generalizing the information obtained. However, the authors consider that it provides new knowledge about the relationship between plant vigour and type of clays. It should be interesting to apply the same analysis to any vineyard growing in soils rich in 2/1 clays. In the future, deeper layers of the soil should be included in order to have a three-dimensional view of clay components variation.

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#### 4. SUPPLEMENTARY DATA

SUPPLEMENT 3.1. Average, maximum, and minimum air temperatures (°C)

Average air temperature: Tmax: maximum temperature; Tmin: minimum temperature; Tx: mean temperature in °C.

VARIABLES	R2	P-VALUE							
NDVI	0.58	0.05							
ELA	0.60	0.05							
Y	0.69	0.001							
TAW	0.49	0.0001							
CLAY	0.18	0.068							
CEC	0.61	0.001							

SUPPLEMENT 3.2. Trunk diameter Pearsons correlations with plant and soil variables

Y: Yield (kg vine<sup>-1</sup>); ELA: Exposed leaf surface (m<sup>2</sup> ha<sup>-1</sup>); TAW: Totally Available Water (mm); Clay (%); CEC: Cation exchange capacity



**SUPPLEMENT 3.3.** Changes in soil temperature evolution according to the depth, phenological stages and vigour condition (2019 and 2020).

A- 0-20 cm 2019; B- 20-40 cm 2019; C- 0-20 cm 2020; D- 20-40 cm 2020. BB: Bud-break; BL: Bloom; V: Veraison; H: Harvest. The vigour zones were defined from the NDVI and trunk diameter values. High vigour: NDVI 0.57-0.61 and trunk diameter: 58.0 mm. Low vigour: NDVI 0.48-0.57 and trunk diameter 36.0 mm. 2019: rainy year; 2020: dry year



Supplement 3.4.: Diffractogram of the clays

Red line: High vigour; blue line: Low vigour. The vigour zones were defined from the NDVI and trunk diameter values. High vigour: NDVI 0.57-0.61 and trunk diameter: 58.0 mm. Low vigour: NDVI 0.48-0.57 and trunk diameter 36.0 mm. Vertical lines indicate the clay types identified. The reported angle is 2 times the angle formed by the entering ray with the horizontal plane of the clay sample, the intensity corresponds to photon counting, it is expressed in count per second (cps)



**SUPPLEMENT 3.5.** Root distribution

A: High Vigour; B: Low Vigour. C: Method for root system analysis (1.0\*1.0 m) and determination of nitrogen and water in the root zone of influence. Area of influence of the vine: 1.0 m depth, 0.5 m both sides of the vine. The vigour zones were defined from the NDVI and trunk diameter values. High vigour: NDVI 0.57-0.61 and trunk diameter: 58.0 mm. Low vigour: NDVI 0.48-0.57 and trunk diameter 36.0 mm. Grid of 5\*5 (0.2\*0.2 m), 25 evaluation quadrats. The points represent the soil samplings performed to create the maps in Figure 6 A and B.

Year/ Variable/ Vigour	<b>Yie</b> (kg vi	eld ne-1)	Bur wei	nch ght	Bunch	n/vine	B ()	₩ g)	El (m² v	<b>_A</b> rine <sup>-1</sup> )	<b>ף'</b> (kg v	₩ ine <sup>-1</sup> )
- HBOUI	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
2014	5.4	3.3	238	243	21	17	1.6	1.5	1.5	1.2	0.7	0.4
2015	8.4*	5.8*	354*	322*	23	21	1.6	1.5	2.0*	1.7*	0.9*	0.5*
2016	7.9*	5.8*	296*	240*	26	24	1.4*	1.1*	2.3*	2.0*	0.6*	0.4*
2017	7.4*	5.4*	284*	209*	25	26	1.6*	1.2*	1.7*	1.3*	0.6*	0.4*
2018	7.0*	5.5*	286*	216*	24	24	1.2*	0.9*	1.2*	0.9*	0.4*	0.2*
2019	5.4*	4.3*	280*	196*	22	23	1.7*	1.5*	1.9*	1.1*	0.7*	0.5*
2020	6.6*	4.7*	248*	161*	26*	30*	1.3*	1.0*	1.5*	1.2*	0.5*	0.3*
2021	7.0*	3.9*	228*	140*	25*	30*	1.4*	1.1*	1.3*	0.8*	0.5*	0.2*
YEAR		k	*			*		*		*	3	*
EFFECT												

SUPPLEMENT 3.6. A	Average values o	f vegetative	and yield	variables fo	r each yea	r and
each vigour zone						

References: Yield (kg vine<sup>-1</sup>); Bunch size (g); Bw: Berry weight (g); ELA: Exposed leaf surface (m<sup>2</sup> vine<sup>-1</sup>); PW: Pruning weight (kg vine<sup>-1</sup>). Significant differences according to the Fisher test. \* < 0.05

Year/ Brix Variable/		Total acidity (g H <sub>2</sub> SO <sub>4</sub> /L)		рН		ApH 1.0 (g L <sup>-1</sup> )		
Vigour	High	Low	High	Low	High	Low	High	Low
2014	19.2*	18.1*	5.2	3.8	3.3	3.5	784*	1161*
2015	23.6	23.8	4.2*	3.6*	3.4	3.5	2021	1743
2016	20.7*	17.6*	4.7	4.1	3.2	3.1	1572*	1917*
2017	22.4*	20.5*	4.5	3.6	3.1	3.2	1876*	1585*
2018	21.5*	19.1*	4.9	4.3	3.3	3.2	1405	1523
2019	22.1*	21.2*	4.3	4.4	3.3	3.4	2098	2110
2020	22.6*	20.2*	4.5	4.5	3.0	2.9	2091*	1692*
2021	17.2*	20.0*	3.9	4.1	3.2	3.1	944*	1694*
YEAR	,	*		*	n	c	,	*
EFFECT						5		

**SUPPLEMENT 3.7.** Average values of berry composition variables for each year and each vigour zone

Reference: Berry composition variables: Brix ( $^{\circ}$ ); Acidity (g H<sub>2</sub>SO<sub>4</sub>/L); ApH1: Total anthocyanins (g L<sup>-1</sup>). Significant differences according to the Fisher test. \* p < 0.05. ns: no significant

# CHAPTER IV

# MANAGEMENT OF PLOT VARIABILITY THROUGH SITE-SPECIFIC MANAGEMENT TECHNIQUES



#### 1. INTRODUCTION PART IV

As mentioned in the previous chapters, the spatial variability of vine vigour is attributable to certain factors of the physical environment, such as soil and topography, in combination with climate factors and management practices. Generally speaking, growers have little information on potential within-plot grape variability since, for example, the quantification of grape composition parameters is not straightforward (Sams et al., 2022). Understanding and knowing the specific causes that generate spatial variability is essential for grape growers to optimize production. The homogenization of productivity improves winery logistics, as it allows better harvest forecast and planning. In addition, the analysis of the temporal variability of plant functioning permits to adjust the inputs throughout the cycle (McBratney et al., 2005). Vegetation index, such as NDVI (Normalized Difference Vegetation Index), as well as other index or tools used in Precision Viticulture (PV), allow to characterize the wiyhin-field variability and to delimit management zones of similar growth and productivity (Sams et al., 2022). In other words, remote sensing, global positioning systems (GPS), geographic information systems (GIS) and other tools can guide growers toward a site-specific management strategy.

The basic concept of site-specific management is the adaptation of inputs, considering that specific requirements of the soil and/or the plant may vary in time and space (Arnó, 2008). The added value of site-specific approach compared to the application of inputs in a uniform manner can be assessed through productive, economic and environmental indicators. The benefits of site-specific management and precision viticulture in terms of production have been the main focus of researchers in the literature. However, environmental issues can also be improved by differential canopy/crop management (Urretavizcaya et al., 2017), phytosanitary treatments, fertilization (Gatti et al., 2022) and irrigation (McClymont et al., 2012, Sanchez et al., 2017, Oldoni et al. 2020). Indeed, the appropriateness of input use has positive consequences, for example, in reducing nitrogen losses due to volatilization or leaching. Regarding economic impacts, it has been reported in Australia that separating grapes

according to their quality would increase economic benefits by 10-30% (Pearse & Hamilton, 2005, Bramley et al., 2011).

This chapter discusses how the differential management of inputs (water and nitrogen) and the change in the leaf:fruit ratio can optimize plant response and production. It is intended to address the productive benefits of site-specific management applied in viticulture.

## 2. EVALUATION OF SITE-SPECIFIC MANAGEMENT TO OPTIMIZE VITIS VINIFERA (TANNAT) PRODUCTION IN

### A VINEYARD WITH HIGH HETEROGENEITY

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#### 2.1 ABSTRACT

#### ENGLISH

Precision viticulture allows to characterize the spatial variability of vineyards and delimit zones with homogeneous management. This study aimed to evaluate the interest of soil and plant site-specific management to increase yields and improve berry quality. During three consecutive seasons, contrasting treatments designed ad hoc for two zones of vigour pre-established by NDVI were tested: high vigour zone (HV) and low vigour zone (LV). The treatments were aimed at reducing water and nitrogen supply and improving microclimatic conditions in the cluster zone in the HV zone. In the LV zone, treatments were aimed at increasing water and nitrogen supply. Leaf removal in the HV zone was the most efficient treatment to improve productivity and quality (sugar and anthocyanins). Moreover, the water restriction improved grape quality, especially in a rainy year. The regulated deficit irrigation strategy applied in the LV zone at specific phenological stages was shown to increase vegetative growth, yield and to improve grape anthocyanins and phenols contents. The benefits of additional nitrogen supply in the LV zone on plant nitrogen status, yield, and berry composition were highly dependent on water availability. Ultimately, this study provided new insights into the relationship between water and nitrogen availability and how this determines vigour and influences yield and grape quality. The deviation from a "Productive Target" pattern resulting from the different soil and plant management was ultimately determined. The use of site-specific techniques could be adjusted on a small production scale, thanks to mapping carried out with precision viticulture technologies.

**Key words:** precision viticulture, sustainable production, vigour, irrigation, fertilisation, leaf removal

#### FRENCH

La viticulture de précision permet de caractériser la variabilité spatiale des vignobles et de délimiter des zones de gestion homogènes. Cette étude visait à évaluer l'intérêt d'une gestion spécifique localisée du sol et de la plante pour augmenter les rendements et améliorer la qualité des baies. Pendant trois saisons consécutives, des traitements contrastés conçus ad hoc pour deux zones de vigueur pré délimitées à partir du NDVI ont été testés : zone de forte vigueur (HV) et zone de basse vigueur (LV). Les traitements visaient à réduire l'apport d'eau et d'azote et à améliorer les conditions microclimatiques dans la zone des grappes dans la zone HV. Dans la zone LV, les traitements visaient à augmenter l'apport en eau et/ou en azote. L'effeuillage dans la zone HV a été le traitement le plus efficace pour améliorer la productivité et la qualité (sucre et anthocyanes). De plus, la restriction en eau a amélioré la qualité du raisin, surtout en année pluvieuse. La stratégie d'irrigation déficitaire appliquée dans la zone LV à des stades phénologiques spécifiques a permis d'augmenter la croissance végétative, le rendement et d'améliorer les teneurs en anthocyanes et en phénols des raisins. Les avantages d'un apport supplémentaire d'azote dans la zone LV sur le statut azoté des plantes, le rendement et la composition des baies dépendaient fortement de la disponibilité en eau. Au final, cette étude a permis de mieux comprendre la relation entre la disponibilité en eau et en azote, son impact sur la vigueur et *in fine* sur le rendement et la qualité du raisin. L'écart par rapport à un modèle de "production ciblée" résultant des différents pratiques culturales sol/plante a été déterminé. Des techniques spécifiques localisées pourraient être mises en place à des échelles de production réduites, grâce aux cartographies issues des technologies de viticulture de précision.

**Mots clés**: viticulture de précision, production durable, vigueur, irrigation, fertilisation, effeuillage.

#### SPANISH

La viticultura de precisión permite caracterizar la variabilidad espacial de los viñedos y delimitar zonas con una gestión homogénea. Este estudio tenía como objetivo evaluar la pertinencia de un manejo sitio-específico del suelo y de la planta para aumentar los rendimientos y mejorar la calidad de la uva. Durante tres temporadas consecutivas, se ensayaron tratamientos contrastados diseñados ad hoc para dos zonas de vigor que fueron preestablecidas por NDVI: zona de vigor alto (HV) y zona de vigor bajo (LV). En la zona HV, los tratamientos tenían como objetivo reducir el aporte de agua y nitrógeno y mejorar las condiciones microclimáticas de la zona de racimos. En la zona de bajo vigor en cambio, los tratamientos se dirigieron a aumentar el suministro de agua y nitrógeno. La eliminación de hojas en la zona HV fue el tratamiento más eficaz para mejorar la productividad y la calidad (azúcar y antocianos). Además, la restricción hídrica mejoró la calidad de la uva, especialmente en un año lluvioso. La estrategia de riego deficitario regulado aplicada en la zona LV en etapas fenológicas específicas demostró poder aumentar el crecimiento vegetativo, el rendimiento y mejorar los contenidos de antocianos y fenoles de la uva. Los beneficios del aporte adicional de nitrógeno en la zona LV sobre el estado nitrogenado de la planta, el rendimiento y la composición de las bayas dependieron en gran medida de la disponibilidad de agua. En definitiva, este estudio aportó nuevos conocimientos sobre la relación entre la disponibilidad de agua y nitrógeno y cómo ésta determina el vigor e influye en el rendimiento y la calidad de la uva. Por último, se determinó la desviación de un patrón de "objetivo productivo" resultante de la diferente gestión del suelo y de la planta. El uso de técnicas específicas para cada lugar pudo ajustarse a pequeña escala de producción, gracias a la cartografía realizada con tecnologías de viticultura de precisión.

**Palabras claves**: viticultura de precisión, producción sostenible, vigor, riego, fertilización, deshojado

#### 2.2 INTRODUCTION

Conventional crop management generally relies on a set of operational decisions which are implemented at the plot scale. Indeed, the soil and canopy management, together with the different inputs (fertiliser, irrigation, phytosanitary products), are applied in a similar way across the plot without considering the within-plot heterogeneity (Arnó, 2008). However, high variability in grape yield and composition within a vineyard (Bramley et al., 2011; Filippetti et al., 2013; Ferrer et al., 2020(a)) may be triggered by non-homogeneous physical and chemical characteristics of the soil, topography, and climate (Jasse et al., 2021). Inadequate soil and plant management at the plot level is ultimately likely to generate both economic loss and environmental issues (Arnó, 2008; Filipetti et al., 2014).

Precision viticulture (PV) combines technologies and methodologies based on data collection and data analysis to optimize production and economic and environmental aspects (Tisseyre et al., 2008; Bramley et al., 2011; Santesteban, 2019). The characterization of the spatial variation within a plot allows the winegrower to apply various management strategies. Within these strategies, homogeneous management zones can be delimited and site-specific management to each zone can be applied. Another management strategy is using VRD (Variable Rate Dosing) technologies that allow achieving a dosage of inputs according to the information generated instantaneously or previously (Tisseyre et al., 2008). The plot spatial variation may concern soil/plant water status (Santesteban, 2019) and soil characteristics (Arnó et al., 2012; Bramley et al., 2011). Topography (Bahat et al., 2021), soil electrical properties (Tardáguila et al., 2011), yield and the Normalized Difference Vegetation Index (NDVI) can ultimately be used to establish management zones. The NDVI is calculated from the red (R) and infrared (IR) wavelengths as follows: NDVI=[IR-R]/[IR+R] (Rouse et al., 1973). Vigour differences (assessed by NDVI) are generally associated with contrasted vegetative growth, yield and grape composition (Filippetti et al., 2013; Ferrer et al., 2020(a); Sams et al., 2022). High vigour is assumed to favor high yield and vegetative growth and the development of fungal diseases that can alter grape composition

(Bramley et al., 2011; Filippetti et al., 2013; Ferrer et al., 2020(a); Gatti et al., 2020). In contrast, low plant vigour often induces low yield, poor vegetative growth and excessive cluster exposure to direct radiation and sunburn issues (McClymont et al., 2012; Ferrer et al., 2020(a)).

When the spatial heterogeneity in plant growth and productivity are due to the variation of physical factors presenting stability over time (Taylor et al., 2010, Arnó et al., 2012; Matese and Di Gennaro, 2015), it is possible to differentiate the management practices (site-specific management) within the same plot year after year. Notably, because water and nitrogen are two of the factors that determine vigour expression, contrasted water and nitrogen supplies may be imposed, depending on soil characteristics, to homogenize plant vigour at the plot scale (Taylor et al., 2010; Martinez-Casasnovas et al., 2012; King et al., 2014). Canopy management, involving, for example, leaf removal to improve the canopy and bunch microclimate, can also be implemented for a site-specific management plan (Pedò et al., 2010; Arrillaga et al., 2021). A selective harvest within each zone to reduce spatial variation in yield and grape composition (Scarlett et al., 2014) and reach different qualities could be another relevant strategy. Lastly, prior to planting the vineyard, soil electroconductivity mapping will guide the winegrower to better reason the vine rows' orientation, the selection of the variety and rootstock and choice of the training system to reduce the heterogeneity later on. Ultimately, PV can improve the environmental and economic sustainability of the vineyards by minimizing environmental impacts and maximizing the oenological potential of the grape (Arnó, 2008).

In Uruguay, the year-to-year fluctuations of rainfall, added to the heterogeneity of soils, can generate local situations of water and nitrogen deficit with consequences on plant physiology and productivity. In this context, PV appears a relevant option to tackle the combined effects of climate (meso and micro) variability and soil heterogeneity at the plot scale (Matese and Di Gennaro, 2015). The present study was aimed at evaluating the impact of site-specific soil and plant management on grapevine vigour, yield and berry composition. Thus, different water or nitrogen supply and leaf removal in the bunch zone were tested over three consecutive seasons within pre-defined zones of high and low vigour. The objective was to develop specific management practices to increase yield and quality and reduce spatial heterogeneity.

#### 2.3 MATERIALS AND METHODS

#### 2.3.1 Experimental site

Vineyard: The experiment was conducted in a commercial vineyard in Canelones, Uruguay (34°36'S,56°14W), over three consecutive seasons (2019–2020–2021). The vineyard of 1.1 ha was planted in 1998 with Vitis vinifera L. cv. Tannat, grafted on SO4 rootstock. The vine spacing was 2.5 m  $\times$  1.2 m (3333 vines ha<sup>-1</sup>). Vines were pruned using a double guyot system, and the shoots were trained to a VSP (vertical shoot positioning) system. The vineyard was not irrigated and received standard fertilization with urea, distributed half pre-flowering and half post-harvest at a total dose of 140 kg of urea (46 % N) fertiliser per ha. This vineyard was characterized by high variability of vine vigour from east to west, and Ferrer et al. (2020(a)) defined three vigour zones, high (HV), medium (MV) and low vigour (LV) (Supplement 4.1). The determination of vigour zones was made by aircraft flight (620 m altitude and speed of 50 m/s) at veraison (January in the southern hemisphere) for three years. High-resolution multispectral images were obtained at ground level (0.2 m). The classes of NDVI (high, medium and low) were consistently located in the same parts of the vineyard each year (Ferrer et al., 2020(a)). Soil physical and chemical characteristics also showed a strong spatial variability, mainly regarding the percentage of clay, clay type and total available water (TAW). The TAW estimated from soil texture and root depth was greater than 180 mm in the HV with a predominance of montmorillonite (expansive clay) and less than 140 mm in the LV with higher content of illite compared to HV. Ultimately, although the vineyard was relatively small, making the application of PV unbeneficial, the high spatial gradient of vigour and its stability over the years made this vineyard of high value to test different management practices according to the vigour.

Treatments: Treatments were carried out depending on the vigour zone to improve the yield and berry quality and reduce their heterogeneity. For this purpose, only the most contrasting vigour areas of the vineyard (HV vs LV) were selected. In the pre-established zones, as described above, HV (NDVI 0.57 to 0.61) and LV (NDVI 0.48 to 0.55), treatments were arranged in a random block design with three replications and 21 vines per replicate. For HV, the treatments are aimed at reducing water and nitrogen supply and improving the microclimatic conditions in the bunch zone. The water restriction (H-W) was implemented from veraison to harvest by covering the soil with polyethylene (white on both sides, 220 micrometers thick, with ultraviolet protection). No nitrogen was applied (0 N unit in the season) in the nitrogen restriction treatment (H-N). During two seasons (2019 and 2020), the winegrower stopped fertilizing with urea on one subplot (rows 5 to 15, where this treatment was randomly installed). Leaf removal (H-L) was applied at the pre-flowering stage by removing 60 % of the leaves. In contrast, for LV, the treatments are aimed at increasing water and nitrogen supply. Additional irrigation was supplied (L+W) compared to the control (LV) to reach 100 % of the climatic demand (ETo) from budburst to flowering and from harvest to leaf fall, and 70 % of ETo from flowering to harvest. For the supplemental nitrogen treatment (L+N), 210 kg urea per ha were supplied (70 kg urea per ha in the form of urea were added prior to flowering to the 140 kg mentioned above). In addition, a treatment combining both water and nitrogen supplements (L+WN) was carried out. These treatments were compared with their respective controls for each vigour zone (HV and LV). All treatments are presented in Table 4.1 and Figure 4.1.

						S	Season	s
Vigour	Treatment	Rainfall allowed	Nitrogen Supplied	Leaves removed	Irrigation supplied	2019	2020	2021
	Control (HV)	1	1	×	×	✓	✓	✓
/igour	Water restriction (H-W)	<b>x</b> 1	~	×	×	~	~	×
High V	Nitrogen restriction (H-N)	~	×	×	×	~	~	×
	Leaf removal (H-L)	1	1	✓	×	✓	✓	✓
	Control (LV)	<ul> <li>✓</li> </ul>	✓	×	×	1	~	✓
onr	Irrigation (L+W)	✓	✓	×	✓	×	~	~
ow vigo	Nitrogen supply (L+N)	~	<b>√</b> √	×	×	~	~	~
	Irrigation+Nitrogen supply (L+WN)	~	<b>√</b> √	×	~	×	×	~

**Table 4.1.** Description of the water, nitrogen and leaf removal treatments in thehigh (HV) and low (LV) pre-delimited vigour zones and years evaluated

<sup>1</sup>No rain from veraison to harvest.



FIGURE 4.1. Plot location of water, nitrogen and leaf removal treatments in the high

(HV) and low (LV) zones.

Pre-defined vigour zones and replications (adapted from Ferrer *et al.,* 2020a) (see Table 4.1 for the treatment description).

#### 2.3.2 Weather measurements

<u>Weather characterization</u>: Meteorological data were collected from a weather station owned by INIA (National Institute of Agricultural Research; 34°40'S, 56°20'W; 10 km from

the experimental site) and managed according to the World Meteorological Organization (WMO) standards, to which added a pluviometer. The following climatic variables were monitored: mean air temperature (Tm, °C), reference Penman–Monteith evapotranspiration (ETo, mm), photosynthetically active radiation (PAR, mmol m<sup>-2</sup> s<sup>-1</sup>), relative air humidity (RH, %) and precipitation (mm).

<u>Microclimate characterization</u>: For the microclimatic data, three HOBO<sup>®</sup> sensors (HOBO<sup>®</sup> U23 ProV2 and HOBO Pendant<sup>®</sup> loggers, USA) were distributed inside the canopy in the bunch zone for each treatment from flowering to harvest (2019 to 2021). The results for the most contrasting years in terms of weather are presented in the 3.1 section (2019 vs 2020). The mean canopy temperature (Tmc) and relative humidity (RHc) were recorded using HOBO<sup>®</sup> U23 ProV2 loggers, while the photosynthetically active radiation in the canopy (PARc) was measured from HOBO Pendant<sup>®</sup> loggers. All variables were recorded at an hourly time step.

#### 2.3.3 Soil and plant measurements

<u>Soil N status</u>: Soil samples were taken at three depths (0–20; 20–40; 40–60 cm) for each replicate in the HV and LV in winter (August) before budburst (2019 to 2021). Nitrate ( $NO_3^{-}$ ), ammonium ( $NH_4^{+}$ ), and organic matter (O.M.) were evaluated each year. The N stock was estimated from the soil analyses combined with a potential mineralization term (Salvo et al., 2014). Nitrogen leaching was not taken into account.

<u>Leaf N status</u>: Leaf nitrogen content was evaluated on 20 whole healthy and exposed leaves (limbs) at veraison. The samples were dried (60 °C, 48 hours) and analyzed in the soil and plant laboratory of the Facultad de Agronomía (Uruguay). Leaf nitrogen was assayed by Kjeldahl's method.

<u>Leaf water status</u>: From flowering to harvest, vine water status at pre-dawn was determined using a pressure chamber (SoilMoisture equipment, Santa Barbara, CA, USA). Nine healthy expanded leaves were taken from each treatment (three leaves per

replicates). Leaves were covered with a plastic bag before cutting the petiole and the measurement was carried out immediately after detaching the leaf from the plant.

<u>Vegetative growth:</u> Vegetative growth was evaluated at veraison. Ten representative shoots (average shoots in terms of length and diameter) were collected for each treatment, and the numbers of primary and secondary leaves were counted. In addition, the number of shoots per plant was counted. The leaf area of each leaf was estimated using the Easy Leaf Area<sup>®</sup> mobile application (Easlon & Bloom, 2014). The plants evaluated had the same number of buds (12 buds). The % lignification was measured in the same ten representative shoots (described above) at harvest. The total length of the shoot and the length of the lignified borer were measured. Pruning weight (PW, g vine<sup>-1</sup>) was measured during winter on the same 63 plants.

<u>Yield:</u> The harvest date was fixed according to the evolution of pH (3.3 to 3.4) and berry weight to prevent dehydration of the berry (avoid weight loss); details of dates are presented in the Supplementary information (Table S 4.2). Yield (Y, kg vine<sup>-1</sup>), the number of bunches (B/vine) and the individual bunch weight (WB) at harvest were determined on 63 plants for each treatment (21 plants for each replicate). As the main bunch disease detected was Botrytis bunch rot (*Botrytis cinerea* Pers.), the percentage of bunch rot (BR, %) was evaluated at harvest. Lastly, the individual berry weight was determined at harvest on samples of 100 berries for each replication.

<u>Grape composition</u>: At harvest, two samples of 100 berries were collected from the central zone of the bunch (Deloire et al., 2019) for each treatment. One sample was crushed using a juicer, Phillips HR2290 (Phillips, Netherlands). The following berry composition variables were determined: total soluble solids (TSS) by refractometer (Atago, Japan); pH by pH-meter (Hanna Instruments, Italy); and acidity by titration (gH2SO4/L), following the protocols established by the OIV (2014). The available yeast nitrogen (YAN, mg/l) content in the musts was determined using formaldehyde quantification (Aerny, 1996). A second sample was used to determine the content of total anthocyanins (A, mg/l) and the total phenol index (TPI) according to the methodology

proposed by Glories and Agustin (1993) as modified by González-Neves et al. (2004). The measurements were performed in duplicate with a spectrophotometer (Shimadzu UV-1240 Mini, Japan) using glass cells for anthocyanin analysis (absorbance at 520 nm) and quartz cells for phenols (absorbance at 280 nm) with a 1 cm optical path length.

#### 2.3.5 Data analyses

<u>Effect of the treatments</u>: Statistical analyses were conducted with the statistical package InfoStat version 2011. Analyses of variance, followed by the Tukey test for means comparison, were conducted to determine the effect of the different treatments on plant and berry variables for each zone of vigour (HV, LV) over the vintages 2019 to 2021.

Derivation from an optimal "Productive Target" pattern: The spatial variations in the HV and LV zones from a "Productive Target" pattern for a sustainable and cost-effective production over vintages (2019 to 2021) of a few selected important productive and economic indicator variables (PW, Y, BR, TSS, YAN, A, and TPI) were analyzed. The definition of this "Productive Target" pattern was based on reported values considered as favorable for reaching a balanced vine (Ravaz Index, 5-7 defined by Ferrer et al. (1997) for Tannat) and high-quality wines for Tannat (González-Neves et al., 2012; Ferrer et al., 2018; Ferrer et al., 2020(b)). The variables considered were categorized into four classes or scores, where score 3 corresponded to the optimal "Productive Target", which includes the following values considered: PW = 650 g, Y = 5 kg/vine, BR = 5 %, TSS = 238 g/l, YAN = 150 mg/l; A = 2540 mg/l and TPI = 80. The level of BR used was proposed by Ky et al. (2012), which established an acceptable tolerance range of 5 % of botrytis berries to avoid severe oenological consequences in the wines.

For each treatment, values in the same score or higher than the Productive Target were considered beneficial for that variable. Conversely, scores below 3 (scores 1 and 2) were assumed to be not beneficial for those selected variables. The values considered for each score and variable were:

PW (g vine<sup>-1</sup>) = Score 1: < 400; Score 2: 400–600; Score 3: 600–800; Score 4: > 800 Y (kg vine<sup>-1</sup>) = Score 1: < 3.5; Score 2: 3.5–5.0; Score 3: 5.0–6.5; Score 4: > 6.5 BR (%) = Score 1: >10; Score 2: 10–5; Score 3: 5–0; Score 4: 0 TSS (g/l) = Score 1: <192; Score 2: 190–215; Score 3: 215–238; Score 4: >238. YAN (mg/l) = Score 1: <100; Score 2: 100–140; Score 3: 140–180; Score 4: >180. A (mg/l) = Score 1: < 1800; Score 2: 1800–2200; Score 3: 2200–2600; Score 4: > 2600. TPI= Score 1: < 25; Score 2: 25–50; Score 3: 50–80; Score 4: >80.

The difference in score for each variable from the optimal "Productive Target" pattern was represented using a spider graph for each treatment (R, I, N, IN, L) and vigour (HV, LV) zone and an average of the three seasons (2019, 2020 and 2021) (Figure 4.5).

#### 2.4 RESULTS

#### 2.4.1 Weather and microclimatic conditions

The monthly water demand (ETo) and supply (rainfall) for the study area are presented in Supplement 4.3. The crop seasons markedly differed in terms of water availability. Notably, 2019 was a rainy year with 885 mm over the season, while 2020 and 2021 were the drier years, with accumulated rainfall over the cropping season of 484 mm and 539 mm, respectively. In addition, the distribution of rainfall within each season varied. In 2019 and 2021, up to 57 % of the rainfall occurred during the ripening period. In contrast, in 2020, 60 % of rainfall occurred from bud break to flowering, while the period from flowering to harvest was drier. Years also differed in terms of reference evapotranspiration (ETo). The accumulated ETo over the season reached 806 mm in 2019, 853 mm in 2021 and up to 903 mm in 2020. Ultimately, the climatic water balance (accumulated rain – accumulated ETo) over the cropping season was positive in 2019 (79 mm) and negative in 2020 (–419 mm) and 2021 (–300 mm).

The effects of the water, nitrogen and leaf removal treatments on the canopy microclimate were evaluated by averaging the daily evolution (from flowering to harvest)

of temperature, relative humidity, and light in the bunch zone for the wet and dry years, respectively 2019 and 2020 (Figure 4.2).

The daily dynamics of mean air temperature (Tm) increased from sunrise to a maximum value at 4 pm for both years (Figure 4.2, 1). The maximum temperature was 25 °C in 2019 (Figure 4.2, 1A), while in 2020, it was 27 °C (Figure 4.2, 1C). For each vigour zone, the mean canopy temperature (Tmc) differed between the treatments in 2019 and 2020 (Figure 4.2, 1). In the HV (Figure 4.2, 1AC), the H-W treatment had the highest average Tmc, with temperatures reaching 30 °C from 11 am to 4 pm. The H-L treatment is the one that presented the lowest average Tmc, similar to the air temperature. The other treatments (HV and H-N) showed intermediate Tmc. In LV (Figure 4.2, 1BD), Tmc was higher than the average air temperature during the two years. However, the L+W treatment had lower Tmc compared to LV and L+N, and it was closer to the air temperature.

The daily dynamics of relative humidity (RH) presented a maximum value at 6 am and a minimum value at 4 pm (Figure 4.2, 3). In 2020, the minimum RH value was 47 %, while in 2019, this minimum RH value was 55 %. High differences between the treatments were observed for the HV zone in 2019 only. The HV and H-N treatments reached the highest RHc (22 % at 4 pm), while H-W and H-L treatments presented similar RHc dynamics compared to the air RH. In the LV (Figure 4.2, 3BD), the LV and L+N treatments showed similar RHc dynamics compared to the air RH for both seasons. In 2020, the L+W treatment resulted in higher RHc (55 %) than LV and LV+N (45 %) at midday.



FIGURE 4.2. Average daily dynamics of weather and microclimatic variables in the bunch

zone.

Evaluation from bloom (November) to harvest (March) for the different treatments (water, nitrogen, leaf removal) applied in the two vigour zones, high vigour (left figures) and low vigour (right figures); and for two years, 2019 (A and B) and 2020 (C and D). The weather (air) and microclimatic (canopy) variables are the average temperature (1); the photosynthetically active radiation (PAR) (2) and relative humidity (%) (3). The vertical lines in graphs correspond to the average interval of confidence.

#### 2.4.2 Soil and plant nitrogen and water status

At the beginning of the trial (2018), N stocks were higher for the HV than the LV (137 and 104 kg ha<sup>-1</sup>, respectively) (Figure 4.3, A). Within each zone, treatments did not differ from each other (p-value  $\leq$  0.05). Nitrogen restriction in the HV lowered N stock for both the second and third seasons (reduction of 30 %). Likewise, the increased N in the LV (Figure 4.3, B) permitted to increase in soil content (32 % higher), reaching similar values to those observed at the HV. The leaf nitrogen content (%Nleaf) at veraison during 2019 did not differ between the treatments within the HV (Figure 3C). In contrast, %Nleaf was 15 % lower for nitrogen restriction in HV compared to the other treatments in 2020. The L+N treatment had a higher %Nleaf (+37 %) than the LV control in 2019 (Figure 4.3, D). In 2020, the L+W treatment was permitted to increase by 60 % the %Nleaf compared to the LV control, while the LV and L+N treatments did not differ from each other (Figure 4.3, D).

The seasonal dynamics of plant water status showed differences between years (Figure 4.4). In 2019, the predawn water potential ( $\Psi$ p) was higher (> -0.46 MPa) compared to 2020 (> -0.85 MPa). The values of  $\Psi$ p from flowering to veraison for HV in 2019 were high (> -0.25 MPa), regardless of the treatments, due to high rain. However, from veraison onwards, H-W differed, from the other treatments, reaching the most negative values (-0.4 MPa at harvest). In 2020,  $\Psi$ p of HV progressively decreased after flowering for all treatments to reach -0.8 MPa at harvest for all treatments. The LV displayed higher levels of water deficit compared to HV. In 2019, the dynamics of  $\Psi$ p were similar for LV and L+N treatments, and those treatments reached similar levels of water deficit, such as H-W (-0.41 MPa at harvest). In 2020, while L+W and L+N treatments reached minimum  $\Psi$ p at harvest of -0.85 MPa, the irrigation treatment (L+W) permitted to maintain the values of  $\Psi$ p above -0.35 MPa through the whole seasons.





A and B: Soil Stock N at 0-40 cm (kg ha<sup>-1</sup>) before budbreak according to the treatments (water, nitrogen, leaf removal) applied for the three cropping seasons (2018-2020). C and D: Percentage of nitrogen in leaves (%Nleaf) at veraison according to the treatments (water, nitrogen, leaf removal) for two cropping seasons. A and C: High vigour; B and D: Low vigour. In both graphs (1, 2), the bars represent the mean values (3 soil or leaf samples per treatment) and the error bars represent standard deviation. Different letters indicate significant differences according to the Tukey test (p-value < 0.05), within each vigour zone and year evaluated.



**FIGURE 4.4.** Changes of the predawn water potential ( $\Psi$ p) according to the treatments (water, nitrogen, leaf removal) applied in the two vigour zones (high vigour, low vigour)

#### for two cropping seasons (2019-2020)

High vigour zone treatments in 2019 (A) and 2020 (C); Low vigour zone treatments in 2019 (B) and 2020 (D). In all graphs, the dots represent the mean values (10 leaf samples per treatment) and the error bars represent standard deviation. \*Asterisks indicate significant differences (p-value< 0.05).

#### 2.4.3 Vegetative growth

Differences in total leaf area (TLA) at veraison were observed between vigour zones, treatments, and years (Table 4.2). The total leaf area was greater for the three years on HV than on LV. The differences between HV and LV were the most pronounced for the years with higher water supply (2019 and 2021). In the HV, the H-L treatment reduced TLA by about 25 %. This decrease in leaf area was mainly due to the lower main leaf area (MLA). It should be noted that this H-L treatment also changed the canopy architecture as leaf removal was specifically performed in the bunch zone. No difference in TLA was observed for other treatments. For LV, the L+W treatment increased TLA in 2020 and 2021 through an increase in both the primary and secondary leaf area (MLA, SLA). The L+N treatment only increased leaf area in 2019 and was similar to other treatments of LV

for the other years. The L+WN treatment did not differ from the L+W treatment for vegetative growth variables.

**Table 4.2.** Average values of vine total leaf area at veraison, cane production in winter and % of lignification at harvest according to treatments (water, nitrogen, leaf removal) applied in the two vigour zones (high vigour, low vigour) for the three cropping seasons.

Treatments	TLA (m <sup>2</sup> vine <sup>-1</sup> )	MLA (m <sup>2</sup> vine <sup>-1</sup> )	SLA (m <sup>2</sup> vine <sup>-1</sup> )	Lignification (%)	PW (g vine <sup>-1</sup> )
		2	2019		
HV	9.3 a	4.7 a	4.5 a	62 b	635
H-W	8.3 a	4.3 ab	4.0 a	58 b	624
H-N	8.2 a	4.8 a	3.4 ab	62 b	501
H-L	6.9 b	3.8 b	3.1 b	87 a	572
LV	3.1 b	1.9 b	1.2 b	49	395
L+W	n.d.	n.d.	n.d.	n.d.	n.d.
L+N	5.2 a	3.4 a	1.9 a	65	415
L+WN	n.d.	n.d.	n.d.	n.d.	n.d.
		2	020		
HV	3.8 a	3.1 a	0.7	60 b	546
H-W	3.5 a	3.0 a	0.5	49 b	505
H-N	3.1 a	2.6 a	0.5	51 b	477
H-L	2.9 b	2.4 b	0.5	85 a	465
LV	1.7 b	1.5 b	0.2 b	53 b	341 b
L+W	5.3 a	4.6 a	0.7 a	74 a	505 a
L+N	1.7 b	1.5 b	0.2 b	68 b	299 b
L+WN	n.d.	n.d.	n.d.	n.d.	n.d.
	1	2	021	1	
HV	7.3 a	5.9 a	1.3	63 b	463
H-W	n.d.	n.d.	n.d.	n.d.	n.d.
H-N	n.d.	n.d.	n.d.	n.d.	n.d.
H-L	5.8 b	4.4 b	1.4	82 a	459
LV	3.5 b	2.7 b	0.8	57 b	172 b
L+W	7.9 a	6.4 a	1.5	66 ab	392 a
L+N	3.9 b	3.0 b	0.9	57 b	213 b
L+WN	7.2 a	5.9 a	1.3	76 a	351 a

TLA: Total leaf area. MLA: Main leaf area. SLA: Secondary leaf area. PW: Pruning Weight. Different letters correspond to significant differences according to the Tukey test (p-value ≤0.05). n.d.: No data available

The % lignification at harvest also showed differences between the vigour zones and treatments. For HV, the H-L treatment presented higher lignification values (> 80 %) compared to the rest of the treatments (<63 %) for the three years evaluated. For LV, the irrigation treatments, L+W and L+WN, permitted to increase in the % of lignification compared to the control (LV).

The pruning weight (PW) varied between the years and between vigor zones. The highest values were recorded in 2019, followed by 2020, and finally in 2021. HV had higher PW values (463 to 635 g vine-1) compared to LV (172 to 395 g vine-1). The treatments modified PW in 2020 and 2021 of LV only, with higher values for L+W (87%) and L+WN treatments compared to LV and L+N.

#### 2.4.4 Yield components

Yield clearly varied between the years and the vigour zones. The yield per vine was higher in 2019 and 2020 compared with 2021 and higher for HV compared to LV except when irrigation was supplied in LV.

In HV, H-L had the lowest yield in spite of higher berry weight for the 3 years. The H-N treatment was as high as HV and H-W in 2019 but as low as H-L in 2020 due to the reduction in berry weight. The incidence of bunch diseases reached up to 8 % for H-N and up to 17 % for HV in 2019, while it was lower than 2.5 % for all other treatments and years in this HV zone.

For LV, the L+N treatment significantly increased yield (16 %) compared to LV in 2019 only. For other years, the L+N treatment did not differ from the LV treatment for any yield variables. In contrast, the two irrigation treatments with or without additional N supply (L+W and L+WN) permitted to increase in the yield per vine (+80 %) compared with the control (LV) through the higher bunch and individual berry weights. Disease incidence was low (<2.2 %) for all treatments of LV, regardless of the year.

**Table 4.3.** Average values of yield components according to the treatments (water,nitrogen, leaf removal) applied in the two vigour zones (high vigour, low vigour) for the

Treatments	Yield (kg vine⁻¹)	Number of bunches	Bunch weight (g)	Berry weight (g)	Part of the cluster affected by bunch rot (%)
			2019		
HV	6.9 a	20.3	344 a	1.72 b	17 a
H-W	6.6 a	21.3	320 a	1.70 b	0.0 c
H-N	6.8 a	22.0	290 ab	1.71 b	7.9 ab
H-L	5.1 b	20.2	259 b	1.85 a	0.9 b
LV	5.3 b	25.7	214 b	1.57	2.2
L+W	n.d.	n.d.	n.d.	n.d.	n.d.
L+N	6.2 a	27.2	227 a	1.60	0.8
L+WN	n.d.	n.d.	n.d.	n.d.	n.d.
			2020		
HV	6.7 a	26.4	251 a	1.34 b	0
H-W	6.5 a	29.3	227 ab	1.33 b	0
H-N	5.6 b	25.5	217 bc	1.18 c	0
H-L	5.2 b	27.2	191 c	1.44 a	0
LV	4.7 b	30.2	161 b	1.02 b	0
L+W	8.3 a	30.1	283 a	1.53 a	0
L+N	4.2 b	31.1	145 b	0.95 b	0
L+WN	n.d.	n.d.	n.d.	n.d.	n.d.
			2021		
HV	5.8 a	25.1	228 a	1.42 b	2.5 a
H-W	n.d.	n.d.	n.d.	n.d.	n.d.
H-N	n.d.	n.d.	n.d.	n.d.	n.d.
H-L	4.6 b	27.1	198 b	1.65 a	0.1 b
LV	4.1 b	30.1	140 b	1.14 b	0.1 b
L+W	7.9 a	29.8	266 a	1.50 a	0.52 a
L+N	4.0 b	29.1	139 b	1.08 b	0.1 b
L+WN	7.9 a	31.3	251 a	1.56 a	0.50 ab

three cropping seasons.

Different letters correspond to significant differences according to the Tukey test (p value ≤0.05). n.d. No data available

#### 2.4.5. Berry composition

Differences between years and between the treatments for each vigour zone were observed for both primary and secondary metabolite concentrations in the berries (Table 4.4).

Table 4.4. Average values of berry composition according to the treatments (water,nitrogen, leaf removal) applied in the two vigour zones (high vigour, low vigour) for thethree cropping seasons.

Treatments	TSS (g/l)	Total acidity (g l-1 sulfuric)	рН	YAN (mg L <sup>-1</sup> )	A (mg/l)	TPI
		20	)19			
HV	213 c	4.4	3.34	147 a	2098 b	46 b
H-W	230 a	4.2	3.32	150 a	2511 a	52 a
H-N	218 bc	4.5	3.31	135 b	2054 b	49 b
H-L	222 b	4.4	3.31	145 a	2646 a	52 a
LV	200	4.2	3.37	109 b	2110	48
L+W	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
L+N	207	4.3	3.33	128 a	2350	44
L+WN	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		20	)20			
HV	226 a	4.5	3.00 a	78 a	2091 b	67 b
H-W	209 b	4.4	2.78 c	75 a	1905 c	69 b
H-N	208 b	4.5	3.04 a	48 b	1568 d	53 c
H-L	225 a	4.4	2.96 b	73 a	2321 a	85 a
LV	203 b	4.5	2.93 b	67 b	1692 c	61 b
L+W	223 a	4.4	3.10 a	125 a	2425 a	77 a
L+N	202 b	4.8	2.80 b	73 b	1914 b	60 b
L+WN	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		20	)21			
HV	172 b	3.9	3.21	104	944 b	36 b
H-W	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
H-N	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
H-L	208 a	3.9	3.20	106	1979 a	48 a
LV	197 b	4.1	3.14	110 d	1694 c	52
L+W	212 a	4.3	3.19	162 b	2110 a	57
L+N	201 b	4.2	3.09	138 c	1938 b	52
L+WN	200 b	4.6	3.10	184 a	2041 ab	50

TSS; Total Soluble Solid. YAN: Yeast assimilable nitrogen. A: total anthocyanins. TPI: total phenol index. Different letters correspond to significant differences according to the Tukey test (p-value  $\leq 0.05$ ). n.d. No data available.

For the primary metabolism, higher total soluble solids and total acidity concentrations were observed in 2019 and 2020 compared to 2021, while the pH and the YAN were the lowest in 2020 in all treatments. The H-W and H-L treatments permitted to increase in the sugar concentration compared to HV, although this increase was not systematic for all years. The pH was, in contrast, lower for H-W (2.78) and H-L (2.97) than HV (3.00) in 2020. The H-N treatment decreased the YAN content compared to all other treatments in HV in 2019 and 2020 (–24 %). In LV, the L+N treatment was permitted to reach higher YAN content (+20 %) than LV in 2019 and 2021 but had no effect on other primary metabolism variables. The YAN increase was even more (+60 %) when irrigation (with or without N) was applied (2020–2021). The L+W treatment also led to a higher sugar concentration and higher pH compared to the other treatments.

Regarding the secondary metabolites, 2019 and 2020 were marked by higher total anthocyanin concentrations (A) and total phenol index (TPI) in the HV zone compared to the LV zone, while the situation was the reverse in 2021.

In HV, H-L was the treatment with the highest A and TPI at harvest for all years. In contrast, the H-N treatment reached similar A and TPI as HV in 2019, but it resulted in lower concentrations than HV in 2020. For the LV, the L+W and L+WN treatments showed the highest A in 2020 and 2021 and also the highest TPI in 2020 compared to all other treatments. To a lesser extent, the L+N treatment also permitted to increase A compared to HV in 2020 and 2021 but had no effect on TPI compared to the control.

### 2.4.6. <u>Relevance of the treatments when compared to an optimal "Productive Target"</u> pattern

For both vigour zones, the treatments applied generally permitted over the three years to improve the score of a few variables compared to the controls (HV, LV), but without systematically reaching the optimal score ( $\geq$  3) (Figure 4.5).

In the high vigour zone, the control HV was sub-optimal for all variables, except for the yield (Y), which reached a maximum score (score 4). Interestingly, treatments such as Leaf removal (H-L) and water restriction (H-W) presented a score of 4 for BR (no disease at all) and equalled the optimal "Productive Target" limit (score 3) for TPI, A and TSS. However, the variables PW and YAN remained sub-optimal (score 2) as for HV. Regarding yield (Y), the H-L presented a lower performance than HV (score 2), while the H-W surpassed the "Productive Target", such as HV (score 4). Similar to HV, the nitrogen reduction treatment (H-N) had higher yields than the "Productive Target", but it had lower scores than the "Productive Target" for the other selected variables. In particular, the H-N treatment reached the worst score (score 1) for YAN.

In the low vigour situation, the control LV reached optimal scores for Y and BR, but all other variables were sub-optimal. The treatment with water supply (L+W) permitted to reach the "Productive Target" for all variables, except the PW variable, whose score was only 2. The contribution of N (L+N) was only permitted to favor the score of YAN compared to LV, while all other variables reached the same score as LV.



**FIGURE 4.5.** Deviation from an optimal 'Productive Target' pattern for the vineyard sustainability and profitability of the different treatments within HV and LV zones over the three years (2019 to 2021).

The optimal 'Productive Target' (score  $\geq$ 3) is delimited by the ruby line (corresponding to score 3) for each of the 7 selected variables. PT: Productive Target; TSS: Total soluble

solid; PW: Pruning Weight; Y: Yield; BR: Bunch root; YAN: Yeast assimilable nitrogen; A: total anthocyanins. TPI: total phenol index. Scores 1 and 2 were considered to be non-optimal.

#### 2.5 DISCUSSION

#### 2.5.1 Changes in water and nitrogen status determine the difference in plant vigour

The productive seasons were climatically different, particularly in water supply (Supplement 4.3). At the time of flowering in 2019, both vigour zones were confronted with a moderate deficit level (Figure 4.4) due to a dry and warm spring (Supplement 4.3). From flowering onward, rainfall generated different water patterns according to vigour zone and treatments. The treatments recovered a good water status in the HV and remained without stress until harvest, except for the H-W treatment, while the  $\Psi p$  for both LV and L+N treatments progressively declined. Inorganic mulches, such as the one used in the H-W treatment, are expected to generate a physical barrier that prevents soil water loss by evaporation and evapotranspiration through the limitation of cover crop development (Hostetler et al., 2007; Ross, 2010). In our study, H-W was more stressed (Figure 4.4, A) than control plants (HV) in 2019, despite the high rainfall. The barrier effect of the plastic mulch, preventing the entry of rainwater, coupled with the dry conditions prior to mulching, may be responsible for the low plant water status measured after flowering. In both seasons, the plastic mulch was placed after seven days without rainfall and high atmospheric demand, which caused soil moisture to drop to moderate to highstress levels until close to harvest. For the LV and L+N, plants appeared only slightly responsive to rainfall events (Figure 4.4, B). When controlled deficit irrigation management (L+W) was applied, plant water status was efficiently maintained at high values of  $\Psi p$  (ranging from -0.2 to -0.4 MPa), according to Ojeda et al. (2002). The higher soil water holding capacity in HV compared to LV could explain these differences in plant water status, as reported by other authors (Bramley et al., 2011; Ferrer et al., 2020(a)).

The applied treatments modified the soil and plant nitrogen availability (Figure 4.3). The H-N treatment reduced foliar N starting from the second productive season

(2020). The absence of effect during the first year of treatment (2019) was likely to be due to the buffering effect of N (Verdenal et al., 2021). The soil N stock for the H-W treatment was also lower compared to HV in 2020. The reduction in soil moisture for this treatment in 2019 (Figure 4.4, A) may have affected the activity of microorganisms and the rate of mineralization of organic matter (Paul, 2007) for the second season. Similarly, in the LV zone, the effect of the treatments was dependent on the water supply of the year. Supplemental N supply (L+N) increased soil N content for all years but not plant nitrogen content. Nitrogen uptake by roots is dependent on soil water availability (Verdenal et al., 2021). Thus, despite the higher N stock in the soil for the L+N treatment compared to LV in 2020, the low water availability did not permit to increase the leaf N content for this treatment. When water was supplied (L+W treatment), the higher humidity permitted to increase the leaf N content in 2020, probably due to the greater microbial activity and N solubility favoring N absorption (Ortega-Heras et al., 2014).

Water conditions and nutrient supply, particularly N, determine the expression of vigour in interaction with other environmental and cultivation management strategies (Chaves et al., 2007). The timing of fertilization and also the type of fertiliser (mineral vs organic) plus the mode of application (soil and foliar application) highly impact the response of the vine to fertilization (Gatti et al., 2020). In our trial, the usual N fertilization was the same for the whole plot. However, the two vigour zones differed in terms of root development, the latter being less abundant and more superficial in the LV compared to HV (data not shown). Supplementary water and N supplies in the LV zone (L+W and L+WN) increased the vegetative growth (Table 4.2) and even exceeded the one observed for HV in 2020. It is well documented that shoots, in particular the secondary shoots (Metay et al., 2014), are the most responsive organs to water and N availability (Chaves et al., 2007; Vrignon-Brenas et al., 2019). Excessive N applications (with water availability) can also generate excessive vigour, altering the microclimate of clusters together with the grape composition and sanitary status (Metay et al., 2014; Soubeyrand et al., 2014). It is clear, based on our results when considering the treatments L+W, L+WN and L+N, that water was the limiting factor and that N application without water availability had no impact on vegetative growth. The H-L treatment and the LV irrigated treatment both
improved pruning weight and lignification, which may be related to a higher source:sink ratio and starch accumulation (Vrignon-Brenas et al., 2019).

#### 2.5.2 Changes in yield and grape composition

The H-L treatment reduced the yield for the three cropping seasons compared to the HV control (Table 4.3). Many authors have reported the effect of pre-flowering leaf removal on yield reduction for several varieties (Palliotti et al., 2011; Arrillaga et al., 2021; Chalfant and Dami, 2021). The restriction of carbohydrate supply due to the removal of photosynthetically active leaves during the flowering period can be critical for berry set and development (Candolfil-Vasconcelos and Koblet, 1990; Frioni et al., 2018). The yield reduction was explained in the present study mainly by the reduction in berry number per bunch in accordance with other studies (Arrillaga et al., 2021). Although yield was lower for the H-L treatment compared to HV, berry weight was consistently higher for the three years in the H-L treatment. The lower number of berries per cluster from the early stages of berry development and the better soil water availability in this vigour zone may have favored the carbon balance and berry growth (Ojeda et al., 2002). The higher water and nitrogen availability over the two seasons of yield elaboration were shown to have a positive impact on carbon gain and on all yield components in several studies (Ojeda et al., 2002; Dos Santos et al., 2003; Vasconcelos et al., 2009; Guilpart et al., 2014). In accordance with those studies, the regulated deficit irrigation strategy in the LV (L+W and L+WN), which increased plant water and N status, permitted one to improve grape yield mainly through an increase in bunch weight (Table 4.3).

Higher light exposure was also concomitant with higher bud fertility (Figure 4.2, Table 4.3), as reported by Sánchez and Dokoozlian (2005). The treatments H-W, H-L, LV and L+N generated an environment with greater luminosity, higher temperature, and lower relative humidity compared to HV (Figure 3.2). Such conditions permitted the reduction of the pressure of pathogenic fungi. The lower leaf area for these treatments (Table 4.2) and the better exposure of the bunches may also have improved the efficacy of chemical control (Molitor et al., 2016). Grey mould incidence was shown to be reduced

on grapevines with lower vegetative and reproductive growth (Valdés-Gómez et al., 2008).

In this trial, the harvest date was fixed according to the evolution of pH (3.3–3.4). In addition, when these pH values were not reached for some years (2020 and 2021), priority was given to avoiding dehydration of the berries (weight loss) for each treatment. It is important to note that the treatments were harvested (Supplement 4.2) at maximum berry weight, thus avoiding a concentration increase in berry components resulting from berry shrivelling. Thus, at maximum bunch weight, H-L treatment improved the accumulation of sugars, anthocyanins, and phenols in HV compared to the HV control (Table 4.4).

The effect of pre-flowering leaf removal on grape composition is controversial in the literature. Some authors indicate an improvement in all or selected grape composition parameters (Palliotti et al., 2011; Gatti et al., 2012; Arrillaga et al., 2021), while others report no change in grape quality (Chalfant and Dami, 2021). In our study, the lower yield for H-L treatment increased the leaf/fruit ratio and increased the light exposure in the bunch zone, which ultimately stimulated the berry growth, sugar accumulation, anthocyanin and phenol contents (Risco et al., 2014, Arrillaga et al., 2021). Using plastic ground cover (H-W) also improved grape composition parameters. Notably, the soluble solids and anthocyanin contents were higher, as reported by other authors (Todic et al., 2008). In contrast, there was no effect of H-W on grape pH or acidity at harvest, in agreement with Hostetler et al. (2007) and Sandler et al. (2009). The effect of plastic ground cover on berry composition was particularly interesting in 2019, which was climatically less favourable (high rainfall during the ripening period). In addition to its impact on plant water status, the plastic cover permitted to increase the light reflection in the cluster zones as mentioned above (Figure 4.2) but also changed the wavelength (Osrečak et al., 2016). Changes in the ratio of red to far-red radiation (R:FR) can occur depending on the type and color of mulch used (Ross, 2010). The material we used presented a high diffuse reflectance (50 %, data not shown) in the wavelength belonging to the red (600 nm), improving the R:FR ratio. Therefore, this mulch could also have

modified the expression or activation levels of the enzymes responsible for the biosynthesis of the molecules (Smart et al., 1988).

Keeping plants under moderate water deficit (L+W) stimulated the accumulation of sugars and anthocyanins (Table 4.4) despite the larger leaf area found compared to LV (Table 4.2). Our results show that additional water supply seems to have played a more critical role in the accumulation of primary and secondary compounds (Ojeda et al., 2002) than bunch exposure. Temperature and light in the canopy zone were lower for L+W compared to LV (Figure 4.2) due to higher vegetative growth (Table 4.2), but the accumulation of sugars and anthocyanins was favored with a regulated deficit irrigation strategy (Table 4.4). In the seasons when water was more available (2019 and 2021), nitrogen supplementation (L+N) also improved the berry composition by increasing the total anthocyanins and YAN (Figure 4.3 and Table 4.3).

## 2.5.3 Valorization of site-specific management

The spatial variability of vineyards is generally due to variations in soil characteristics (Taylor et al., 2010; Arnó et al., 2012; Brillante et al., 2016), which affect the availability of water and nutrients. Such spatial variability was characterized by our vineyard in previous work (Ferrer et al., 2020(a)) (Supplement 4.1), thus permitting us to set a boundary for two vigour zones over eight successive years of observations.

We proposed a strategy based on site-specific management, where differential cultivation techniques (irrigation, fertilization, leaf removal) are applied according to vigour zones to optimise production and quality and increase input use efficiency (McClymont et al., 2012). Water management, either with a regulated deficit irrigation strategy (L+W, L+WN) or with the use of plastic covers (H-W), allowed to achieve adequate yields with improvement in grape composition parameters (Table 4.4), reduce the incidence of bunch diseases and reach "Productive Target". Other studies, in agreement with our results, confirm that water management through irrigation scheduling by vigour zone and in combination with cover crops can improve water use

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efficiency and promote both yield and grape composition (McClymont et al., 2012). Nitrogen reduction in an HV determined that the productive and compositional variables moved away from the "Productive Target" pattern (Figure 4.5). The treatments H-L and L+W were the closest to the "Productive Target" for the Tannat variety and the treatments that also favored the within subplot and between year homogenization of all the key variables evaluated (PW, Y, TSS, BRIX, YAN, A and TPI). In addition, it should be noted that L+W treatment was able to achieve higher yields than targeted with the high berry quality levels setting in the "Productive Target".

The benefits of site-specific management can be evaluated from an environmental and economic point of view. Reasoning the inputs where and when needed brings environmental advantages compared to more 'traditional and uniform' management and reduces production costs (Arnó et al., 2008). A few examples include reduced pollution due to less nutrient loss (Bongiovanni & Lowenberg-Deboer, 2004), reduced water use, and less pesticide application (Hedley, 2015), among others. Economic benefits may be more difficult to quantify. In our study, treatments showed differences compared to a "Productive Target" situation necessary to obtain good quality wines (Figure 4.5). H-L and L+W treatments showed the potential to produce good quality wines even with higher yields and better bunch health than HV and LV. In addition, both yield and composition parameters were more homogeneous, a situation that is desirable for winegrowers. Without intervention using management techniques, wines that would be produced from the control grapes (HV and LV) would be impossible to sell under the category of quality wines according to Uruguayan regulations because of their low alcohol level (less than 12 %). Therefore, their sale price would be automatically significantly lower than that of quality wine (USD 2.5 vs USD 7.2; 750 ml bottle). In rainy years, the hydric reduction treatment improved the sanitary condition of the clusters, improving the composition parameters and, therefore, the wine produced. Although nylon can be used for several seasons, the installation, maintenance, and removal costs are high, and the environmental impact of using non-degradable plastics must also be considered (Hostetler et al., 2007).

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To conclude, while a 1 ha plot can be considered homogeneous from a topographic and soil-climatic aspect, this study has demonstrated high variability of soil, production parameters and grape composition. It provided knowledge on the relationships between water and nitrogen availability, plant vigour and their impact on grape quality. Applying precision viticulture technologies, we were able to experimentally apply site-specific management strategies that improved plant productivity (yield and berry composition) and reduced heterogeneity at the plot level. This approach could be used by winegrowers on a larger scale to determine micro-terroirs and thus generate the application of site-specific techniques to obtain the potential for productivity.

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# 2.7 SUPPLEMENTARY DATA



**SUPPLEMENT 4.1**. Map of NDVI values depicting the three vigour zones based on the average NDVI over the period from 2015 to 2017.

Vigour	Treatment / Seasons	2019	2020	2021
High	Control (HV)	6th of March	10th of March	05th of March
	Water restriction (H-W)	04th of March	04th of March	N.T.
	Nitrogen restriction (H- N)	01st of March	05th of March	N.T.
	Leaf removal (H-L)	04th of March	10th of March	05th of March
Low	Control (LV)	6th of March	05th of March	05th of March
	Irrigation (L+W)	N.T.	10th of March	05th of March
	Nitrogen supply (L+N)	6th of March	04th of March	05th of March
	Irrigation+Nitrogen supply (L+WN)	N.T.	N.T.	05th of March

SUPPLEMENT 4.2. Harvest date for each year and treatment

N.T.: No treatment

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**SUPPLEMENT 4.3.** Monthly weather data records for three cropping seasons.

Rainfall (mm) and Reference evapotranspiration ETo (mm). The green rectangles represent the cropping cycles (Sep - Mar).

# CHAPTER V

# THE SYNCHRONY OF BERRY GROWTH AND SUGAR ACCUMULATION RELIES ON THE INTERACTIVE EFFECTS OF ENVIRONMENT AND MANAGEMENT PRACTICES



## 1. ABSTRACT

#### ENGLISH

This study aimed to characterize grape ripening dynamics for different climatic situations and crop management strategies (leaf:fruit ratio; water and nitrogen). Meteorologically, the 8 years were organized into three groups according to water availability: rainy years (2014, 2015 and 2019), dry years (2016, 2018 and 2020) and intermediate years (2017 and 2021). Differences in water availability marked differences in berry weight, Brix and total soluble solids per berry at harvest. Berry weight and total soluble solids were lower in dry years compared to rainy years. In turn, these variables were higher in high vigour (HV) compared to low vigour (LV) plants. In contrast, Brix remained constant in both year groups and in both vigors. The impacts of specific-site management for the two vigour zones (HV, LV) were addressed for two contrasted rainy (2019) or dry (2020) years. For HV, leaf removal before flowering (H-L) increased berry size, Brix and total soluble solids per berry in both years. Water restriction at veraison (H-W) did not modify berry size but increased Brix and total soluble solids per berry in rainy years and decreased them in dry years. Finally, nitrogen restriction (H-N) only modified berry weight, Brix and total soluble solids per berry in the dry year. For LV, supplemental nitrogen (L+N) did not modify berry parameters in any of the year groups evaluated. The addition of water (L+W) in a dry year increased berry weight, soluble solids accumulation and Brix.

The berry population was then normalized to address the kinetics of sugar and water accumulation, while reducing the natural asynchrony and heterogeneity of berry development. The dynamics of water and sugar accumulation in the berry could be fitted through a bi-linear adjustment model where two phases were clearly distinguished. Phase I was characterized by an increment of berry volume, resulting from both sugars and water accumulations. During phase II, sugars continued to accumulate with no further increase in berry volume except in the shrivelling years (2021 and 2019). The growth/sugar loading ratio (slope) during phase I was rather stable for both vigour zones (HV vs. LV), different climatic situations and vineyard management treatments (water/nitrogen supply or restriction, leaf removal): it only decreased one year for HV

compared to LV, and for treatments with either N restriction (H-N) or water supply (L+W). The other parameters of the model (durations of phase I and II) displayed more variations with climatic conditions (rainy vs. dry) and management treatments. For dry years, a longer duration of phase I and a shorter duration of phase II were observed compared to rainy years. Water supply or restriction treatments (L+W; H-W) resulted in comparable phase I and II modifications as wet/dry year. Leaf removal (H-L) shortened Phase II compared to the control (HV) only for wet year. The effect of nitrogen supply or restriction (L+N; H-N) on the durations of phases I and II highly relied on the climatic conditions (wet/dry year) with no consistent clear trends. Ultimately, the results indicated a clear role of water and carbon supply at early stages of berry formation (from bloom, "BL", to veraison, "V") on the ripening dynamic. Higher water availability (WA BL\_V) favored high berry volume at veraison, which reached ca. 70% of their final size, with subsequent shorter phase I and longer phase II. Higher leaf: fruit ratio, which was negatively correlated to the water availability before bloom, had the reverse effect, increasing P.I at the expense of P.II. Initial reserves at budburst (trunk starch content) and photosynthetic activity also impacted the duration of phase I. Higher availability of carbon during the inflorescence formation stages (bud break to bloom) seemed to decrease the apparent asynchrony between water and carbon accumulation.

The changes in the duration of the phases I and II due to the climatic characteristics of the year or cultivation techniques trigger new questions to address. Notably, the apparent accumulation of sugars at constant berry growth deserves more study to determine whether it may be due to a disconnection of the two processes or rather to an increase of the asynchrony of berry development within the population. Knowing more about the physiological responses of these processes to edaphoclimatic factors and management practices would be useful to better reason sampling and/or harvesting strategies and reach maximum berry volume with maximum sugar accumulation while avoiding berry shriveling.

#### FRENCH

Cette étude visait à caractériser la dynamique de maturation du raisin pour différentes situations climatiques et stratégies de gestion de la culture (rapport feuille/fruit ; eau et azote). Sur le plan météorologique, les 8 années ont été regroupées en trois groupes selon la disponibilité en eau : années pluvieuses (2014, 2015 et 2019), années sèches (2016, 2018 et 2020) et années intermédiaires (2017 et 2021). Les différences de disponibilité en eau ont marqué des différences dans le poids des baies, le Brix et les solides solubles totaux par baie à la récolte. Le poids des baies et les solides solubles totaux étaient plus faibles dans les années sèches par rapport aux années pluvieuses. De même, ces variables étaient plus élevées chez les plantes à haute vigueur (HV) que chez celles à faible vigueur (LV). En revanche, le Brix est resté constant dans les deux groupes d'années et dans les deux vigueurs. Les impacts de la gestion spécifique du site pour les deux zones de vigueur (HV, LV) ont été abordés pour deux années contrastées pluvieuses (2019) ou sèches (2020). Pour HV, l'effeuillage avant la floraison (H-L) a augmenté la taille des baies, le Brix et les solides solubles totaux par baie pour les deux années. La restriction hydrique à la véraison (H-W) n'a pas modifié la taille des baies mais a augmenté le Brix et les solides solubles totaux par baie lors des années pluvieuses et les a diminués lors des années sèches. Enfin, la restriction en azote (H-N) n'a modifié le poids des baies, le Brix et les solides solubles totaux par baie que l'année sèche. Pour LV, l'ajout d'azote (L+N) n'a pas modifié les paramètres des baies dans aucun des groupes d'années évalués. L'ajout d'eau (L+W) au cours d'une année sèche a augmenté le poids des baies, l'accumulation de solides solubles et le Brix.

La population de baies a ensuite été normalisée pour tenir compte de la cinétique de l'accumulation de sucre et d'eau, tout en réduisant l'asynchronie et l'hétérogénéité naturelles du développement des baies. La dynamique de l'accumulation d'eau et de sucre dans la baie a pu être ajustée par un modèle d'ajustement bi-linéaire où deux phases ont été clairement distinguées. La phase I a été caractérisée par une augmentation du volume des baies, résultant à la fois de l'accumulation de sucres et d'eau. Au cours de la phase II, les sucres ont continué à s'accumuler sans que le volume des baies n'augmente davantage, sauf pendant les années de flétrissement (2021 et

2019). Le rapport croissance/charge en sucre (pente) pendant la phase I a été plutôt stable pour les deux zones de vigueur (HV vs LV), les différentes situations climatiques et les traitements de gestion du vignoble (apport ou restriction d'eau/azote, effeuillage) : il n'a diminué qu'une seule année pour HV par rapport à LV, et pour les traitements avec restriction d'azote (H-N) ou apport d'eau (L+W). Les autres paramètres du modèle (durées de la phase I et II) ont montré plus de variations avec les conditions climatiques (pluvieux vs sec) et les traitements de gestion. Pour les années sèches, une durée plus longue de la phase I et une durée plus courte de la phase II ont été observées par rapport aux années pluvieuses. Les traitements d'apport ou de restriction d'eau (L+W; H-W) ont entraîné des modifications comparables des Phases I et II pour les années humides et sèches. L'effeuillage (H-L) a raccourci la phase II par rapport au contrôle (HV) uniquement pour les années humides. L'effet de l'apport ou de la restriction d'azote (L+N; H-N) sur les durées des phases I et II dépendait fortement des conditions climatiques (année humide/année sèche) sans qu'aucune tendance claire ne se dégage. En fin de compte, les résultats ont indiqué un rôle clair de l'approvisionnement en eau et en carbone aux premiers stades de la formation des baies (de la floraison "BL" à la véraison, "V") sur la dynamique de maturation. Une plus grande disponibilité en eau (WA BL\_V) a favorisé un volume élevé de baies à la véraison, qui ont atteint environ 70% de leur taille finale, avec une phase I plus courte et une phase II plus longue. Un rapport feuille/fruit plus élevé, qui était négativement corrélé à la disponibilité en eau avant la floraison, a eu l'effet inverse, augmentant P.I aux dépens de P.II. Les réserves initiales au débourrement (teneur en amidon du tronc) et l'activité photosynthétique ont également eu un impact sur la durée de la phase I. Une plus grande disponibilité du carbone pendant les étapes de formation des inflorescences (du débourrement à la floraison) a semblé diminuer l'asynchronisme apparent entre l'accumulation d'eau et de carbone.

Les changements dans la durée des phases I et II dus aux caractéristiques climatiques de l'année ou aux techniques de culture soulèvent de nouvelles questions. Notamment, l'accumulation apparente de sucres à croissance constante des baies mérite d'être étudiée plus avant pour déterminer si elle est due à une déconnexion des deux processus ou plutôt à une augmentation de l'asynchronie du développement des baies au sein de la population. Une meilleure connaissance des réponses physiologiques de ces

processus aux facteurs édaphoclimatiques et aux pratiques de gestion serait utile pour mieux raisonner les stratégies d'échantillonnage et/ou de récolte et atteindre un volume maximal de baies avec une accumulation maximale de sucres tout en évitant le flétrissement des baies.

#### SPANISH

Este estudio tuvo como objetivo caracterizar la dinámica de maduración de la uva para diferentes situaciones climáticas y diferentes estrategias de manejo del cultivo (relación hoja:fruto; agua y nitrógeno). Meteorológicamente, los 8 años se agruparon en tres grupos en función de la disponibilidad hídrica: años lluviosos (2014, 2015 y 2019), años secos (2016, 2018 y 2020) y años intermedios (2017 y 2021). Las diferencias en la disponibilidad de agua marcaron diferencias en el peso de baya, Brix y sólidos solubles totales por baya en la cosecha. El peso de baya y los sólidos solubles totales fueron menores en los años secos en comparación con los años lluviosos. A su vez, estas variables fueron mayores en las plantas de vigor alto (HV) en comparación con las de vigor bajo (LV). Por el contrario, el Brix se mantuvo constante en ambos grupos de años y en ambos vigores. Los impactos del manejo específico para cada sitio para las dos zonas de vigor (HV, LV) se abordaron en dos años bien contrastantes en oferta hídrica: lluvioso (2019) vs. secos (2020). Para HV, la eliminación de hojas antes de la floración (H-L) aumentó el tamaño de baya, Brix y sólidos solubles totales por baya en ambos años. La restricción de agua en el envero (H-W) no modificó el tamaño de baya, pero aumentó los Brix y los sólidos solubles totales por baya en los años lluviosos y los disminuyó en los años secos. Por último, la restricción de nitrógeno (H-N) sólo modificó el peso de la baya, los Brix y los sólidos solubles totales por baya en el año seco. Para LV, el nitrógeno suplementario (L+N) no modificó los parámetros de las bayas en ninguno de los grupos de años evaluados. La adición de agua (L+W) en un año seco aumentó el peso de las bayas, la acumulación de sólidos solubles y los grados Brix.

A continuación, se normalizó la población de bayas para abordar la cinética de acumulación de azúcar y agua, reduciendo al mismo tiempo la asincronía natural y la heterogeneidad del desarrollo de las bayas. La dinámica de acumulación de agua y azúcar

en la baya pudo ajustarse mediante un modelo de ajuste bilineal en el que se distinguían claramente dos fases. La fase I se caracterizó por un incremento del volumen de la baya, resultado tanto de la acumulación de azúcares como de agua. Durante la fase II, los azúcares siguieron acumulándose sin que aumentara más el volumen de la baya, excepto en los años de marchitamiento (2021 y 2019). La relación crecimiento/carga de azúcar (pendiente) durante la fase I fue bastante estable para ambas zonas de vigor (HV frente a LV), las diferentes situaciones climáticas y los tratamientos de gestión del viñedo (suministro o restricción de agua/nitrógeno, deshojado): sólo disminuyó un año para HV en comparación con LV, y para los tratamientos con restricción de N (H-N) o suministro de agua (L+W). Los otros parámetros del modelo (duraciones de las fases I y II) mostraron más variaciones con las condiciones climáticas (lluvioso frente a seco) y los tratamientos de gestión. En los años secos se observó una mayor duración de la fase I y una menor duración de la fase II en comparación con los años lluviosos. Los tratamientos de suministro o restricción de agua (L+W; H-W) dieron lugar a modificaciones de las fases I y II comparables a las de los años húmedos/secos. La eliminación de hojas (H-L) acortó la fase II en comparación con el control (HV) sólo para el año húmedo. El efecto del suministro o restricción de nitrógeno (L+N; H-N) sobre las duraciones de las fases I y II dependió en gran medida de las condiciones climáticas (año húmedo/seco), sin tendencias consistentes. En última instancia, los resultados indicaron el claro rol de la disponibilidad de agua y carbono en las fases tempranas de formación de la baya (desde la floración, BL, hasta el envero, V) sobre la dinámica de maduración. Una mayor disponibilidad de agua (WA BL\_V) favoreció un alto volumen de bayas en el envero, que alcanzaron aproximadamente el 70 % de su tamaño final, con una subsiguiente fase I más corta y una fase II más larga. Una mayor relación hoja:fruto, que se correlacionó negativamente con la disponibilidad de agua antes de la floración, tuvo el efecto contrario: aumentó la P.I a expensas de la P.II. Las reservas iniciales en el momento de la brotación (contenido de almidón en el tronco) y la actividad fotosintética también influyeron en la duración de la fase I. Una mayor disponibilidad de carbono durante las etapas de formación de la inflorescencia (desde la brotación hasta la floración) pareció disminuir la asincronía aparente entre la acumulación de agua y carbono.

Los cambios en la duración de las fases I y II debidos a las características climáticas del año o a las técnicas de cultivo suscitan nuevas cuestiones que abordar. En particular, la acumulación aparente de azúcares con un crecimiento constante de las bayas merece más estudio para determinar si puede deberse a una desconexión de los dos procesos o más bien a un aumento de la asincronía del desarrollo de las bayas dentro de la población. Conocer mejor las respuestas fisiológicas de estos procesos a los factores edafoclimáticos y a las prácticas de gestión sería útil para razonar mejor las estrategias de muestreo y/o recolección y alcanzar el máximo volumen de bayas con la máxima acumulación de azúcares, evitando al mismo tiempo el marchitamiento de las bayas.

#### 2. INTRODUCTION

Grape development can be described through the dynamics of berry growth and the accumulation of primary (sugar and organic acids) and secondary metabolites (anthocyanins, tannins and aromatic precursors), which together determine the yield and oenological potential of the grape. Berry growth follows a sigmoidal curve pattern divided into three stages: a first stage of rapid green growth, a stationary phase and, finally, a phase of berry ripening (Coombe, 1992, Wang et al., 2003, Thomas et al., 2008). The ripening process starts with berry softening (stage called "veraison"), with the loss of chlorophyll (Coombe & McCarthy, 2000, Castellarin et al., 2015, Bigard et al., 2019), and then is characterized by cell expansion (Ojeda et al., 1999), accumulation of sugars, anthocyanin synthesis and degradation of organic acids concentration (Robinson & Davies, 2000, Deluc et al., 2007).

Berry growth and composition are determined by genotype (Liu et al., 2007, Dai et al., 2011, Wang et al., 2018). The concentrations of primary and secondary metabolites vary during berry development (Coombe, 1992) and are affected by environmental conditions and cultural practices (Nuzzo and Matthews, 2006, Sadras et al., 2007). Water availability plays a key role in berry development and influences grape composition. Ojeda et al. (2001) demonstrated that moderate or severe deficits applied from flowering can modify berry size due to their impact on cell expansion during the first phase of berry development. Plant vigour is another factor affecting berry development and biochemical composition, mainly due to the modification of the leaf:fruit ratio and carbohydrate availability and the influence of canopy development on grape microclimate. Plants with excessive vigour have larger berry size (Cortell et al., 2005), higher yield (Bramley et al., 2011), lower sugar concentration and higher pH (Kliewer & Dokoozlian, 2005, Cortell, 2006, Vance, 2012). On the other hand, low vigour is associated with small berries, low yields and more exposed clusters. Although the impact of low vigour on berry composition depends on canopy dimensions and microclimate, a few authors have reported positive correlations between low vigour and phenolic compound accumulation (Peña-Neira et al., 2004, Cortell et al., 2005). While nitrogen supply has generally minor effect on berry primary metabolism (Lasa et al., 2012, Miliordos et al., 2022), it impacts

the secondary metabolism, especially the anthocyanin metabolism (Soubeyrand et al., 2014, Keller, 2020). Lastly, other practices such as thinning and leaf removal modify the cluster microclimate by increasing the grapes' exposure to light (Yue et al., 2021, Wang et al., 2022) and improve the anthocyanins and phenols accumulation in berry.

Berry water flows determine final berry size as 75-85% of berry weight at harvest is made of water (Ribéreau-Gayon et al., 2006). Water flow is also the driving force that allows the import and accumulation of sugar in the berry (Rebucci et al., 1997, Dreier et al., 2000). Depending on the stage of fruit development, water enters the berry via xylem or via phloem (Ollat et al., 2002, Keller et al., 2017). During the green stage, water mainly arrives via the xylem tracheids (Greenspan et al., 1996, Choat et al., 2009), involving a first wave of growth by cell expansion. From veraison onwards, the water flow through the xylem decreases and the phloem becomes the predominant pathway for water and solutes entering the berry (Greenspan et al., 1994, Dai et al., 2010, Keller et al., 2014). Phloem discharge increases the berry osmotic potential, which in turn increases water flow (Xie et al., 2009), thus generating a second wave of cell growth. During the second phase of berry growth and onwards, xylem was shown to remain functional, intact and unoccluded (Rogiers et al., 2006, Tilbrook & Tyerman, 2009, Bondada et al., 2017). Although controversial, some authors suggested xylem reflux to the plant could occur after the phloem discharge stops (Keller et al., 2015, Keller et al., 2017). As berries have low or no photosynthetic capacity, the sugars come from leaf photosynthesis. Sucrose produced by photosynthesis reaches the berries (Zhang et al., 2006) where invertase enzymes break it into glucose and fructose (Takayanagi & Yokotsuka, 1997) which accumulate in vacuoles. During the phloem discharge period, the concentration of sugars in the berry increases proportionally with berry growth (Nuzzo & Matthews, 2006) to reach a maximum of 1.1 M at a glucose/fructose ratio of 1 (Houel et al., 2015). Sugar loading and berry growth cease from this threshold. Then, sugar concentration generally increases due to berry water loss. Berry stomatal density is very low (less than 1 stomata per mm<sup>2</sup>). In addition, during ripening, stomata become partially or totally occluded by wax, losing their function (Hardie et al., 1996). Thus, berry transpiration depends primarily on cuticular conductance (Blanke & Leyhe, 1987) and, as ripening progresses, there is an overall decrease in transpiration (Rogiers et al., 2004, Greer & Rogiers, 2009).

The ripening process is coordinated individually in each berry and at the cluster level does not occur simultaneously (Robinson & Davis, 2000). It has been reported that berry development within a bunch is highly asynchron (Kontoudakis et al., 2011, Rolle et al., 2015, Bigard et al., 2019). Grapes collected at harvest are composed of a combination of grapes that are at different stages of ripening. In other words, over-ripen berries coexist with ripen berries and also un-ripen berries (Kontoudakis et al., 2011, Rio Segade et al., 2013). The asynchrony of berry development originates straight from flowering and can increase as berries develop due to a wide range of complex genotypic, environmental and crop management interactions (Reshef et al., 2017). Berries positions within the cluster influence their microclimatic environment, notably their light and temperature exposures. Indeed, exposed berries can be up to 17 °C warmer than shaded berries, which are usually at air temperature (Spayd et al., 2002, Tarara et al., 2008). Due to this asynchrony, populations of berries require 40 to 50 days to reach the maximum concentration of 1.1 M hexoses vs. 26 days for an individual berry (Davies & Robinson, 1996, Coombe & McCarthy, 2000, Famiani et al., 2016, Shahood et al., 2020). Moreover, the onset of ripening is considered in many studies as the color change of berries (Parker et al., 2011, Toffali et al., 2011) when this phenomenon is known to occur later than sugar loading (Sadras et al., 2012, Castellarin et al., 2015). Ultimately, the high heterogeneity and asynchrony in berry development, added to the confusion in the onset of ripening in the literature, impairs the understanding about the processes involved in berry development and their responses to abiotic constraints. Synchronization of berries improves knowledge of the processes occurring at the berry level. One strategy to achieve synchronization is to sort berries according to sugar content (Nelson et al., 1963, Shahood et al., 2019). Grapevine berries do not accumulate sugar before softening. The basal total soluble solids level measured at softening is around 6º Brix (Keller & Shrestha, 2014, Zhang et al., 2006). The plateau of sugar loading, which is reached for individual berries at 1.1 M hexoses (McCarthy & Coombe, 1999, Rogiers et al., 2006, Shahood et al., 2020), corresponds to 18-20º Brix. Normalizing the stages of berry development within the range of 6 to 20º Brix allows synchronization of ripening processes from data obtained from a heterogeneous population of berries.

The present study was aimed to characterizing the kinetics of sugar and water accumulation of average berries coming from a few population samples under different climatic conditions, vigour and crop management (nitrogen, water, leaf/fruit ratio) after the average berries were synchronized over the period from 6 and 20 °Brix and normalized from the maximum volume.

#### 3. MATERIALS AND METHODS

#### **3.1 STUDY SITE AND TREATMENTS**

The experiments were carried out in a commercial vineyard in Canelones, Uruguay (34° 36 S, 56° 14 W). The vineyard consisted of 1.1 ha of Vitis vinifera L. cv. Tannat, grafted on SO4 rootstock and planted in 1998. The vineyard was on a gradual slope of 1-2% (north /south). The rows were oriented north-south, with vine density of 3333 plant ha-1 (2.5 m x 1.2 m). Plants were pruned using a double guyot system and the shoots were trained to a VSP (vertical shoot positioning). This vineyard was characterized by high variability of vine vigour from east to west. Ferrer et al. (2020 (a)) defined three vigour zones in this vineyard named as high (H), medium (M), and low vigour (L) zones. The determination of the zones was made by aircraft flights (620 m altitude) at veraison (January in the southern hemisphere) during three years (2015, 2016 and 2017), using the Normalized Difference Vegetation Index (NDVI). High resolution multispectral images were obtained on the ground (0.2 m). Three ranges of NDVI were set, corresponding to 0.57 to 0.61 for H, 0.55 to 0.57 for M and 0.55 to 0.48 for L (Supplement 1). These three classes of NDVI were consistently located in the same parts of the vineyard year after year (Ferrer et al., 2020 (a)). Management practices (hereafter called "control treatment") were similar on the all vineyard whatever the zone. The vineyard was not irrigated, and received standard fertilization with urea, distributed half pre-flowering and half post-harvest at a total dose of 140 kg of urea (46% N) fertilizer per hectare.

The first experiment was conducted for eight consecutive years (2014-2021). For this experiment, only the most contrasting vigour zones of the vineyard (H vs L) were selected. In the pre-established zones described above, H and L control treatments were

arranged in a random block design with three replicates and 21 plants per replicate, i.e. 63 plants per vigour condition.

The second experiment was conducted in two contrasted climatic years (2019 and 2020). Different treatments were set according to vigour zone (H and L) to improve berry yield and quality and reduce berry heterogeneity compared to the control treatments. For this purpose, 63 plants per treatment were selected in three replicates (21 plants per replicate). Three different treatments applied in H were aimed at reducing water (H-W) and nitrogen supply (H-N) or improve microclimatic conditions in the bunch zone (H-L), while treatments in L were aimed at increasing water (L+W) and nitrogen supply (L+N). In H zone, reduced water supply treatment (H-W) was implemented from veraison to harvest by covering the soil with polyethylene (white surface on both sides, 220 micrometres thick, with ultraviolet protection, Tashiro Takata®, Uruguay). No nitrogen was applied (0 N unit in the season) in the nitrogen restriction treatment (H-N). Approximately 60% of the leaves in the bunch zone were removed before flowering for the leaf removal treatment (H-L). In L zone, additional irrigation was supplied for the treatment (L+W) to reach 100% of the climatic demand (ETo) from budburst to bloom and from harvest to leaf fall, and 70% of ETo from bloom to harvest. For the supplemental nitrogen treatment (L+N), 210 kg urea per ha were supplied (70 kg urea per ha in form of urea were added prior to flowering to the 140 kg mentioned above).

#### **3.2 WEATHER AND MICROCLIMATE MEASUREMENTS**

The microclimatic environment, including minimum temperature (Tm, °C), maximum temperature (TM, °C), photosynthetically active radiation (PAR, ), relative humidity (RH%) was measured with HOBO<sup>®</sup> sensors (HOBO<sup>®</sup> U23 ProV2 loggers, USA). Average maximum temperatures (TMx) were calculated. Three sensors were distributed inside the canopy in the bunch zone, for each treatment from flowering to harvest (2014 to 2020). Rainfall was recorded in the vineyard. Water availability (WA) was determined as the sum of rainfall plus supplemental water inputs for the L+W treatment. The leaf water vapor pressure deficit (VPD) was calculated according to FAO guidelines, using microclimatic data (temperature and relative humidity).

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The all-microclimatic parameters were organized into three categories according to the following phenological stages: budbreak to bloom (BB\_BL; September to November), bloom to veraison (BL\_V; November to January) and veraison to harvest (V\_H; January-March). The average values for each phenological category were used. For the water variable (WA), the sum of rainfall or irrigation water during the phenological period was used. In addition, the number of days with average temperatures above 30° C (ND30) during the period from veraison to harvest was counted.

#### **3.3 PLANT MEASUREMENTS**

#### 3.3.1 Nitrogen, carbon and water status

SPAD index, which can be considered as a proxy of nitrogen content, was recorded. SPAD measurements (MC-100, Apogee Instruments Inc. Logan, UT, USA) were carried out weekly from flowering to harvest on ten sun-exposed and expanded leaves, selected from ten plants per replicate (30 leaves per treatment).

During winter of the two contrasted climatic years (August 2019 and 2020), trunk diameter (TD) was evaluated using a digital caliper (Neiko 01407  $\pm$  0.2 mm) at 10 cm above the grafting point. Wood samples from the trunk of four plants per replicate (12 plant per treatment) were collected. Samples were extracted from the trunk at 20 cm above the grafting point using a 4.0 mm drill bit to approximately half of the trunk depth. The samples were dried for 48 hours at 60 °C. Then, they were grounded with a mesh smaller than 0.1 mm. On a 20 mg sub-sample, trunk starch (ST) was extracted with an ethanol solution (80% v/v) for subsequent enzymatic quantification with spectrophotometry at 340 nm as proposed by Gomes et al. (2003).

Leaf photosynthetic activity (Photo) was also recorded during the 2019 and 2020 seasons using an infrared gas analyzer (Licor-6400<sup>®</sup>, LI-COR Biosciences, Inc., Lincoln Nebraska). Measurements were performed between 9:00 AM and 11:00 AM on nine healthy, exposed adult leaves per treatment, at four different times of the cycle (flowering, bunch closure, veraison and pre-harvest). The photosynthetically active radiation inside Licor chamber was set at 950 µmol photons m-2s-1.

From flowering to harvest, leaf predawn water potential ( $\Psi$ p) was determined using a pressure chamber (SoilMoisture equipment, Santa Barbara, CA, USA). Nine healthy expanded leaves were taken from each treatment (three leaves per replicates). The measurements were carried out between 2:00 AM and 4:00 AM immediately after detaching the leaf from the plant. A water stress index (WSI) was calculated from the sum of all water potentials over the seasons as Myers (1988). As WSI is expressed in absolute values (MPa day-1). Thus, higher WSI correspond to more severe water deficit.

#### 3.3.2 Vegetative growth, yield and berry sample

Exposed Leaf Area (ELA, in m<sup>2</sup> ha<sup>-1</sup>) was assessed at veraison on 9 vines per treatment according to the method of Carbonneau (1995). Yield per plant (Y, kg vine<sup>-1</sup>) at harvest was determined on 63 plants for each treatment (21 plants for each replicate) for all years (2014 to 2021). Leaf:fruit ratio was calculated.

After veraison (stage 34 EyL), berry sampling was carried out every week to determine the evolution of berry weight and composition. The individual berry weight (Bw, g) was determined on one sample of 100 berries for each replicate (300 berries per treatment). Berries were collected from the central zone of the bunch (Deloire et al., 2019) for each treatment. The sample was crushed using a juicer, Phillips HR2290 (Phillips, Netherlands). The sugar content (Total Soluble Solids, TSS, g/l) was determined by refractometer (Atago, Japan) following the protocols established by the OIV (2014). In addition, during 2019 and 2020, on the first sampling date (mid-January), 30 berries per replicate (90 berries per treatment) were collected individually. Berries were collected randomly from different cluster position, one berry per cluster. Sugar content and berry weight was determined for each berry using a hand-held refractometer. Sugar content was then classified into six categories of 2.0 °Brix range each.

Bunch harvest date was fixed when berry pH reached 3.3 - 3.4. When these pH values were not reached in some years, berries were harvested before any dehydration (weight loss) was observed.

#### **3.4 DATA ANALYSIS**

Berry development at the population level was analysed within the range between 6 and 20 ° Brix. The lower limit of this range (6 °Brix) was assumed to correspond to the beginning of berry sugar loading, and the upper limit was assumed to correspond to the arrest of phloem sugar discharge (1.1 Molar equivalent to 18-20 °Brix, Houel et al. 2015). Brix values outside the above range were not considered in this analysis. Indeed, values above 20 °Brix are associated with a concentration effect due to berry dehydration rather to supplemental sugar loading. For each sampling date, berry volume was estimated from the ratio of berry weight to berry density (D, g l-1). Berry density was estimated following the methodology proposed by Vila et al. (2009).

The normalization of berry volume corresponded to the ratio between the berry volume at each sampling date and the maximum volume reached during the entire period. This calculation was performed for each replicate in each year evaluated. This was represented by the following equation (Equation 1):

$$NBv_n = \frac{Bv_n}{Bv_{max}} \quad (1)$$

where,

n is the sampling date

Bv: is the berry volume value at a date n

[Bv] \_max is the maximum volume reached during ripening for each treatment and evaluated year

NBv\_n is the berry volume normalized at a date n

The sugar content (total soluble solids, TSS) per berry was calculated following the methodology proposed by Deloire (2011). TSS was determined at each sampling date both for average berries (TSS, mg berry-1) and normalized berries (mg ml-1NBv).

Statistical analyses were performed with OriginPRO 9.1 software (OriginLab Corporation, Northampton, MA, USA) and RStudio (2023). A bi-linear model was fitted to explain the relationship between NBv and TSS. Five parameters corresponding to the

break point (BP), the slope, the duration of phases I and II and the total duration of the two phases (TDP) were obtained from this model. Phase I, corresponded to a simultaneous sugar accumulation and volume increase (water accumulation), while Phase II was associated to accumulation of sugars at constant volume (no supplemental accumulation of water). In addition, analyses of variance were performed, followed by Fisher's test for the comparison of means.

Distribution curves were performed in Rstudio, based on the relative frequency of the number of berries in each Brix category and berry weight at the beginning of ripening (2019 and 2020). The Kolmogorov-Smirnov test (ks.test, stats package) determined whether the distributions were statistically different. The results of the ks.test were represented in a "network plot" (package: igraph), where the nodes represent the treatments, and the node connections indicate whether the distributions are different. In addition, the integral variable (I Brix and I Bw) was calculated to quantify the dispersion in the distributions. The distributions with a higher integral show a greater dispersion of the values obtained.

The effect of the different vigours zones (H vs L) for the series of years (2014 to 2021) and the effect of the treatments for each vigour zone for the years 2019 and 2020 were addressed. Correlations between weather and micro-climatic (WA BB\_Bl; WA Bl\_V; WA V\_H; Tm Bl\_V; Tm V\_H; VPD Bl\_V; VPD V\_H), plant variables (WSI Bl\_V; WSI V\_H; leaf/fruit ratio; SPAD, TD; TS; Photo), berry weight (Bw) and model parameters (slope; BP; TDP, I Brix, I Bw) were determined by principal component analysis (PCA). The relationship between the model parameters and the variables evaluated (mentioned above) was determined using Pearson's correlation coefficient (ggplot2), only the significative correlation was presented (p-value < 0.05).

#### 4. <u>RESULTS</u>

## 4.1. Berry development response to climate and vigour condition

The water supply showed strong interannual and monthly variability (Figure 5.1). The years 2014, 2015 and 2019 (Group 1), were characterized as wet with total rainfall

during the growing period higher than 550 mm. Nevertheless, rainfall distribution throughout the years were different. In 2014, 60% of the rainfall occurred between veraison and ripening (V\_H). In 2015, rainfall was the highest before bloom (BB\_BI) while in 2019, rainfalls were more homogenously distributed over the three periods. Years 2016, 2018 and 2020 (Group 2) were classified as dry years with a total rainfall below 450 mm. In 2016, rainfall was the highest during the BI\_V period (54%). In 2018 and 2021, the period with the highest rainfalls was BB\_BI (51% and 50%, respectively). The third group of years, including 2017 and 2021 (Group 3) were classified as intermediate (with precipitation between 450 and 550 mm). In this group of years, the highest rainfall occurred during the V\_H period (48% and 37% for 2021 and 2017 respectively).





The phenological periods are BB\_BL: budbreak to bloom. BL\_V: bloom to veraison. V\_H: veraison to harvest. The numbers indicate the accumulated rainfall for each period evaluated.

Yields varied between years and between vigours (Table 1.1). The H zone was systematically characterized by higher yield (ranging 6.7 to 8.3 kg.plant<sup>-1</sup>) compared to L zone (yield: 3.9 to 6.8 kg.plant<sup>-1</sup>) (p-value<0.05), except in 2014 when yield was affected by sanitary conditions in high vigor plants (data not show). Also, the years 2015, 2016, 2017 and 2018 were characterized by the highest yields in both vigor zones. The lowest yields were observed in 2014 and 2019 on the H zone, and in 2020 and 2021 on the L

zone. The leaf:fruit ratio was similar between years and vigours (ranging 0.31 to 2.03), except in rainy years and 2020 was higher in H compared to L. The rainy years presented a higher Bw and a higher sugar accumulation (Bw: 1.56 g; TSS: 310 mg.berry<sup>-1</sup>), followed by the intermediate years (Bw: 1.38 g; TSS per berry: 251 mg.berry<sup>-1</sup>) and, finally, the dry years (Bw: 1.16 g; TSS per berry: 244 mg.berry<sup>-1</sup>). Bw and TSS were systematically higher in the H zone compared to L for all years except for Bw during rainy years (2014 and 2015), which didn't show any differences between vigor zones. In addition, there were no statistical differences of Brix between the vigor zones.

Year	Vigour	Yield (kg.plant <sup>-1</sup> )	Leaf:fruit ratio	<b>Bw</b> (g)	Brix	TSS per berry (mg.berry <sup>-1</sup> )		
RAINY YEARS								
2014	High	6.7	1.73*	1.62	19.0	301*		
	Low	5.7	0.96*	1.50	18.0	255*		
2015	High	8.3*	1.48*	1.54*	22.2	343*		
	Low	6.8*	1.01*	1.46*	20.0	309*		
2019	High	6.9*	1.35*	1.69 *	22.0	348*		
	Low	5.3*	0.61*	1.59 *	21.2	306*		
	DRY YEARS							
2016	High	7.5*	0.98	1.40 *	20.8	266*		
	Low	5.8*	0.82	1.08 *	20.0	208*		
2018	High	7.0*	1.02	1.24 *	21.0	293*		
	Low	5.4*	1.04	0.95 *	19.0	231*		
2020	High	6.7*	0.58*	1.29 *	22.6	279*		
2020	Low	4.7*	0.38*	1.02 *	20.2	189*		
INTERMEDIATE YEARS								
2017	High	7.2*	1.17	1.62 *	22.4	323*		
2017	Low	5.4*	1.10	1.25 *	20.0	239*		
2021	High	7.1*	1.06	1.45 *	19.2	230*		
	Low	3.9*	0.94	1.20 *	20.2	212*		
Significance (p-value 0.05)								
Rainy vs Dry		*	ns	*	ns	*		
Rainy vs Intermediate		ns	ns	*	ns	ns		
Dry vs Intermediate		ns	ns	*	ns	ns		

TABLE 5.1. Plant and berry variables at harvest according to vigour and group of years

\* Indicates significant differences between vigour zones according to Fisher (p-value 0.05).

Changes in berry volume as a function of sugar accumulation in standardized berries allowed to distinguish two clear phases in a bilinear model (Figure 5.2, Table 5.2).

The first phase was characterized by an increase in sugar content and berry volume, while during the second stage sugar content kept increasing at constant berry volume. For the years 2015 (rainy) and 2017 (intermediate) the bilinear adjustment could not be performed as sampling for these years started later and/or showed high variability (i.e., 2017).



FIGURE 5.2. Bilinear fit of normalized berry volume as a function of sugar content per berry (TSS) for the different treatments and groups of years (rainy, dry and intermediate).

Each point represents a sample of 100 berries. Second x-axis (red) represents the days after veraison (DPV).

Year	Vigour	Slope (mg <sup>-1</sup> )	R <sup>2</sup>	Breaking point	Phase	Phases duration (days)		
				TSS	1	п		
				(mg.NBerry⁻¹)	I	11	IDP	
RAINY YEARS								
2014	High	0.0034 a	0.82	158 * a	27 a	12 b	39	
	Low	0.0040 a	0.97	147 * a	25 a	13 b	38	
2015	High	n.d	n.d	n.d	n.d	n.d	n.d	
	Low	n.d	n.d	n.d	n.d	n.d	n.d	
2019	High	0.0029 b	0.92	140 b	21 b	27*a	48	
	Low	0.0026 b	0.96	142 b	23 b	24*a	47	
DRY YEARS								
2016	High	0.0030	0.87	169 a	41* a	8* b	49 a	
	Low	0.0033	0.96	173 a	36* a	13* b	49 a	
2018	High	0.0031	0.95	155 * b	33*b	11* a	44 b	
	Low	0.0028	0.83	168 * b	30* b	15* a	45 b	
2020	High	0.0029*	0.91	165 * b	38 a	11*ab	49 a	
	Low	0.0041*	0.97	157 * b	38 a	9 * ab	47 a	
INTERMEDIATE YEARS								
2017	High	n.d	n.d	n.d	n.d	n.d	n.d	
	Low	n.d	n.d	n.d	n.d	n.d	n.d	
2021	High	0.0047	0.89	128 *	32	13	45	
	Low	0.0045	0.92	132 *	30	12	42	
Significance								
Rainy vs	dry years	n.s	-	<0.0001	< 0.0001	0.0004	0.013	

**TABLE 5.2.** Parameters of the bilinear adjustment between berry volume and sugar content for the different treatments and groups of years (rainy, dry and intermediate).

\* Indicates significant differences between vigour zones according to Fisher (p-value 0.05). Letters indicate differences between years of the same climate group (Fisher, p-value 0.05). Slope: growth/sugar loading ratio. Phase I: accumulation of sugars simultaneously with the accumulation of sugars. Phase II: Sugar accumulation at constant volume. TDP: Total Duration Phases. Sugar accumulation over the range 6 - 20 °Brix.

The variations of the parameters among the groups of years were addressed considering only the wet and dry groups, as solely one year of the intermediate group could be fitted. Globally, all model parameters except the slope, which corresponded to the ratio of berry volume:sugar accumulation, varied among the wet and dry years (Table 5.2). The slope ranged about 0.0032 mg<sup>-1</sup>. However, it should be noted that berry volume at 6-10 °Brix (initial point of the slope) represented on average 75% of the maximum berry volume for wet years vs 60% for dry years. The breaking point (BP) at the end of berry volume increment (end of Phase I), was reached at lower TSS (146 mg.NBerry<sup>-1</sup> TSS) under wet years compared to dry years (164 mg.NBerry<sup>-1</sup> TSS). Lastly, the total duration

of phases I and II (TDP) was lower for rainy years (43 days) compared to dry years (47 days), mainly due o the lower duration of Phase I, as Phase II tended in contrast longer for wet years.

The parameters of the model also differentially varied between H and L and between years, depending on the group of years.

For the rainy years (Figure 5.2 AD, Table 5.2) there were no differences between the vigour zones for any of the parameters. However, differences were observed between the years in this group: 2014 had a higher Slope, Breaking Point, and longer duration of Phase I compared to 2019.

For dry years (Figure 5.2 BE, Table 5.2), the slope was higher for L than H in 2020. BP was also higher for L compared to H in 2018, while it was the reverse in 2020. The total duration of sugar accumulation did not differ between the two vigour zones, although the duration of each phase showed differences between the vigours. H displayed a longer Phase I and lower Phase II than L in 2016 and 2018. In contrast, Phase II tended to longer for the H zone in 2020. BP and durations of Phases I and II also varied among years in this group. BP was higher in 2016 compared to 2018 and 2020. TDP was higher in 2016 and 2020 compared to 2018, mainly due to a longer Phase I. For the intermediate year, only BP differed between vigours, with higher value for L than H.

An exploratory analysis of the results (principal component analysis, PCA), including all weather and microclimatic variables (WA BB\_BL; WA BL\_V; WA V\_H; Tm BL\_V; Tm V\_H; VPD), plant water status variables (WSI BL\_V; WSI V\_H), berry weight at harvest (Bw) and the berry development model parameters (Slope; BP; TDP) for all years (2014 - 2021) and vigour situations, is presented in Figure 5.3 A. The two PCA axes accounted for 63.9% of the variability (axis 1: 41.3% and axis 2: 22.6%). The years were grouped in the same way as described above, forming three distinct groups on axis 1. On the right were the years of group 1 (rainy, 2014, 2015 and 2019) and on the left were the years of group 2 (dry years, 2016, 2018 and 2020). The Group 1 of years was found to be associated with high water availability during the fruit set to veraison period (WA BL\_V)
or during ripening (WA V\_H), high atmospheric demand from bud break to bloom (VPD BB\_BI) and high berry weight (Bw). On the other hand, the years of Group 2, were associated with higher mean temperature during ripening (TM\_V\_H), higher water deficit from fruit set to veraison (WSI\_BL\_V) or during ripening (WSI\_V\_H), higher vapor pressure deficit (VPD V\_H), higher breakpoint (BP) and higher TDP. Intermediate years (2017, 2021) were between those two groups on axis 1. On the axis 2, water availability from bud break to bloom (WA BB\_BI) was opposite to leaf:fruit ratio. The years with the highest pre-flowering water availability (WA BB\_BL) were in the upper part of axis 2 (2015, 2018, 2020 and 2021). Those years also tended to display lower leaf:fruit ratio (Table 5.1). The years 2014, 2016, 2017 and 2019, showed the opposite trend.

The correlations between the model parameters (slope, TDP and BP) and the weather, plant and berry variables were analyzed for all years at global level (Fig. 5.3 B). Significant correlations (higher 0.45) were obtained only for variables related to water statut (WA V\_H, WSI BL\_V, WSI V\_H) and berry weight (Bw). Correlations between the Slope and those variables were low (r<sup>2</sup><0.19, p-value > 0.05). However, high correlations (r<sup>2</sup>>0.5, p-value < 0.05) were for the two other parameters. Total Notably, duration of Phases I and II (TDP) showed negative correlation with water availability (WA V\_H) and positive correlation with the Water Stress Index (WSI BL\_V and WSI V\_H). The BP was positively correlated with WSI V\_H and negative whit Bw.



FIGURE 5.3. Principal component analysis for the evaluated variables and all years (2014

## to 2021)

A- Principal component analysis (PCA) for all evaluated variables and all years (2014 to 2021). Weather and microclimatic variables: WA BL\_V: Water availability during the period bloom to veraison; WA V\_H: Water availability during the period veraison to harvest; VPD BB\_BL: Vapour pressure deficit during the period bud break to bloom; VPD BL\_V: Vapour pressure deficit during bloom to veraison; VPD V\_H: Vapour pressure deficit during the period veraison to harvest; Tm BL\_V: Mean temperature during the period bloom to veraison and Tm BL\_V: Mean temperature during the period veraison to harvest, Plant variables: WSI BL\_V: Water stress index from bloom to veraison; WSI V\_H: Water stress index from veraison to harvest, leaf/fruit ratio; Berry development variable: Bw: Berry weight (g); Slope: growth/sugar loading ratio (mg-1); BP: break point (mg.Nberry-1); TPD: Total duration of phases (phase I + phase II, days). B-Pearson correlation coefficient for all years.

# 3.2.<u>BERRY DEVELOPMENT RESPONSE TO NITROGEN, WATER AND LEAF REMOVAL</u> TREATMENTS FOR A WET AND DRY YEAR

For two contrasting years in terms of water supply (wet group 1: 2019 and dry group 2: 2020), different water supply/restriction, nitrogen and leaf removal treatments were applied according to vigour condition. As observed previously, yield was higher when the rain or the vigour were higher (Table 5.3). Yield also varied among the treatments for each year and vigour conditions. In the H zone, H-L had the lowest yield in both years, while increasing berry weight. The H-N maintained yield in a rainy year but reduced yield in a dry year compared to H. Water restriction (H-W) did not change yield. The L+N treatment increased yield (16 %) compared to L in 2019. The treatment with additional water (L+W) applied during the dry year resulted in an 80% higher yield per vine than the control, due at least partly, to higher berry weight. None of the treatments modified the leaf:fruit ratio, except the L+W treatment which reduced this ratio for the dry year (2020). Water restriction (H-W) during the wet year or water supply (L+W) during the dry year increased sugar concentration compared to the controls. However, water or nitrogen restriction (H-W, H-N) during dry year had the reverse effect leading to lower Brix values.

During rainy year (2019), the average growth/sugar loading ratio (Slope) was 0.0028 mg<sup>-1</sup>, with no differences between treatments of high and low vigour zones, except for the H-N treatment for which this ratio was lower (Figure 5.4 A; Table 5.4). The break point (BP) varied by about 8 mg.NBerry<sup>-1</sup> between the treatments: it was higher for the defoliated treatment (H-L) in H zone, while it was lower for the N supply treatment (L+N) in L zone. The total duration of Phases I and II (TDP) decreased compared to the control in H zone, both for H-W treatment (-5 days) and for H-L treatment (-2 days), mainly due to the shortening of Phase II. Despite no difference in TDP was observed for other treatments in H (H-N) and L (L+N) zones compared to the controls, the durations of Phases I and II were different, with a more prolonged Phase I (and shorter Phase II) for H-N and a more prolonged phase 2 (and shorter Phase I) for L+N.

Treatmonte	Y Leaf/fruit (kg/vine) ratio		Bw	DDIV					
Treatments			(g)	DRIA					
2019 (Rainy)									
Н	7.0 a	1.36 a	1.72 b	22.0 b					
H-W	7.0 a	1.25 b	1.70 b	23.2 a					
H-N	6.9 a	1.20 b	1.71 b	22.2 b					
H-L	4.9 b	1.01 b	1.85 a	22.6 b					
L	5.3 b	0.61 b	1.57	21.2					
L+W	n.d.	n.d.	n.d.	n.d					
L+N	6.3 a	0.84 a	1.60	21.2					
2020 (Dry)									
Н	6.7 a	0.59	1.34 b	22.6 a					
H-W	6.5 a	0.57	1.33 b	21.0 b					
H-N	5.6 b	0.54	1.18 c	20.8 b					
H-L	5.2 b	0.57	1.44 a	22.4 a					
L	4.7 b	0.37 b	1.02 b	20.2 b					
L+W	8.4 a	0.80 a	1.53 a	22.3 a					
L+N	4.3 b	0.50 b	0.95 b	20.1 b					

**TABLE 5.3.:** Plant and grape composition at harvest by treatment and contrasted years (rainy vs. dry)

Contrasting years: 2019 (rainy) vs 2020 (dry). Average values for samples of 300 berries. Different letters indicate significant differences between treatments for each year according to Fisher (p-value 0.05).

During dry year (2020), the slope ranged from 0.0029 mg<sup>-1</sup> in H zone to 0.041 mg<sup>-1</sup> in L zone for the control treatments (Figure 5.4 BD; Table 5.4). While the slope did dot vary between the treatments in H zone, it decreased under water supply (L+W) in L zone. The break point differentially varied between treatments compared to the controls for both zones. It increased by 18 mg.NBerry<sup>-1</sup> for H-L and H-W, while it decreased by 10 to 25 mg.NBerry<sup>-1</sup>, respectively for L+W and H-N (Table 5.4). Lastly, the total duration of phases 1 and 2 (TDP) varied among the treatments for the two zones. In H zone, TDP was shorter for H-W (ca. -5 days), due to the shortening of phase 2. In L zone, TDP increased (ca. 4 days) when water was supplied for L+W. This increase was explained by a higher duration of phase 2 that was not compensated by the shortening of phase 1. As observed in 2019, TDP for H-N was similar to the control, in spite of variations of the durations of the two phases (shorter phase 1 and longer Phase II).



FIGURE 5.4. Bilinear fit of sugar accumulation as a function of berry volume normalized

by vigour, treatment and year.

A: High vigour 2019. B: High vigour 2020. C: Low Vigour 2019. D: Low vigour 2020. Each point represents a sample of 100 berries. Second x-axis (red) represents the days after veraison (DPV).

<b>TABLE 5.4.</b> Parameters of the bilinear adjustment of normalized sugar accumulation for
the different treatments for contrasting years (2019 and 2020)

Trootmonts	Slope P2		Breaking point	Phases duration (days)			
realments	(mg <sup>-1</sup> )	<b>N</b> -	TSS (mg.Nberry <sup>-1</sup> )	I	П	TDP	
			2019 (Rainy)				
Н	0.0029 a	0.92	140 b	21 b	27 a	48 a	
H-W	0.0028 a	0.96	142 b	22 b	21 c	43 c	
H-N	0.0023 b	0.83	143 b	25 a	24 b	49 a	
H-L	0.0030 a	0.92	150 a	22 b	24 b	46 b	
L	0.0026	0.96	142 a	24 a	23 b	47	
L+W	n.d	n.d	n.d	n.d	n.d	n.d	
L+N	0.0032	0.83	130 b	21 b	26 a	47	
			2020 (Dry)				
Н	0.0029	0.91	165 b	38 a	11 b	49 a	
H-W	0.0030	0.95	181 a	39 a	6 c	45 b	
H-N	0.0033	0.85	158 c	36 b	17 a	53 a	
H-L	0.0033	0.93	185 a	39 a	10 b	49 a	
L	0.0041 a	0.91	157 b	38 a	9 b	47 b	
L+W	0.0033 b	0.94	167 a	33 b	17 a	50 a	
L+N	0.0042 a	0.96	159 b	36 a	9 b	45 b	

Slope: growth/sugar loading ratio. Phase I: accumulation of sugars simultaneously with the accumulation of sugars. Phase II: Sugar accumulation at constant volume. TDP: Total duration phase. Sugar accumulation range 6 - 20°Brix. Different letters indicate significant differences between treatments for each year and each vigor condition according to Fisher (p-value 0.05) n=3

Sugar concentration in individual berries ranged from 4.0 to 16.0 Brix for the same sampling date, which allowed grouping berries into six classes (4.0-6.0; 6.0-8.0; 8.0-10.0; 10.0-12.0; 12.0-14.0 and 14.0-16.0 °Brix) (Figure 5.5). Berry distribution at the onset of ripening was modified according to year, viguor and treatments. The year effect marked a more left-skewed distribution in 2019 and more centered during 2020 (p-value: < 0.01). The vigor's influence on the distributions depended on the year's climatic characteristics. The distribution of berries was more skewed to the left at high vigor, while at low vigor it was more centered (Figure 5.5 AB; p-value: < 0.05) for 2019. During 2020, there were no differences between vigor (Figure 5.5 CD).



FIGURE 5.5. Berry distribution at the beginning of ripening according to sugars

## accumulation in 2019 and 2020.

Lines and nodes represent the relative frequency for each brix category. High vigor condition: black (high control); red (nitrogen restriction); blue (water restriction); green (leaf removal). Low vigor treatments: gray (low control), violet (nitrogen supply) and orange (water supply). A: High vigor treatments in 2019. B: Low vigour treatments in 2020. C: High vigour treatments in 2020. D: Low vigour treatments in 2020. The x-axis represents Brix categories, increasing by 2 Brix. Straight brackets indicate that the value is included, and curved brackets indicate that it is not included.

The nodes in the network plots represent the treatments, and the node connections indicate whether the distributions are different.

The treatments also modified the degree of maturity at the onset of ripening as a function of the climatic characteristics of the year (rainy vs. dry). For 2019, the treatment with water restriction (H-W) was the treatment that, for the same date, presented the greatest advance in ripening, with the highest percentage of berries with Brix above 10.0° (41%). These changes in the distribution made H-W different from the control (H) and the rest of the treatments. In addition, the H-W and H-N treatments had the greatest dispersion in the distributions (> integral). The other treatments (H-L and H-N) showed no change in the ripening distribution with respect to the control. In H-L, 46% of the

observations were between 6.0 and 8.0 °Brix and 48% between 8.0 and 12.0 °Brix, indicating a lower dispersion in the observations. While in H-N, the highest proportion of berries were between 8.0 and 10.0 °Brix (32%), and 51% were below 8.0 °Brix. In the low vigor, N supply did not differ from the control in the distribution or dispersion of observations. In both treatments (L, L+N), 57% of the berries had values between 10.0 and 12.0 °Brix.

In dry year (2020), all treatments in zone H (H-N, H-W and H-L) showed a delayed ripening compared to the control. Although the control presented a greater advance in ripening, it was the one that presented the greatest variability in the observations. For the H-N treatment, 51% of the observations were between 4.0 and 10.0 °Brix (Figure 5.5). The variability of observations was lower for treatments H-W and H-L, with observations concentrated between 8.0 and 12.0 °Brix (66% and 80%, respectively). For the low vigor zone, treatments differed in the degree of maturity. Water supply (L+W) showed a skewed distribution to the right with 74% of berries between 10.0 and 14.0 °Brix. These results indicate an acceleration of ripening with greater dispersion in the distribution in the L+W treatment. The L+N treatment showed a delay in ripening and less dispersion compared to the control. The L+N showed a slight leftward bias with the highest proportion of berries between 6 and 8 Brix (37%).

Berry weight at the onset of ripening ranged from 0.5 to 1.8 g for the same sampling date. The year effect marked a lower dispersion in the distributions in 2019 (pvalue: < 0.01). The influence of treatments seems not to have modified the distribution of berries except for the irrigation treatment (L+W). This treatment (Figure 5.6 C) in a dry year presented a rightward bias (with higher berry weights) unlike the control situation (p-value: < 0.01).



FIGURE 5.6. Berry distribution at the beginning of ripening according to berry weight in

#### 2019 and 2020.

Lines represent the relative frequency for each berry weight. High vigour condition: black (high control); red (nitrogen restriction); blue (water restriction); green (leaf removal). Low vigour treatments: gray (low control), violet (nitrogen supply) and orange (water supply). A: High vigour treatments in 2019. B: Low vigour treatments in 2020. C: High vigour treatments in 2020. D: Low vigour treatments in 2020. The x-axis represents berry weight.

The PCA analysis (Figure 5.7 A) for all treatments, weather and micro climatic variables, plant and berry variables for the two contrasting years (2019 vs. 2020) explained 66.7% of the total variance. Axis 1 explained 47.6% of the variance and was mainly related to weather and microclimatic variables (TMx V\_H; ND30; VPD BL\_V; VPD V\_H and PAR V\_H) and plant variables (WSI V\_H; leaf/fruit ratio and Bw). On axis 2 (19.1%), water deficit before veraison (WSI BL\_V) was opposite to photosynthesis (Photo) and trunk diameter (TD). Total duration of Phase I and II (TDP), break point (BP), integral of Brix, integral of Bw, on the right, were correlated. Slope was opposite of the rest of the model parameters. The rainy (2019) and dry (2020) years were grouped opposite each other on axis 1. The treatments within each dry/wet group were also spread along axis 2.

For both years, treatments of low zone were mostly correlated with water deficit (WSI BL\_V), except L+W in 2020 (dry year).



## Figure 5.7. Principal component analysis for the evaluated variables, treatments, and

## contrasting years (2019 vs. 2020).

Microclimate variables: WA BL\_V: Water availability during the period from bloom to veraison; WA V\_H: Water availability during the period from veraison to harvest; VPD BB\_BL: vapor pressure deficit during the period from bud break to bloom; VPD BL\_V: vapor pressure deficit during the period from bud bloom to veraison; VPD V\_H: vapor pressure deficit during the period from veraison to harvest. Plant variables: WSI BL\_V: Water stress index from bloom to veraison; WSI V\_H: Water stress index from bloom to veraison; ST: Starch in trunk: mg gMS<sup>-1</sup>; Photo: photosynthesis ( $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>); Slope: Slope (mg<sup>-1</sup>); BP: Break point (mg<sup>-1</sup>); TDP: Total duration phases: (phase I + phase II, days). B-Pearson correlation coefficient for all years.

The correlations between the variables and the model parameters for the two years (2019, 2020) are presented in Figure 5.7 B. As nitrogen status at harvest (SPAD), did not significantly correlate with the model parameters, they were not shown in the table. High correlations (r<sup>2</sup>> 0.5) were observed between the parameters of the modeland the other weather/microclimatic and plant variables. Indeed, the growth/berry sugar loading (Slope) was negatively correleted with trunk diameter (TD), photosynthesis (Photo) and berry weight (Bw) at debut of maduration. Slope was positively correlated with days with temperature above 30 °C (ND30) and leaf water vapor pressure deficit (VPD Bl\_V). Total duration phases (TDP) is negatively correlated with PAR V H, VPD Bl V, VPD V H, WSI V H,

strach in trunk (ST) and TD. The dispersion in berry weight at the beginning of ripening (I Bw) showed positive correlations with microclimatic parameters (PAR V\_H, VPD BI\_V, VPD V\_H), water stress (WSI V\_H) and plant reserves (ST). Similarly, dispersion in sugar accumulation was favored by VPD V\_H, WSI V\_H and ST.

## 5. DISCUSSION

The potential berry size highly depends on the early stages of fruit formation when cell division and cell enlargement occur (Ojeda et al., 1999; Ristic and Iland, 2005). This stage is susceptible to climatic factors such as temperature and water and assimilates supplies (Ebadi et al., 1996; Ojeda et al., 2001). Similarly, to other studies (Ollat et al., 2002; Ojeda et al., 2002), water availability impacted berry growth in our study (Figure 4). High water availability from bloom to veraison (WA Bl V) during rainy years (2014, 2015, and 2019) increased berries growth, which reached at the end of the green growth stage 70% of the maximum weight vs 40% of the maximum weight for dry years (2016, 2018 and 2020) (Figure 3 and 4). Part of these differences between groups may be due to the late berry measurements for two of the rainy years (2014 and 2015), which only started 15 DAS vs 0-5 DAS for all other years. For dry years, berries volume doubled during the ripening period, as reported in other studies (Coombe, 1992). The water deficit can accelerate the senescence of plant tissues (Prieto et al., 2010), including mesocarp cells (Bonada et al., 2013). Temperature is another factor affecting the onset and rate of cell death (Bonada et al., 2013). Yet, the integrity of the cell membrane conditions the water fluxes in and out of the berry (Tilbrook and Tyerman, 2009). Ultimately, the higher temperature in the bunch zone due to water deficit and leaf senescence during dry years may have promoted berry shrivelling, especially in L. In contrast, berry water content remained rather stable during rainy years, as Bonada et al. (2013) reported for wellwatered grapevine plants. The functionality of vascular tissues and water balance in the berry have implications on sugar flow toward the berries and their impacts on berry size (Zhang et al., 2017). Differences in sugar accumulation were also observed between wet and dry years. For rainy years, total duration of sugar loading was between 39-48 days, while for dry years sugar loading duration was 10 days longer (Table 5.1). Severe water

deficit can cause a blockage of ripening, associated with the blockage of photosynthesis with an impact on sugar accumulation (Deloire et al., 2005).

Sugar transport occurs by mass flow that transports water and solutes simultaneously through phloem vessels (Keller et al., 2017). According to Shahood et al., (2020), after reaching the maximum sugar accumulation (1.1 M), phloem discharge does not continue and the possible uncoupling between water and sugar fluxes is due to statistical biases of a heterogeneous berry population. The maximum berry weight was reached earlier during rainy years than dry years, resulting in a greater apparent uncoupling between water and sugar fluxes. The stability of maximum berry weight while sugar continued to accumulate could have resulted from the maintenance of water flow from the plant to the berry at this late ripening stage (Greer and Rogiers, 2011; Keller et al., 2014) or may be explained by an increase in berry heterogeneity at the population level (Shahood et al., 2020). In this latter case, the apparent stability of berry weight would rather rely on the mix of still-growing berries with non-growing or shrivelling berries.

Normalization of berry volume and sugar accumulation allowed us to characterize the grape ripening in two phases through a bilinear fit, the Phase I corresponding to a simultaneous water and sugar accumulation, with BP corresponding to the sugar content at which berry weight was maximum, and the Slope parameter corresponding to the water:sugars accumulations ratio. Phase II followed this phase, corresponding to sugar accumulation at constant berry volume. Phase I was shorter in wet years and Phase II was longer compared to dry years. Therefore, higher WA favoured a greater apparent asynchrony between water and sugar loading into the berry. In addition to WA, the leaf:fruit ratio also affected the duration of the phases (Figure 5.4) by lengthening the duration of Phase I and shortening Phase II. As the leaf:fruit ratio was negatively correlated with the WA before bloom (WA BB\_BI, Figure 5.4 A), this latter may indicate the early water deficit experienced by the plant. Variations of breakpoint were highly related to the variations of Phase I. Although the durations of the phases and the breakpoint differed according to the type of year (wet/dry), it is noteworthy that there were no clear differences for each groups of years between the high/low vigour zones (Table 1). Ultimately the water:sugar accumulation ratio (Slope) was relatively stable, indicating a strong genotypic effect.

Then, the impact of different management practices including water supply/restriction, nitrogen supply, or leaf removal on the parameters of the model were tested for two contrasted years (wet/dry). Similarly, to the global analysis on the 8 years, water limitation (H-W) decreased the duration of phase II, while water supply (L+W) had the reverse effect. The moderate water deficits strategy proposed in this trial (L+W) slightly delayed the total duration of berry ripening (+4 days) but ultimately permitted to increase both berry size and sugar accumulation (Figure 5.5). The higher carbohydrate supply (ST, Photo) was positively correlated to P.I (and BP). Starch reserves are strongly mobilized from perennial structures to growing organs from budburst to flowering (Zufferey et al., 2015). Then, leaf photosynthesis ensures carbon feeding (Mullins et al., 1992; Hieke et al., 2002). Our result suggests that high sugar availability may favor the synchrony between berry growth and sugar loading similarly to water deficit, maybe by reducing the asynchrony of development between the berries. Sugars play a major role in the induction of inflorescences during the first year (year n), flower initiation during the second year (year n+1), meiosis during sexual reproduction (year n+1) and berry sugar loading (Lebon et al., 2008). A higher carbon pool at these stages could favour the synchrony of flower formation along the bunch. Lastly, as observed over 8 years, the water:sugar accumulation ratio (Slope) only poorly varied between the treatments applied during the wet/dry years (Table 5.2).

Nitrogen and carbon are two macronutrients that determine plant vigour (Miliordos et al., 2022) and affect grape development (Keller 2010). In our study, only the N restriction treatment (H-N) impacted the parameters of the berry development model, and in opposite ways for the wet and dry years. Additionally, the restriction of N reduced the weight of the berries and Brix at harvest but only for the wet year (Supplement 3). Ultimately, no correlation was observed between SPAD and model parameters. The role of nitrogen on sugar accumulation is controversial. Assimilation of NO<sub>3</sub><sup>--</sup> and NH<sub>4</sub><sup>+</sup> is regulated by internal factors (C metabolism) and external factors, such as water availability (Keller and Koblet, 1995). Adverse environmental conditions (drought) can

restrict photosynthetic activity and reduce N assimilation and thus amino acid synthesis (Stefanello et al., 2020). Our results indicate that sugar accumulation and berry growth dynamics are more determined by water availability and carbon metabolism (photosynthesis and ST) than by nitrogen availability (Figure 5.7 B).

Lastly, the pre-bloom leaf removal treatment in this trial increased bunch exposure and reduced bunch disease incidence as reported by several articles (Poni et al., 2006; Palliotti et al., 2011; Pereyra et al., 2022). An increase in berry weight and TSS was also observed for both years, associated for the wet year only to a lower P.II and total ripening duration (-2 days) compared to the control. Leaf removal at this stage decreases the proportion photosynthetic active leaves. Therefore, carbon allocation to berries production decreases, favoring fruit abscission (Frioni et al., 2018). Higher photosynthetic efficiency of remaining leaves has been reported under such treatments (Poni et al., 2006; Risco et al., 2013). This double effect (more efficient leaves and fewer fruits per bunch) would explain sugars' higher and faster accumulation in the remaining berries (Kliewer 1970; Arrillaga et al., 2020). Non defoliated plants reach veraison with large, old and potentially also shaded leaves that contribute less to photosynthesis (Poni et al., 1994) and also shade the clusters. In contrast, higher secondary leaves promote the accumulation of sugars in the fruit and starch in the wood after veraison (Vasconcelos and Koblet, 1990). Thus, leaf removal and yield reduction in this treatment were able to fasten sugar accumulation, as also reported by Previtali et al., (2021).

## 6. CONCLUSIONS

This study addressed the dynamics of berry growth and sugar accumulation as a function of the influence of climate and cultivation techniques during eight consecutive seasons.

Modification of the leaf:fruit ratio through pre-bloom leaf removal systematically increased berry size and sugar content regardless of the type of year. The role of nitrogen in berry growth was highly dependent on the year's water conditions. Under water restriction, the berry accumulated more sugar compared to the control, especially in wet

years. Deficit irrigation had the highest Bw, TSS and Brix values compared to all other treatments.

The dynamics of berry growth and sugar accumulation showed a bi-linear behavior that differed according to the climatic characteristics of the year. Sugar and water accumulation (slope) was a genetically determined factor. The water availability of the year on one side and plant carbohydrate status on the other side were the factor that mostly modified the duration of phase I and phase II in an apparent opposite way. Indeed, low water availability, such as higher carbon supply (resulting from higher leaf:fruit ratio, higher reserves pool or leaf photosynthetic rate), seemed to favor the synchrony between berry growth and sugar loading. In contrast, the role of nitrogen on berry ripening was not straightforward and highly depended on water availability during the year.

The role of carbohydrates on early berry development and their participation in berry population variability at the whole plant and bunch level should be further investigated.

# 7. SUPPLEMENTARY INFORMATION

Yield Leaf area Leaf:fru									
Year Vigour		(kg/vine)	$(m^2/vine)$	ratio					
RAINY YEARS									
2014	High	6.7	8.50*	0.30					
2014	Low	5.7	3.54*	0.34					
2015	High	8.3*	9.79*	0.23					
2015	Low	6.8*	6.78*	0.23					
2010	High	6.9*	9.34*	0.27*					
2019	Low	5.3*	3.13*	0.21*					
		DRY YEAR	S						
2016	High	7.5*	7.34*	0.31					
	Low	5.8*	4.72*	0.36					
2010	High	7.0*	6.90*	0.18					
2018	Low	5.4*	5.55*	0.16					
2020	High	6.7*	3.87*	0.22					
2020	Low	4.7*	1.77*	0.27					
		INTERMEDIATE	YEARS						
2017	High	7.2*	8.46*	0.27					
	Low	5.4*	5.87*	0.24					
2021	High	7.1*	7.38*	0.25					
2021	Low	3.9*	3.61*	0.22					

# SUPPLEMENT 5.1. Plant parameters grouped by type of year (rainy, dry and

intermediate)

\* Indicates significant differences between treatments for each year according to Fisher (p-value 0.05).

**SUPPLEMENT 5.2.** Plant parameters for the different treatments for contrasting years (2019 and 2020)

Treatments	Y (kg vine⁻ ¹)	Leaf (m² vine-1)	WA (mm)	TD (mm)	ST	Photo	SPAD	
		2019	(Rainy)					
HV	7.0*	9.3*	474	54	74	12.4*	24	
H-W	7.0*	8.3*	234	55	61	14.1*	30	
H-N	6.9*	8.2*	474	54	51	9.8*	22	
H-L	4.9*	6.9*	474	53	56	12.8*	25	
LV	5.3	3.1*	474	43	52	7.3*	22	
L+W	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
L+N	6.3	5.2*	474	41	46	10.1*	25	
	2020 (Dry)							
HV	6.7*	3.8*	160	55	132*	10*	24	
H-W	6.5*	3.5*	100	57	126*	13*	24	
H-N	5.6*	3.1*	160	54	100*	6*	22	

H-L	5.2*	2.4*	160	56	141*	10*	24
LV	4.7*	1.7*	160	40	92*	6*	23
L+W	8.4*	5.3*	302	45	128*	14*	25
L+N	4.3*	1.7*	160	42	114*	9*	23

\* Indicates significant differences between treatments for each year according to Fisher (p-value 0.05).

SUPPLEMET 5.3.: Statistical values for distribution curves

Trootmonts	2019						
meatments	HIGH VIGOR						
2019 (Rainy)		D/p	Н	H-W	H-N	H-L	
Н	60.8	Н	0.002	0.33	0.15	0.09	
H-W	74 3	H-W	0.50	0.0003	0.42	0.33	
H-N	71.3	H-L	0.86	0.0002	0.58		
	71.5 E4 Q		L	OW VIGOR			
П-L	54.0	D/p	L	L+N	L+W		
L	66.1	L		0.11	n.d.		
I +\//	71 4	L+N	0.70		n.d.		
L + N	n d	L+VV	n.d.	n.d.			
	n.u.			2020			
2020 (	Dry)			2020			
Н	158		H	igh vigor			
H-W	75.4	D/p	Н	H-W	H-N	H-L	
H-N	81.4	H-W	0.0001	0.46	0.49	0.46	
H	61 /	H-N	0.0003	0.10	0.20	0.10	
11-6	01.4	H-L	0.0007	0.24	0.75		
L	82.2	LOW VIGOR					
L+W	92.1	D/p	L	L+N	L+W		
		L		0.27	0.44		
L+N	69.4	L+N	0.01	0.0001	0.42		

Treatments: H: High vigor control; H-W: High vigor water restriction; H-N: High vigor nitrogen restriction; H-L: High vigor leaf removal; L: Low vigor control; L+W: Low vigor water supply; L+N: Low vigor nitrogen supply. Integral under the distribution curve. D: Kolmogorov-Smirnov test statistic value. P-value corresponding to the pair of comparisons. n.d.; No data



SUPPLEMENT 5.4. Evolution of berry weight and sugar accumulation for 2019 according to vigor and treatments



**SUPPLEMENT 5.5.** Evolution of berry weight and sugar accumulation for 2019 according to vigor and treatments

# **CHAPTER VI**

# Summary- Conclusions-

Perspectives

## 1. ENGLISH

The term vigour is a concept that refers to the vegetative growth and yield of the vine. These two components of vigour are usually not spatially homogeneous. The heterogeneity of vigour can be evaluated at different scales, i.e., at the level of regions, vineyards within a region, at the vineyard level, or at the level of the shoots of a plant. Indeed, a very high spatial variability of vigour can lead to yield losses and alterations in the bunches' health and the grape's qualitative components. Reaching plant balance (in terms of source/sink ratio) allows winegrowers to optimize yields with adequate grape quality and maintain sufficient carbon reserves for the following season.

Although the concept of spatial variability of vigour is not new to grape growers, the level of vigour heterogeneity in a vineyard is poorly adressed. However, this variability can be easily quantified with the development of new technologies and precision agriculture tools applied in viticulture. Remote sensing (thermal cameras, multispectral imaging), GPS (global positioning system), data analysis and information interpretation systems, variable application equipment (VRA), yield monitoring systems, real-time data, etc. make it possible to characterize the natural variability of vigour and adapt cultivation practices accordingly. Among remote sensing techniques, aerial or satellite images processed from red and infrared reflectance to generate vegetative indices such as NDVI (Normalized Difference Vegetative Index) have been widely used to assess regional heterogeneity in plot vigour of both annual and perennial crops.

According to the literature (Bramley & Hamilton, 2004, van Leeuwen et al., 2018; Gatti et al., 2021), intra-plot variations in vegetative growth, root system, yield, grape ripening and berry health, among others, depend on variations in soil characteristics. Indeed, the soil's physical, chemical and biological properties, such as temperature, depth, texture, structure, and microorganism activity, determine the volume of soil explored by the roots, the level of available nutrients, and the soil's capacity to store water. In addition, the climate in interaction with the soil is a key factor of plant functioning. Soil-climate parameters influence vine growth, vigour, physiology and ripening. Climatic variability of critical parameters such as temperature and water availability determine differences in grape production and quality from year to year.

The spatial variability (within a plot) of grape yield and composition is associated with stable seasonal physical characteristics (soil and topography) interacting with seasonal abiotic and biotic conditions (climate, water and nitrogen availability, presence of diseases) and the agronomic management strategy. Water and nutrients (mainly nitrogen) are well-known factors conditioning plant development, growth and yield. Water availability is one of the most critical factors affecting vine vegetative development. In case of water deficit, aerial and root growth may be limited due to decreased cell turgor and increased resistance to root penetration in dry soils. Ultimately, the physical characteristics of the soil, such as its texture, structure and depth, are important factors to consider because of their effects on root temperature and on the supply of water and minerals to the plant.

Knowledge of the specific causes that generate spatial variability of vigour is important for grape growers to optimize production and reduce costs. The objective of the present study was to identify the physical factors responsible for intra-plot heterogeneity of vigour in a representative vineyard in southern Uruguay during eight consecutive seasons. This vineyard planted with cv. Tannat (red) was selected for its significant east-west variability in plant vigour. In this plot, the Ravaz index averaged around 14, indicating that plant vigour was outside the optimal range for this variety (6-10). In addition, the coefficient of yield variation at the plot level was 47%. Vigour was assessed at veraison (January in the southern hemisphere) during three years (2015, 2016 and 2017) using the normalized difference vegetation index (NDVI), calculated from aerial imagery (altitude 620 m and speed 50 m/s). High-resolution (0.2 m) multispectral images were obtained over the ground to define three vigour zones: high (HV), medium (MV) and low (LV). Each NDVI class (high, medium and low) was systematically located in the same areas of the vineyard each year. In winter 2020, trunk diameter was assessed to corroborate the interannual stability of vigour, and positive correlations were established between NDVI and vegetative growth parameters (pruning weight, leaf area and trunk diameter). In winter 2015, a complete description of soil physical and chemical

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parameters was carried out. Samples were taken at three depths (0-20, 20-40 and 40-60 cm) in which Bray phosphorus, pH, exchangeable calcium, magnesium, potassium and sodium, organic matter (OM), nitrate, cation exchange capacity (CEC), % sand (Sa), clay (Cl) and silt (Si) were determined at 252 points in the plot. Six auger samples were taken for each vigour zone. At each sampling point, horizons, depth, texture, consistency, structure, bulk density and % organic matter were determined. To identify and quantify the different clay fractions, a mixed soil sample was taken at a depth of 20-30 cm for each vigour zone (HV and LV). The clay fractions were analyzed by X-ray diffractometry (XRD). On the basis of physicochemical characteristics, in particular CEC (14.5-33.7 cmol<sup>+</sup> kg<sup>-1</sup>) and total cations (12.6-33.5 cmol<sup>+</sup> kg<sup>-1</sup>), the soil was classified as a vertical Argiudoll. In addition, an eight-year database (2014-2021) was used, including climatic data (rainfall, temperature, relative humidity and light), vegetative growth (total and exposed leaf area; leaf nitrogen and potassium contents; pruning weight), yield (number of bunches per plant, bunch weight, berry weight), bunch health and grape composition (total soluble solids, acidity, pH, anthocyanins and total phenols contents).

During the eight seasons evaluated, the HV zone was associated with higher levels of leaf area, pruning weight, yield (higher berry and bunch weight) and bunch disease incidence than the LV zone. These characteristics of the HV zone were the result of a deeper and more structured soil, with higher CEC, organic matter content, nitrogen reserves and clay content, and abundance of montmorillonite clay. These latter factors promoted higher potassium availability and soil water retention (TAW) in the HV zone compared to the LV zone. These soil properties in the HV zone proved to be more conducive to root growth, particularly fine roots, with better vertical as well as horizontal distribution. The greater root growth, coupled with the greater soil depth, allowed plants in the HV zone to better absorb water and nutrients. On the other hand, the LV zone was distinguished by a lower TAW level, resulting from a shallower and more compact soil and a greater presence of illite, which negatively conditioned root growth. Limitation of water content in the LV zone may also have reduced nitrogen availability to the plant by negatively impacting microbial activity and uptake of this nutrient. Indeed, leaf nitrogen content was lower for LV compared to HV.

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Although vegetative growth and yield were associated with edaphic parameters, the year's climatic conditions enhanced or attenuated these differences depending on the zone of vigour. Rainy years stimulated vegetative growth in both vigour zones. However, yields decreased in the HV zone when excessive rainfall in the month prior to harvest favored bunch rot. In dry years, the swelling properties of montmorillonite in the HV zone buffered the effects of water deficit on plant growth and yield compared to the LV zone. Berry weight and composition at harvest also varied in the HV and LV zones as a function of weather conditions, water and nitrogen availability, and source/sink ratio. Differences in berry weight at harvest between vigour zones were highly related to water availability. The lower water holding capacity of the LV zone soils resulted in a decrease in berry weight (-22%) compared to the HV zone. The sugar and acid content of berries depended mainly on air and canopy temperature in both zones and on rainfall in the LV zone (lower sugars and acids in the LV zone compared to HV zone). Anthocyanin and phenol contents showed significant variations between the HV and LV zones. However, these differences were not constant from year to year, as they depended mainly on the climatic and microclimatic conditions of the year and, in particular, on water availability (higher contents in dry years).

An analysis of the dynamics of growth and sugar accumulation in berries from veraison to the sugar loading plateau showed marked differences between wet years (> 550 mm rainfall) and dry years (< 450 mm rainfall). In wet years, the total duration of sugar loading ranged from 39 to 48 days, whereas, in dry years, this duration was extended by about 10 days. By normalizing berry population volume and sugar accumulation, grape ripening could be fitted by a bilinear adjustment with two distinct phases. A phase I corresponding to a simultaneous accumulation of water and sugars was delimited, followed by a phase II associated with sugar accumulation at constant berry volume. The separation between these phases (BP), which indicates the sugar content at which the maximum volume is reached, was parameterized, as well as the slope of phase I (slope), which represents the ratio between water and sugar accumulation in the berries. The water/sugar accumulation ratio (slope) was found to be relatively stable, indicating strong genotypic determinism. However, in wet years, phase I was shorter and phase II longer than in dry years. Thus, greater water availability seemed to favor the

apparent asynchrony between water and sugar accumulation in the berry. The stability of maximum berry volume, while sugar continues to accumulate, could be due to the maintenance of water flow from plant to berry and/or an increase in berry volume heterogeneity at the population level.

Traditionally, plots are often managed uniformly without considering the natural variability of resource availability. The same amount of inputs (water and nitrogen) is applied at the plot level, regardless of the likely unequal access to resources among plants in the plot. Uniform irrigation applied to a heterogeneous plot (block with variable vigour) is likely to generate inefficient water use and to reinforce plot heterogeneity. The same conclusion could be reached with uniform canopy or fruit load management in a plot with high leaf/fruit ratio heterogeneity. Characterization of intra-plot variability is useful both to exploit vineyard heterogeneity and, conversely, to seek uniformity. For example, identifying areas with different harvest dates, sugar/acid ratios and/or secondary metabolite profiles can help generating wine products of different quality. It is also possible to reduce intra-zone heterogeneity by locally adapting inputs and soil/plant management practices to the variability observed in the vineyard. Such site-specific management approach improves the use of resources by applying what is needed in each zone.

This study showed that variation in yield and vigour at the plot level was mainly caused by variability in soil characteristics at the vineyard level and that it was exacerbated by the year's climatic conditions, thus generating two well-defined vigour zones (HV, LV). As mentioned above, the stability over time of vigour and yield variations related to soil heterogeneity allows to separate differentiated management zone within a single plot. Site-specific management can then be applied by adapting the inputs to the specific soil and/or plant needs over time. Such approach aims to reduce heterogeneity in plant vigour. Vineyard-specific management may include differential canopy management, phytosanitary treatments, fertilization, irrigation, cluster thinning and leaf removal, among other practices. The interannual stability of the contrasted vigour zones in this trial made it possible to test differential management of inputs and source/sink ratios. Since water and nitrogen availability were identified as key factors of vigour heterogeneity, these two inputs were integrated into a specific localized management plan to optimize production while promoting environmental sustainability.

The grower initially managed the selected vineyard in the same way throughout the plot. It is a vineyard in which no irrigation was applied. In addition, it received a standard fertilization with urea (46% N), half pre-flowering and half post-harvest, with a total dose of 140 kg per hectare. Plant cover management was controlled in the row with herbicides, and in the inter-row (a mixture of grasses and asteraceae) by periodic mowing. In the pre-established areas and during three seasons (2019, 2020 and 2021), a randomized block design with three replications and 21 vines per replication (63 plants per treatment) was delimited for the treatments carried out. The cultural techniques applied in this study were carried out according to vigour zone to improve yield, grape quality and reduce heterogeneity. In the case of HV, the treatments aimed to reduce water and nitrogen inputs and to improve the microclimate in the bunch zone. Water restriction (H-W) was applied from veraison to harvest by covering the soil with polyethylene. In the nitrogen restriction treatment (H-N), no nitrogen was applied (0 N units during the season) for two consecutive seasons (2019 and 2020). Leaf removal (H-L) in the bunch zone was applied at the pre-flowering stage by removing 60% of the leaves. In LV, on the other hand, treatments aimed to increase vegetative growth and yield with water and nitrogen supplementation. Supplemental irrigation (L+W) was provided compared to the control (LV) to achieve 100% of climatic demand (ETo) from bud break to flowering and from harvest to leaf drop, and 70% of ETo from flowering to harvest. For the additional nitrogen (L+N) treatment, 210 kg urea per hectare was supplied (to the 140 kg mentioned above, 70 kg urea per hectare was added in the form of urea before flowering).

The H-W treatment created a physical barrier that prevented soil water loss by evaporation and transpiration by limiting cover crop development in the inter-row. This caused a decrease in soil moisture to moderate or low levels until near harvest, especially in wet years. The lower soil moisture accounted for the lower soil nitrogen availability for this treatment by affecting the rate of organic matter mineralization. This treatment did not reduce vegetative growth or yield, but it generated a better microclimate with more light, higher temperature and lower relative humidity in the cluster zone. Those microclimatic changes significantly reduced the pressure of pathogenic fungi and positively modified the compositional parameters of the grapes (soluble solids and anthocyanins). The effect of plastic ground cover on berry composition was particularly interesting in 2019 (rainy year). This treatment shortened the duration of phase II of berry growth and sugar accumulation.

The H-N treatment showed a strong negative impact on vegetative growth from the second year of production (2020). N restriction reduced berry weight and Brix at harvest, but only in the wet year. Growth and berry sugar accumulation model parameters were oppositely influenced in the wet and dry years. Phase II duration decreased in wet years and was longer in dry years. Plant response to this treatment was highly dependent on water availability during the season.

Leaf removal in the cluster zone before flowering lowered the source/sink ratio and negatively affected the sink. The H-L treatment reduced yield in all three growing seasons compared to the HV control due to a reduction in the number of berries per cluster. Restriction of carbohydrate supply due to the removal of photosynthetically active leaves during the flowering period may have affected the carbon allocation to growing berries, promoting fruit abscission. Although yield was lower in the H-L treatment compared to HV, berry weight was consistently higher due to less competition. In addition, this treatment increased pruning wood weight and its lignification, which may be related to a greater accumulation of reserve starch. The change in source/sink ratio increased bunch exposure to light and reduced bunch compactness, which ultimately reduced the incidence of bunch diseases. Changes in the cluster microclimate also stimulated berry growth and accumulation of sugar, anthocyanins, and phenols. In wet years, this treatment shortened the duration of phase II compared to the control. Thus, leaf removal and yield reduction in this treatment may have accelerated sugar accumulation associated with decreased yield and increased photosynthetic efficiency of the remaining leaves.

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In zone LV, the effect of treatments depended on the water supply of the year. Additional N supply (L+N) increased soil N content in all years, but not plant N content. Thus, despite the higher soil N content for the L+N treatment compared to LV in 2020, low water availability did not increase foliar N content. In this vigour zone, water was the limiting factor and N application without water availability had no impact on vegetative growth. In seasons with higher water availability (2019 and 2021), nitrogen supplementation (L+N) also positively influenced berry composition by increasing the must's total anthocyanin and nitrogen content.

When deficit irrigation (L+W) was applied during ripening, plant water status was maintained at  $\Psi$ p values ranging between -0.2 and -0.4 MPa. The higher soil moisture allowed an increase in leaf N content, thus stimulating vegetative growth. Improved plant water and nitrogen status resulted in improved grape yield, mainly through increased bunch weight. Temperature and light in the canopy zone were lower in L+W than in LV, but a regulated deficit irrigation strategy favored the accumulation of sugars and anthocyanins. Water supply (L+W) increased the duration of phase II, slightly delaying total berry ripening time (+4 days).

Although a 1 ha plot can be considered homogeneous from a topographic, edaphic and climatic point of view, this study showed great variability in soils, production parameters and grape composition. New information was provided on the interaction of the soil-plant-environment system. In particular, the dominant role of water availability in the first place and soil nitrogen availability in the second place in conditioning plant vigour. The effect of nitrogen on plant response was highly dependent on soil water availability. These two inputs and other soil characteristics led to greater exploration of vine roots, which favored wood and grape production. New knowledge was also gained about the relationship between plant vigour and clay type. The predominance of montmorillonite over illite was an important factor for soil fertility in the HV zone. The gradient of vine vigour and yield between the two zones remained stable over the years, regardless of climatic conditions. This indicates that soil characteristics can mitigate or enhance the effects caused by climatic conditions. The determination (possibly by remote sensing) of the vigour zones of a plot is a prerequisite for proposing specific soil and crop management practices that optimize the use of resources and ensure the economic and environmental sustainability of wine production. The application of precision viticulture technologies has made it possible to improve plant productivity (yield and berry composition) and reduce heterogeneity at the plot level.

In future research, it would be desirable to apply a larger-scale spatial variability approach to determine microterroirs and thus generate the application of site-specific techniques to obtain differentiated products in terms of grape quality. In turn, analyses conducted in vineyards rich in 2/1 type clays will confirm the hypotheses put forward in this work on the role of clay type in determining vigour. A more detailed analysis of the existing heterogeneity in the vineyard plot, but especially at the cluster level, will allow the refinement of specific management strategies to achieve optimal sugar and anthocyanin production and accumulation. In addition, future approaches should consider the soil-plant-atmosphere complex and, in particular, the role of the various plant subsystems in this complex interaction. An example would be the role of carbon stocks in establishing variability at the cluster level, which has implications for water and sugar accumulation during ripening at the berry level.

## 2. <u>FRENCH</u>

Le terme de vigueur est un concept qui fait référence à la croissance végétative et au rendement de la vigne. Ces deux composantes de la vigueur ne sont généralement pas homogènes dans l'espace. L'hétérogénéité de la vigueur peut être corrigée à différentes échelles, c'est-à-dire au niveau des régions, des vignobles d'une même région, d'un vignoble ou au niveau des rameaux d'une plante. Une variabilité spatiale de la vigueur très élevée peut en effet engendrer des pertes de rendement, une altération de l'état sanitaire des grappes et des composantes de qualité des raisins. La gestion localisée de l'équilibre des plantes (en termes de rapport source/puits) permet aux viticulteurs d'optimiser le rendement avec une qualité de raisin adéquate et de maintenir un niveau élevé de stock de carbone nécessaire pour la saison suivante.

Bien que le concept de variabilité spatiale de vigueur ne soit pas nouveau pour les viticulteurs, il reste en général difficile d'évaluer le niveau d'hétérogénéité de vigueur au

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vignoble. Avec le développement des nouvelles technologies et outils d'agriculture de précision appliqués à la viticulture, cette variabilité peut être toutefois facilement quantifiée. Les capteurs à distance (caméras thermiques, images multispectrales), le GPS (système de positionnement global), les systèmes d'analyse des données et d'interprétation de l'information, les équipements d'application à débit variable (VRA), les systèmes de suivi des rendements, les données en temps réel, etc. permettent de caractériser la variabilité naturelle de vigueur et d'adapter les pratiques culturales en conséquence. Parmi les techniques de télédétection, les images aériennes ou satellitaires traitées à partir de la réflectance rouge et infrarouge pour générer des indices végétatifs tels que le NDVI (Normalised Difference Vegetative Index) ont été largement utilisées pour évaluer l'hétérogénéité régionale de la vigueur parcellaire des cultures annuelles et pérennes.

D'après la littérature (Bramley & Hamilton 2004, Tardáguilla et al. 2011, van Leeuwen et al. 2018, Gatti et al. 2021), les variations intra-parcellaire de la croissance végétative, du système racinaire, du rendement, de la maturation du raisin et de la santé des baies, entre autres, dépendent des variations des caractéristiques du sol. En effet, les propriétés physiques, chimiques et biologiques du sol, telles que la température, la profondeur, la texture, la structure et l'activité des micro-organismes, déterminent le volume de sol exploré par les racines, le niveau de nutriments disponibles et la capacité du sol à stocker l'eau. De plus, le climat en interaction avec le sol est un facteur déterminant du fonctionnement des plantes. Les paramètres pédoclimatiques influencent la croissance, la vigueur, la physiologie et la maturation des raisins de la vigne. La variabilité climatique de paramètres critiques tels que la température et la disponibilité en eau détermine les différences de production et de qualité du raisin d'une année à l'autre.

La variabilité spatiale (au sein d'une même parcelle) du rendement et de la composition des raisins est associée à des caractéristiques physiques saisonnières stables (sol et topographie) qui interagissent avec les conditions abiotiques et biotiques saisonnières (climat, disponibilité de l'eau et de l'azote, présence de maladies) et la stratégie de gestion agronomique. L'eau et les nutriments (principalement l'azote) sont

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des facteurs bien connus qui conditionnent le développement, la croissance et le rendement des plantes. La disponibilité en eau est l'un des facteurs les plus impactant pour le développement végétatif de la vigne. En cas de déficit hydrique, la croissance aérienne et racinaire peut être limitée en raison de la diminution de la turgescence cellulaire et de la résistance accrue à la pénétration des racines dans les sols secs. Enfin, les caractéristiques physiques du sol, notamment sa texture, sa structure et sa profondeur, sont des facteurs importants à prendre en compte en raison de leurs effets sur la température des racines et sur l'apport d'eau et de minéraux à la plante.

La connaissance des causes spécifiques qui génèrent la variabilité spatiale de vigueur est importante pour les viticulteurs afin d'optimiser la production et de réduire les coûts. La présente étude avait pour objectif d'identifier les facteurs physiques responsables de l'hétérogénéité intra-parcellaire de vigueur d'un vignoble représentatif du sud de l'Uruguay au cours de huit saisons consécutives. Ce vignoble implanté avec le cv. Tannat (rouge) a été sélectionné en raison de sa grande variabilité est-ouest de la vigueur des plantes. Pour cette parcelle, l'indice Ravaz était en moyenne d'environ 14, ce qui indique que la vigueur de la plante était en dehors de la plage optimale établie pour cette variété (6-10). En outre, le coefficient de variation du rendement au niveau de la parcelle était de 47%. La vigueur de cette parcelle a été évaluée à la véraison (janvier dans l'hémisphère sud) pendant trois années (2015, 2016 et 2017) à partir de l'indice de végétation par différence normalisée (NDVI), calculé à partir d'images aériennes (altitude de 620 m et vitesse de 50 m/s). Des images multispectrales à haute résolution (0,2 m) ont été obtenues au sol pour définir trois zones de vigueur : haute (HV), moyenne (MV) et basse (LV). Chaque classe NDVI (haute, moyenne et basse) a été systématiquement localisée dans les mêmes zones du vignoble chaque année. En hiver 2020, le diamètre du tronc a été évalué pour corroborer la stabilité interannuelle de la vigueur, et des corrélations positives ont été ajustées entre le NDVI et les paramètres de croissance végétative (poids de la taille, surface foliaire et diamètre du tronc). En hiver 2015, une description complète des paramètres physiques et chimiques du sol a été réalisée. Des échantillons ont été prélevés à trois profondeurs (0-20, 20-40 et 40-60 cm) où le phosphore de Bray, le pH, le calcium, le magnésium, le potassium et le sodium échangeables, la matière organique (MO), les nitrates, la capacité d'échange cationique

(CEC), le % de sable (Sa), d'argile (CI) et de limon (Si) ont été déterminés en 252 points de la parcelle. Six prélèvements à la tarière ont été réalisés pour chaque zone de vigueur. A chaque point de prélèvement, les horizons, la profondeur, la texture, la consistance, la structure, la densité apparente et le pourcentage de matière organique ont été déterminés. Pour identifier et quantifier les différentes fractions d'argile, un échantillon de sol mixte a été prélevé à une profondeur de 20-30 cm pour chaque zone de vigueur (HV et LV). Les fractions d'argile ont été analysées par diffractométrie aux rayons X (XRD). Sur la base des caractéristiques physico-chimiques, en particulier la CEC (14,5 - 33,7 cmol<sup>+</sup> kg<sup>-1</sup>) et les cations totaux (12,6 - 33,5 cmol<sup>+</sup> kg<sup>-1</sup>), le sol a été classé comme un Argiudoll vertical. Une base de données de huit ans (2014-2021) a été utilisée, comprenant les données climatiques (précipitations, température, humidité relative et luminosité), la croissance végétative (surface foliaire totale et exposée ; azote et potassium foliaires ; poids de la taille), le rendement (nombre de grappes par plante, poids de la grappe, poids des baies), l'état sanitaire de la grappe et la composition du raisin (solides solubles totaux, acidité, pH, anthocyanes et phénols totaux).

Au cours des huit saisons évaluées, la zone HV a été associée, par rapport à la zone LV, à des niveaux supérieurs de surface foliaire, de poids de taille, de rendement (poids des baies et poids des grappes plus élevé) et d'incidence des maladies des grappes. Ces caractéristiques de la zone HV résultaient d'un sol plus profond et plus structuré, avec des taux supérieurs de CEC, de teneur en matière organique, de stock d'azote et de teneur en argile ainsi qu'une abondance d'argile de type montmorillonite. Ces derniers facteurs ont favorisé une plus grande disponibilité du potassium et une plus grande rétention d'eau du sol (TAW) dans la zone HV par rapport à la zone LV. Ces propriétés du sol dans la zone HV se sont avérées plus propices à la croissance des racines, en particulier les racines fines, avec une meilleure distribution à la fois verticale et horizontale. La croissance accrue des racines, ajoutée à la profondeur du sol supérieure, a permis aux plantes de la zone HV de mieux absorber l'eau et les nutriments. La zone LV, par contre, s'est distinguée par un niveau de TAW inférieur, résultant d'un sol moins profond et plus compact et d'une plus grande présence d'illite, ce qui a conditionné négativement la croissance des racines. La limitation de la teneur en eau dans la zone LV peut également réduire la disponibilité de l'azote pour la plante en ayant un impact négatif sur l'activité microbienne et l'absorption de ce nutriment. Cette situation s'est traduite par une teneur en azote foliaire inférieure à celle des plantes HV.

Bien que la croissance végétative et le rendement aient été associés aux paramètres du sol, les conditions climatiques de l'année ont soit renforcé, soit atténué ces différences selon la zone de vigueur. Les années pluvieuses ont stimulé la croissance végétative dans les deux zones de vigueur. Toutefois, les rendements ont diminué dans la zone HV lorsque les précipitations excessives du mois précédant la récolte ont favorisé la pourriture des grappes. Lors des années sèches, les propriétés de gonflement de la montmorillonite dans la zone HV ont amorti les effets du déficit hydrique sur la croissance et le rendement des plantes comparé à la zone LV. Le poids des baies et leur composition à la récolte ont également varié dans les zones HV et LV en fonction des conditions climatiques du millésime, la disponibilité en l'eau et en l'azote ainsi que le rapport source/puits. Les différences de poids des baies à la récolte entre les zones de vigueur étaient également liées à la disponibilité en eau. La plus faible capacité des sols à retenir l'eau dans la zone LV a entraîné une diminution du poids des baies (-22 %) par rapport à la zone HV. La teneur en sucre et en acides des baies dépendait principalement de la température de l'air et de la canopée dans les deux zones et de la pluviométrie pour la zone LV (sucres et acides plus forts dans la zone HV). Les teneurs en anthocyanes et en phénols ont montré des variations significatives entre les zones HV et LV, mais ces différences n'étaient pas constantes d'une année sur l'autre car très dépendantes des conditions climatiques et microclimatiques de l'année, et notamment de la disponibilité en eau (teneurs supérieures les années sèches).

Une analyse des dynamiques de croissance et d'accumulation des sucres dans les baies de la véraison au plateau de chargement en sucre a montré des marquées entre les années humides (> 550 mm de précipitations) et les années sèches (< 450 mm de précipitations). Pour les années humides, la durée totale du chargement en sucre était comprise entre 39 et 48 jours, tandis que pour les années sèches, cette durée était rallongée d'environ 10 jours. La normalisation du volume de la population de baies et de l'accumulation de sucre a permis de caractériser la maturation du raisin en deux phases au moyen d'un ajustement bilinéaire. La Phase I, correspondant à une accumulation

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simultanée d'eau et de sucres, était suivie d'une Phase II correspondant à l'accumulation de sucres à volume de baies constant. La séparation entre ces phases (BP), indiquant la teneur en sucre à laquelle le volume maximal est atteint, ainsi que la pente de la Phase I (Slope), représentant le ratio entre l'accumulation d'eau et de sucre dans les baies, ont été déterminées. Le rapport d'accumulation eau/sucre (Slope) s'est avéré être relativement stable, indiquant un fort déterminisme génotypique. Par contre, lors des années humides, la Phase I était plus courte et la Phase II plus longue par rapport aux années sèches. Une plus grande disponibilité en eau semble ainsi favoriser l'asynchronisme apparent entre l'accumulation d'eau et de sucre dans la baie. La stabilité du volume maximal des baies, alors que le sucre continue de s'accumuler, pourrait être due au maintien du flux d'eau de la plante vers la baie et/ou à une augmentation de l'hétérogénéité du volume des baies au niveau de la population.

Traditionnellement, les parcelles sont généralement gérées de manière uniforme sans tenir compte de la variabilité naturelle de disponibilité des ressources. La même quantité d'intrants (eau et azote) est appliquée au niveau de la parcelle, indépendamment de l'accès probablement inégal aux ressources entre les plantes de la parcelle. Une irrigation uniforme appliquée sur une parcelle hétérogène (bloc à vigueur variable), en plus de générer une utilisation inefficace de l'eau, renforce l'hétérogénéité de la parcelle. La même conclusion pourrait être tirée avec une gestion uniforme de la canopée ou de la charge en fruits sur une parcelle présentant une forte hétérogénéité du rapport feuilles/fruits. La caractérisation de la variabilité intra-parcellaire est utile soit pour exploiter l'hétérogénéité du vignoble ou, au contraire, de rechercher l'uniformité. Ainsi, l'identification de zones ayant des dates de récolte, des rapports sucre/acidité et/ou des profils de métabolites secondaires différents peut permettre de générer des produits vinicoles de qualité différents. La réduction de l'hétérogénéité intra-parcellaire est par ailleurs possible en adaptant localement les intrants et les pratiques de gestion du sol et des plantes à la variabilité observée dans le vignoble. Cette approche de gestion par zone ou par site permet de maximiser l'utilisation des ressources en appliquant ce qui est nécessaire dans chaque zone.

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Cette étude a montré que la variation du rendement et de la vigueur au niveau de la parcelle était principalement causée par la variabilité des caractéristiques du sol au niveau du vignoble et exacerbée par les conditions climatiques, générant deux zones de vigueur bien définies (HV, LV). Comme mentionné ci-dessus, la stabilité dans le temps des variations de vigueur et de rendement liées à l'hétérogénéité du sol permet d'établir des zones de gestion différenciées dans une même parcelle. Par ailleurs, la gestion spécifique d'un site est basée sur un dosage variable des intrants du système, en tenant compte du fait que les exigences spécifiques du sol et/ou des plantes peuvent varier dans le temps et l'espace. Cette approche vise également à réduire l'hétérogénéité de la vigueur des plantes. La gestion spécifique localisée au vignoble peut inclure une gestion différentielle de la canopée, des traitements phytosanitaires, de la fertilisation, de l'irrigation, de l'éclaircissage des grappes et de l'effeuillage entre autres pratiques. La stabilité interannuelle des zones de vigueur contrastées dans cet essai, a permis de tester des gestions différenciées des intrants et du rapport source/puits. L'eau et la disponibilité de l'azote ayant été identifiés comme des facteurs déterminants dans l'établissement de la vigueur, ces deux intrants ont été intégrés dans un plan de gestion spécifique localisée visant à optimiser la production tout en favorisant la durabilité environnementale.

Le vignoble sélectionné était initialement conduit par le vigneron de la même manière sur l'ensemble de la parcelle. Il s'agit d'un vignoble où aucune irrigation n'est appliquée. En outre, il reçoit une fertilisation standard à l'urée (46% N), répartie pour moitié en préfloraison et pour moitié en post-récolte, avec une dose totale de 140 kg d'engrais par hectare. La gestion des mauvaises herbes était contrôlée dans le rang avec des herbicides, et l'inter-rang est un mélange de graminées et d'astéracées, contrôlé par une fauche périodique. Dans les zones préétablies et pendant trois saisons (2019, 2020 et 2021), un plan en bloc randomisé avec trois réplications et 21 vignes par réplication (63 plantes par traitement) a été délimité pour les traitements réalisés. Les techniques culturales mises en œuvre dans cette étude ont été réalisées en fonction de la zone de vigueur pour avoir un impact sur le rendement, la qualité du raisin et réduire l'hétérogénéité. Dans le cas de HV, les traitements visaient à réduire l'apport d'eau et d'azote et à améliorer les conditions microclimatiques dans la zone des grappes. La restriction d'eau (H-W) a été appliquée de la véraison à la récolte en couvrant le sol avec du polyéthylène. Dans le traitement de restriction de l'azote (H-N), aucun apport d'azote n'a été effectué (O unité N au cours de la saison) pendant deux saisons consécutives (2019 et 2020). L'effeuillage (H-L) a été appliqué au stade de la préfloraison en enlevant 60% des feuilles, dans le but de réduire le rendement et d'améliorer l'exposition des grappes. Pour le LV, en revanche, les traitements visaient à augmenter la croissance végétative et le rendement avec un apport d'eau et d'azote. Une irrigation supplémentaire (L+W) a été fournie par rapport au contrôle (LV) pour atteindre 100 % de la demande climatique (ETO) du débourrement à la floraison et de la récolte à la chute des feuilles, et 70 % de l'ETo de la floraison à la récolte. Pour le traitement d'azote supplémentaire (L+N), 210 kg d'urée par hectare ont été fournis (aux 140 kg mentionnés ci-dessus, 70 kg d'urée par hectare ont été ajoutés sous forme d'urée avant la floraison).

Le traitement H-W a créé une barrière physique qui a empêché la perte d'eau du sol par évaporation et transpiration en limitant le développement de la culture de couverture dans l'inter-rang. Cette situation a entraîné une baisse de l'humidité du sol à des niveaux modérés à faibles jusqu'à l'approche de la récolte, en particulier les années pluvieuses. La plus faible humidité du sol explique la plus faible disponibilité de l'azote du sol pour ce traitement en affectant le taux de minéralisation de la matière organique. Ce traitement n'a pas affecté la croissance végétative et le rendement. A son tour, il a généré un environnement avec une lumière plus forte, une température plus élevée et une humidité relative plus faible au niveau du bouquet. Ces changements du microclimat des grappes ont réduit de manière significative la pression des champignons pathogènes et modifié positivement les paramètres de composition des raisins (solides solubles et anthocyanes). L'effet de la couverture plastique du sol sur la composition des baies a été particulièrement intéressant en 2019 (année pluvieuse). Dans ce traitement, la durée de la Phase II de la croissance des baies et de l'accumulation de sucre a été raccourcie.

Le traitement H-N a montré un fort impact négatif sur la croissance végétative à partir de la deuxième année de production (2020). La restriction de N a réduit le poids des baies et le Brix à la récolte, mais seulement pour l'année humide. Les paramètres du modèle de croissance et d'accumulation de sucre dans les baies ont été influencés de manière opposée lors des années humides et sèches. La durée de la Phase II a diminué
pendant les années humides et a été plus longue pendant les années sèches. La réponse des plantes à ce traitement a été fortement influencée par la disponibilité en eau pendant l'année.

L'élimination des feuilles dans la zone des grappes avant la floraison a non seulement modifié le rapport source/sink en raison de l'élimination des feuilles, mais a également affecté le sink. Le traitement H-L a réduit le rendement pendant les trois saisons de croissance par rapport au contrôle HV, ce qui s'explique par une réduction du nombre de baies par grappe. La restriction de l'approvisionnement en hydrates de carbone due à l'élimination des feuilles photosynthétiquement actives pendant la période de floraison a affecté l'allocation du carbone aux baies en croissance, favorisant l'abscission des fruits. Bien que le rendement ait été plus faible pour le traitement H-L par rapport à HV, le poids des baies était systématiquement plus élevé étant donné la concurrence plus faible. De plus, ce traitement a augmenté le poids du bois de taille et sa lignification, ce qui serait lié à une plus grande accumulation d'amidon de réserve. La modification du rapport source/sink a permis d'augmenter l'exposition des grappes à la lumière et de réduire la compacité des grappes, ce qui a finalement réduit l'incidence des maladies des grappes. Les changements du microclimat de la grappe ont également stimulé la croissance des baies, l'accumulation de sucre, d'anthocyanes et de phénols. Lors des années humides, ce traitement a raccourci la durée de la Phase II par rapport au contrôle. L'effeuillage et la réduction du rendement dans ce traitement ont donc pu accélérer l'accumulation de sucre associée à une diminution du rendement et à une augmentation de l'efficacité photosynthétique des feuilles restantes.

Dans la zone LV, l'effet des traitements dépendait de l'approvisionnement en eau de l'année. L'apport supplémentaire de N (L+N) a augmenté la teneur en N du sol pour toutes les années, mais pas la teneur en azote des plantes. Ainsi, malgré l'azote du sol plus élevé pour le traitement L+N par rapport au LV en 2020, la faible disponibilité en eau n'a pas permis d'augmenter la teneur en azote foliaire pour ce traitement. Dans cette zone de vigueur, l'eau était le facteur limitant et l'application de N sans disponibilité d'eau n'avait aucun impact sur la croissance végétative. Pendant les saisons où la disponibilité en eau était plus élevée (2019 et 2021), la supplémentation en azote (L+N) a également

influencé positivement la composition des baies en augmentant la teneur totale en anthocyanes et en azote dans le moût.

Lorsque l'irrigation déficitaire (L+W) a été appliquée pendant la maturation, l'état hydrique des plantes a été maintenu à des valeurs  $\Psi$ p (entre -0,2 et -0,4 MPa). L'humidité plus élevée du sol a permis une augmentation de la teneur en N foliaire, stimulant ainsi la croissance végétative. L'amélioration du statut hydrique et azoté de la plante a permis d'améliorer le rendement des raisins, principalement par une augmentation du poids des grappes. La température et la lumière dans la zone de la canopée étaient inférieures pour L+W par rapport à LV, mais l'accumulation de sucre et d'anthocyanes a été favorisée par une stratégie d'irrigation déficitaire régulée. L'apport d'eau (L+W) a augmenté la durée de la Phase II, retardant légèrement la durée totale du mûrissement des baies (+4 jours).

Bien qu'une parcelle de 1 ha puisse être considérée comme homogène d'un point de vue topographique, pédologique et climatique, cette étude a montré une grande variabilité des sols, des paramètres de production et de la composition des raisins. De nouvelles informations ont été fournies sur l'interaction du système sol-planteenvironnement. En particulier, le rôle dominant de la disponibilité en eau d'abord et de la disponibilité en azote du sol ensuite dans l'établissement de la vigueur des plantes. L'effet de l'azote sur la réponse des plantes dépendait fortement de la disponibilité en eau du sol. Ces deux apports, associés à d'autres caractéristiques du sol, ont conduit à une exploration accrue des racines de la vigne, ce qui a favorisé la production de bois et de raisins. De nouvelles connaissances ont également été apportées sur la relation entre la vigueur des plantes et le type d'argile. La prédominance de la montmorillonite sur l'illite était un facteur important pour la fertilité du sol dans la zone HV. Le gradient de vigueur de la vigne et de rendement entre les deux zones est resté stable au fil des ans, indépendamment des conditions climatiques. Cela indique que les caractéristiques du sol permettent d'atténuer ou de renforcer les effets causés par les conditions climatiques. La détermination (possible par télédétection) des zones de vigueur dans une parcelle est une condition préalable pour proposer des pratiques spécifiques de gestion des sols et des cultures afin d'optimiser l'utilisation des ressources et d'assurer la durabilité économique et environnementale de la production viticole. En appliquant des

technologies de viticulture de précision, il a été possible d'améliorer la productivité des plantes (rendement et composition des baies) et de réduire l'hétérogénéité au niveau des parcelles.

Dans les recherches futures, il serait souhaitable d'appliquer une approche de variabilité spatiale à plus grande échelle pour déterminer les micro-terroirs et ainsi générer l'application de techniques spécifiques au site pour obtenir des produits différenciés en termes de qualité du raisin. A son tour, l'analyse effectuée dans les vignobles riches en argiles de type 2/1 permettra de confirmer les hypothèses émises dans ce travail sur le rôle du type d'argile dans la détermination de la vigueur. Une analyse plus poussée de l'hétérogénéité existante dans la parcelle de vigne, mais surtout au niveau des grappes, permettra d'affiner davantage les stratégies de gestion spécifiques pour obtenir une production et une accumulation de sucre et d'anthocyanes optimales. En outre, les approches futures doivent prendre en compte le complexe sol-plante-atmosphère et en particulier le rôle des différents sous-systèmes végétaux dans cette interaction complexe. Un exemple serait le rôle des stocks de carbone dans l'établissement de la variabilité au niveau de la grappe qui a des répercussions au niveau de la baie sur l'accumulation d'eau et de sucre pendant la maturation.

# 3. <u>SPANISH</u>

El término *vigor* es un concepto que hace referencia al crecimiento vegetativo y al rendimiento de la vid. Estos dos componentes del vigor no suelen ser homogéneos espacialmente. La heterogeneidad del vigor puede evaluarse a diferentes escalas, es decir, a escala de regiones; de viñedos dentro de una región, de un viñedo o dentro de una misma planta. En efecto, una variabilidad espacial muy elevada del vigor puede provocar pérdidas de rendimiento, alteraciones del estado sanitario de los racimos y de los componentes cualitativos de la uva. La búsqueda del equilibrio de la planta (en términos de relación fuente/sumidero) permite a los viticultores optimizar el rendimiento con una calidad de uva adecuada y mantener un nivel elevado de reservas de carbono necesarias para la temporada siguiente.

Aunque el concepto de variabilidad espacial del vigor no es nuevo para los viticultores, en general sigue siendo difícil evaluar a nivel de un viñedo la heterogeneidad del vigor. Sin embargo, con el desarrollo de nuevas tecnologías y herramientas de agricultura de precisión aplicadas en la viticultura, esta variabilidad puede ser cuantificada fácilmente. Sensores remotos (cámaras térmicas, imágenes multiespectrales), GPS (sistema de posicionamiento global), sistemas de análisis de datos e interpretación de la información, equipos de aplicación variable (VRA), sistemas de seguimiento del rendimiento, datos en tiempo real, etc. permiten caracterizar la variabilidad natural del vigor y adaptar las prácticas de cultivo en consecuencia. Entre las técnicas de teledetección, las imágenes aéreas o por satélite procesadas a partir de la reflectancia roja e infrarroja para generar índices vegetativos como el NDVI (índice vegetativo de diferencia normalizada) se han utilizado ampliamente para evaluar la heterogeneidad regional en el vigor de las parcelas tanto de cultivos anuales como perennes.

Según la bibliografía (Bramley y Hamilton, 2004, Tardáguilla et al., 2011; van Leeuwen et al., 2018, Gatti et al., 2021), las variaciones intraparcela en el crecimiento vegetativo, el sistema radicular, el rendimiento, la maduración de la uva y la sanidad de las bayas, entre otras, dependen de las variaciones en las características del suelo. En efecto, las propiedades físicas, químicas y biológicas del suelo, como la temperatura, la profundidad, la textura, la estructura y la actividad de los microorganismos, determinan el volumen de suelo explorado por las raíces, el nivel de nutrientes disponibles y la capacidad del suelo para almacenar agua. Además, el clima en interacción con el suelo es un factor determinante en el funcionamiento de las plantas. Los parámetros edafoclimáticos influyen en el crecimiento, el vigor, la fisiología y la maduración de la vid. La variabilidad climática de parámetros críticos como la temperatura y la disponibilidad de agua determinan las diferencias en la producción y la calidad de la uva de un año a otro.

La variabilidad espacial (dentro de una parcela) del rendimiento y la composición de la uva está asociada a características físicas estacionales estables (suelo y topografía) que interactúan con las condiciones abióticas y bióticas estacionales (clima, disponibilidad de agua y nitrógeno, presencia de enfermedades) y la estrategia de gestión

agronómica. El agua y los nutrientes (principalmente el nitrógeno) son factores bien conocidos que condicionan el desarrollo, el crecimiento y el rendimiento de las plantas. La disponibilidad de agua es uno de los factores más importantes que afectan al desarrollo vegetativo de la vid. En caso de déficit hídrico, el crecimiento aéreo y radicular puede verse limitado debido a la disminución de la turgencia celular y al aumento de la resistencia a la penetración de las raíces en suelos secos. Por último, las características físicas del suelo, como su textura, estructura y profundidad, son factores importantes que hay que tener en cuenta por sus efectos en la temperatura de las raíces y en el suministro de agua y minerales a la planta.

El conocimiento de las causas específicas que generan la variabilidad espacial del vigor es importante para que los viticultores optimicen la producción y reduzcan costes. El objetivo del presente estudio fue identificar los factores físicos responsables de la heterogeneidad intraparcelaria del vigor en un viñedo representativo del sur de Uruguay durante ocho temporadas consecutivas. Este viñedo plantado con cv. Tannat (tinta) fue seleccionado por su gran variabilidad este-oeste en cuanto al vigor de la planta. En esta parcela, el índice de Ravaz se situó en torno a 14 de media, lo que indica que el vigor de la planta estaba fuera del intervalo óptimo para esta variedad (6-10). Además, el coeficiente de variación del rendimiento a nivel de parcela fue del 47 %. El vigor fue evaluado en el envero (enero en el hemisferio sur) durante tres años (2015, 2016 y 2017) mediante el índice de vegetación de diferencia normalizada (NDVI), calculado a partir de imágenes aéreas (altitud 620 m y velocidad 50 m/s). Se obtuvieron imágenes multiespectrales de alta resolución (0,2 m) sobre el terreno para definir tres zonas de vigor: alto (HV), medio (MV) y bajo (LV). Cada clase de NDVI (alto, medio y bajo) se localizó sistemáticamente en las mismas zonas del viñedo cada año. En invierno de 2020, se evaluó el diámetro del tronco para corroborar la estabilidad interanual del vigor y se establecieron correlaciones positivas entre el NDVI y los parámetros de crecimiento vegetativo (peso de poda, superficie foliar y diámetro del tronco). En invierno de 2015, se llevó a cabo una descripción completa de los parámetros físicos y químicos del suelo. Se tomaron muestras a tres profundidades (0-20, 20-40 y 40-60 cm) en las que se determinó el fósforo Bray, el pH, el calcio, magnesio, potasio y sodio intercambiables, la materia orgánica (MO), el nitrato, la capacidad de intercambio catiónico (CEC), el % de

arena (Sa), arcilla (Cl) y limo (Si) en 252 puntos de la parcela. Se tomaron seis muestras de barrena para cada zona de vigor. En cada punto de muestreo se determinaron los horizontes, la profundidad, la textura, la consistencia, la estructura, la densidad aparente y el porcentaje de materia orgánica. Para identificar y cuantificar las diferentes fracciones de arcilla, se tomó una muestra de suelo mixto a una profundidad de 20-30 cm para cada zona de vigor (HV y LV). Las fracciones de arcilla se analizaron por difractometría de rayos X (DRX). Sobre la base de las características fisicoquímicas, en particular la CEC (14,5-33,7 cmol<sup>+</sup> kg<sup>-1</sup>) y los cationes totales (12,6-33,5 cmol<sup>+</sup> kg<sup>-1</sup>), el suelo se clasificó como un Argiudoll vertical. Además, se utilizó una base de datos de ocho años (2014-2021), que incluía datos climáticos (precipitaciones, temperatura, humedad relativa y luz), crecimiento vegetativo (superficie foliar total y expuesta; nitrógeno y potasio foliares; peso de poda), rendimiento (número de racimos por planta, peso del racimo, peso de la baya), sanidad del racimo y composición de la uva (sólidos solubles totales, acidez, pH, antocianos y fenoles totales).

Durante las ocho temporadas evaluadas, la zona HV se asoció con niveles más altos de área foliar, peso de poda, rendimiento (mayor peso de la baya y del racimo) e incidencia de enfermedades del racimo que la zona LV. Estas características de la zona HV eran el resultado de un suelo más profundo y estructurado, con mayor CEC, contenido de materia orgánica, reservas de nitrógeno y contenido de arcilla, y abundancia de arcilla montmorillonita. Estos últimos factores promovieron una mayor disponibilidad de potasio y retención de agua en el suelo (TAW) en la zona HV en comparación con la zona LV. Estas propiedades del suelo en la zona HV resultaron ser más propicias para el crecimiento de las raíces, en particular las raíces finas, con una mejor distribución tanto vertical como horizontal. El mayor crecimiento de las raíces, unido a la mayor profundidad del suelo, permitió a las plantas de la zona HV absorber mejor el agua y los nutrientes. La zona LV, por su parte, se distinguía por un menor nivel de TAW, resultado de un suelo menos profundo y más compacto y de una mayor presencia de illita, que condicionaba negativamente el crecimiento de las raíces. La limitación del contenido de agua en la zona LV también puede reducir la disponibilidad de nitrógeno para la planta al repercutir negativamente en la actividad microbiana y la absorción de este nutriente. El resultado fue un menor contenido de nitrógeno en las hojas que en las plantas HV.

Aunque el crecimiento vegetativo y el rendimiento se asociaron a los parámetros edáficos, las condiciones climáticas del año potenciaron o atenuaron estas diferencias en función de la zona de vigor. Los años lluviosos estimularon el crecimiento vegetativo en ambas zonas de vigor. Sin embargo, los rendimientos disminuyeron en la zona HV cuando las lluvias excesivas del mes anterior a la cosecha favorecieron la podredumbre del racimo. En años secos, las propiedades de hinchamiento de la montmorillonita en la zona HV amortiguaron los efectos del déficit hídrico sobre el crecimiento y el rendimiento de las plantas en comparación con la zona LV. El peso y la composición de las bayas en el momento de la cosecha también variaron en las zonas HV y LV en función de las condiciones climáticas de la cosecha, la disponibilidad de agua y nitrógeno y la relación fuente/fosa. Las diferencias en el peso de las bayas en la cosecha entre zonas de vigor también se relacionaron con la disponibilidad de agua. La menor capacidad de retención de agua de los suelos de la zona LV se tradujo en una disminución del peso de las bayas (-22 %) en comparación con la zona HV. El contenido en azúcares y ácidos de las bayas dependió principalmente de la temperatura del aire y de la canopia en ambas zonas y de las precipitaciones en la zona LV (azúcares y ácidos más elevados en la zona HV). Los contenidos de antocianos y fenoles mostraron variaciones significativas entre las zonas HV y LV, pero estas diferencias no fueron constantes de un año a otro, ya que dependían en gran medida de las condiciones climáticas y microclimáticas del año y, en particular, de la disponibilidad de agua (mayores contenidos en los años secos).

Un análisis de la dinámica de crecimiento y acumulación de azúcares en las bayas desde el envero hasta la meseta de carga de azúcares mostró marcadas diferencias entre los años húmedos (> 550 mm de precipitaciones) y los años secos (< 450 mm de precipitaciones). En los años húmedos, la duración total de la carga de azúcar osciló entre 39 y 48 días, mientras que, en los años secos, esta duración se prolongó unos 10 días. Normalizando el volumen de la población de bayas y la acumulación de azúcar, la maduración de la uva se pudo categorizar en dos fases mediante un ajuste bilineal. Se determinó una fase I que corresponde a una acumulación simultánea de agua y azúcares, seguida de una fase II asociada a la acumulación de azúcares a volumen constante de baya. Se determinó la separación entre estas fases (BP), que indica el contenido de azúcar

al que se alcanza el volumen máximo, así como la pendiente de la fase I (pendiente), que representa la relación entre la acumulación de agua y azúcar en las bayas. La relación de acumulación de agua/azúcar (pendiente) resultó ser relativamente estable, lo que indica un fuerte determinismo genotípico. Sin embargo, en los años húmedos, la fase I fue más corta y la fase II más larga que en los años secos. Así pues, una mayor disponibilidad de agua parece favorecer la aparente asincronía entre la acumulación de agua y azúcar en la baya. La estabilidad del volumen máximo de bayas, mientras el azúcar sigue acumulándose, podría deberse al mantenimiento del flujo de agua de la planta a la baya y/o a un aumento de la heterogeneidad del volumen de bayas a nivel de población.

Tradicionalmente, las parcelas suelen gestionarse de manera uniforme sin tener en cuenta la variabilidad natural de la disponibilidad de recursos. Se aplica la misma cantidad de insumos (agua y nitrógeno) a nivel de parcela, independientemente del probable acceso desigual a los recursos entre las plantas de la parcela. El riego uniforme aplicado a una parcela heterogénea (bloque con vigor variable), además de generar un uso ineficiente del agua, refuerza la heterogeneidad de la parcela. Se podría llegar a la misma conclusión con una gestión uniforme de la canopia o de la carga de frutos en una parcela con una elevada heterogeneidad en la relación hoja/fruto. La caracterización de la variabilidad intraparcelaria es útil tanto para explotar la heterogeneidad del viñedo como, por el contrario, para buscar la uniformidad. Por ejemplo, la identificación de zonas con diferentes fechas de cosecha, relaciones azúcar/ácido y/o perfiles de metabolitos secundarios puede ayudar a generar productos vitivinícolas de diferente calidad. También es posible reducir la heterogeneidad intraparcelaria adaptando localmente los insumos y las prácticas de gestión del suelo y de las plantas a la variabilidad observada en el viñedo. Este planteamiento de gestión por zonas o lugares específicos permite aprovechar al máximo los recursos aplicando lo necesario en cada zona.

Este estudio demostró que la variación en el rendimiento y el vigor a nivel de parcela estaba causada principalmente por la variabilidad en las características del suelo a nivel de viñedo y exacerbada por las condiciones climáticas del año, lo que genera así dos zonas de vigor bien definidas (HV, LV). Como ya se ha mencionado, la estabilidad en el tiempo de las variaciones de vigor y rendimiento relacionadas con la heterogeneidad

del suelo permite establecer zonas de gestión diferenciadas dentro de una misma parcela. Además, la gestión específica del lugar se basa en una dosificación variable de los insumos del sistema, teniendo en cuenta que las necesidades específicas del suelo y/o de las plantas pueden variar en el tiempo y en el espacio. Este enfoque también pretende reducir la heterogeneidad en el vigor de las plantas. La gestión específica de los viñedos puede incluir la gestión diferencial del dosel, los tratamientos fitosanitarios, la fertilización, el riego, el aclareo de racimos y el deshojado, entre otras prácticas. La estabilidad interanual de las zonas de vigor contrastadas en este ensayo permitió probar una gestión diferenciada de los insumos y de las relaciones fuente/fosa. Dado que el agua y la disponibilidad de nitrógeno se identificaron como factores clave en el establecimiento del vigor, estos dos insumos se integraron en un plan de gestión localizado específico destinado a optimizar la producción al tiempo que se promovía la sostenibilidad medioambiental.

El viñedo seleccionado fue manejado inicialmente por el viticultor de la misma manera en toda la parcela. Se trata de un viñedo en el que no se aplica riego. Además, recibe una fertilización estándar con urea (46 % de N), la mitad en prefloración y la otra mitad en poscosecha, con una dosis total de 140 kg por hectárea. La gestión de la cobertura vegetal se controló en la fila con herbicidas, y en la entrefila (mezcla de gramíneas y asteráceas) mediante siegas periódicas. En las zonas preestablecidas y durante tres campañas (2019, 2020 y 2021), se delimitó un diseño de bloques al azar con tres repeticiones y 21 cepas por repetición (63 plantas por tratamiento) para los tratamientos realizados. Las técnicas culturales aplicadas en este estudio se realizaron en función de la zona de vigor para incidir en el rendimiento, la calidad de la uva y reducir la heterogeneidad. En el caso del HV, los tratamientos tenían por objeto reducir los aportes de agua y nitrógeno y mejorar las condiciones microclimáticas de la zona de gestión. La restricción hídrica (H-W) se aplicó desde el envero hasta la cosecha cubriendo el suelo con polietileno. En el tratamiento de restricción de nitrógeno (H-N), no se aplicó nitrógeno (O unidades de N durante la temporada) durante dos temporadas consecutivas (2019 y 2020). El deshojado (H-L) se aplicó en la fase de prefloración eliminando el 60 % de las hojas, con el objetivo de reducir el rendimiento y mejorar la exposición de los racimos. En LV, en cambio, los tratamientos se dirigieron a aumentar el crecimiento

vegetativo y el rendimiento con suplementación de agua y nitrógeno. Se proporcionó riego suplementario (L+W) en comparación con el control (LV) para alcanzar el 100 % de la demanda climática (ET) desde la brotación hasta la floración y desde la cosecha hasta la caída de las hojas, y el 70 % de la ETo desde la floración hasta la cosecha. Para el tratamiento con nitrógeno adicional (L+N), se suministraron 210 kg de urea por hectárea (a los 140 kg antes mencionados, se añadieron 70 kg de urea por hectárea en forma de urea antes de la floración).

El tratamiento H-W creó una barrera física que impidió la pérdida de agua del suelo por evaporación y transpiración al limitar el desarrollo del cultivo de cobertura en la entrefila. Esto provocó una disminución de la humedad del suelo a niveles moderados o bajos hasta cerca de la cosecha, especialmente en años húmedos. La menor humedad del suelo explica la menor disponibilidad de nitrógeno del suelo para este tratamiento al afectar a la tasa de mineralización de la materia orgánica. Este tratamiento no afectó al crecimiento vegetativo ni al rendimiento. A su vez, generó un ambiente con más luz, más temperatura y menos humedad relativa en el racimo. Estos cambios en el microclima de los racimos redujeron significativamente la presión de las uvas (sólidos solubles y antocianos). El efecto de la cobertura plástica del suelo sobre la composición de las bayas fue especialmente interesante en 2019 (año lluvioso). En este tratamiento, se acortó la duración de la fase II de crecimiento de las bayas y de acumulación de azúcar.

El tratamiento H-N mostró un fuerte impacto negativo en el crecimiento vegetativo a partir del segundo año de producción (2020). La restricción de N redujó el peso de las bayas y los grados Brix en la cosecha, pero sólo en el año húmedo. Los parámetros del modelo de crecimiento y acumulación de azúcar en baya se vieron influidos de forma opuesta en los años húmedos y secos. La duración de la fase II disminuyó en los años húmedos y fue mayor en los años secos. La respuesta de las plantas a este tratamiento fue muy dependiente de la disponibilidad de agua durante la temporada.

La eliminación de hojas en la zona del racimo antes de la floración no sólo modificó la relación fuente/sumidero debido a la eliminación de hojas, sino que también afectó al sumidero. El tratamiento H-L redujo el rendimiento en las tres temporadas de cultivo en comparación con el control HV, lo que se debió a una reducción del número de bayas por racimo. La restricción del suministro de carbohidratos debido a la eliminación de las hojas fotosintéticamente activas durante el periodo de floración afectó a la asignación de carbono a las bayas en crecimiento, promoviendo la abscisión del fruto. Aunque el rendimiento fue inferior en el tratamiento H-L en comparación con el HV, el peso de las bayas fue sistemáticamente superior debido a la menor competencia. Además, este tratamiento aumentó el peso de la madera de poda y su lignificación, lo que estaría relacionado con una mayor acumulación de almidón de reserva. El cambio en la relación fuente/sumidero aumentó la exposición de los racimos a la luz y redujo la compacidad de los racimos, lo que en última instancia redujo la incidencia de las enfermedades de los racimos. Los cambios en el microclima del racimo también estimularon el crecimiento de las bayas y la acumulación de azúcar, antocianinas y fenoles. En los años húmedos, este tratamiento acortó la duración de la fase II en comparación con el control. Por tanto, la eliminación de hojas y la reducción del rendimiento en este tratamiento pueden haber acelerado la acumulación de azúcar asociada a una disminución del rendimiento y a un aumento de la eficiencia fotosintética de las hojas restantes.

En la zona LV, el efecto de los tratamientos dependió del suministro de agua del año. El suministro adicional de N (L+N) aumentó el contenido de N del suelo en todos los años, pero no el contenido de N de la planta. Así, a pesar del mayor contenido de N en el suelo para el tratamiento L+N comparado con el LV en 2020, la baja disponibilidad de agua no incrementó el contenido de N foliar. En esta zona de vigor, el agua fue el factor limitante y la aplicación de N sin disponibilidad de agua no tuvo ningún impacto en el crecimiento vegetativo. En las temporadas con mayor disponibilidad de agua (2019 y 2021), la suplementación con nitrógeno (L+N) también influyó positivamente en la composición de las bayas al aumentar el contenido total de antocianos y nitrógeno en el mosto. Cuando se aplicó riego deficitario (L+W) durante la maduración, el estado hídrico de la planta se mantuvo en valores de  $\Psi$ p (entre -0,2 y -0,4 MPa). La mayor humedad del suelo permitió aumentar el contenido de N en las hojas, lo que estimuló el crecimiento vegetativo. La mejora del estado hídrico y nitrogenado de la planta se tradujo en una mejora del rendimiento de la uva, principalmente a través de un aumento del peso del racimo. La temperatura y la luz en la zona del dosel fueron inferiores en L+W que en LV, pero la acumulación de azúcares y antocianinas se vio favorecida por una estrategia de riego deficitario regulado. El suministro de agua (L+W) aumentó la duración de la fase II, retrasando ligeramente el tiempo total de maduración de las bayas (+4 días).

Aunque una parcela de 1 ha puede considerarse homogénea desde el punto de vista topográfico, edafológico y climático, este estudio mostró una gran variabilidad en los suelos, los parámetros de producción y la composición de la uva. Se aportó nueva información sobre la interacción del sistema suelo-planta-medio ambiente. En particular, el papel dominante de la disponibilidad de agua en primer lugar y de la disponibilidad de nitrógeno del suelo en segundo lugar a la hora de establecer el vigor de las plantas. El efecto del nitrógeno sobre la respuesta de las plantas dependió en gran medida de la disponibilidad de agua en el suelo. Estas dos aportaciones, junto con otras características del suelo, propiciaron una mayor exploración de las raíces de la vid, lo que a su vez favoreció la producción de madera y uva. También se han adquirido nuevos conocimientos sobre la relación entre el vigor de la planta y el tipo de arcilla. El predominio de la montmorillonita sobre la illita fue un factor importante para la fertilidad del suelo en la zona HV. El gradiente de vigor de la vid y de rendimiento entre las dos zonas se mantuvo estable a lo largo de los años, independientemente de las condiciones climáticas. Esto indica que las características del suelo pueden mitigar o potenciar los efectos causados por las condiciones climáticas. La determinación (posible mediante teledetección) de las zonas de vigor de una parcela es un requisito previo para proponer prácticas específicas de gestión del suelo y del cultivo que optimicen el uso de los recursos y garanticen la sostenibilidad económica y medioambiental de la producción vitivinícola. La aplicación de tecnologías de viticultura de precisión ha permitido mejorar la productividad de las plantas (rendimiento y composición de las bayas) y reducir la heterogeneidad a nivel de parcela.

En futuras investigaciones, sería deseable aplicar un enfoque de variabilidad espacial a mayor escala para determinar los *microterroirs* y generar así la aplicación de técnicas específicas para cada lugar con el fin de obtener productos diferenciados en términos de calidad de la uva. A su vez, los análisis realizados en viñedos ricos en arcillas de tipo 2/1 confirmarán las hipótesis planteadas en este trabajo sobre el papel del tipo de arcilla en la determinación del vigor. Un análisis más detallado de la heterogeneidad existente en la parcela de viñedo, pero especialmente a nivel de racimo, permitirá perfeccionar las estrategias de gestión específicas para lograr una producción y acumulación óptimas de azúcar y antocianos. Además, los futuros planteamientos deberán tener en cuenta el complejo suelo-planta-atmósfera y, en particular, el papel de los distintos subsistemas vegetales en esta compleja interacción. Un ejemplo sería el papel de las reservas de carbono en el establecimiento de la variabilidad a nivel de racimo, que tiene implicaciones a nivel de baya para la acumulación de agua y azúcar durante la maduración.

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