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Melissa Baiochi

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Mémoire de fin d'études

Presented to obtain the diploma of Agronomic Engineering

Option: Production Végétale Durable (PVD)

**Analysis of the impact of canopy height, soil cover, and watering
on the ecophysiological functioning of a sugar-less variety
(VDQA-G5)**



by Melissa BAIACHI

Year of defense: 2023

Hosting institution: UMR LEPSE
(INRAE/Institut Agro Montpellier)



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Analysis of the combined impact of canopy structure, soil covering practices and irrigation on the carbon, water and nitrogen functioning of a sugar-less variety (VDQA-G5)



by Melissa BAIOSCHI

Year of defense: 2023

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and Martha VIOLET**

ABSTRACT

Climate change poses a significant threat to viticulture, especially in vulnerable regions like the Mediterranean. Rising temperatures and increased drought events are affecting grape quality and wine production. Berries ripen earlier and tend to accumulate more sugars, producing wines with high alcohol content and low acidity. The excessive use of phytosanitary products, primarily fungicides, raises environmental concerns. To address these challenges, strategies such as vineyard management modifications and the selection of new grape varieties are being explored. The variety VDQA-G5 is a fungus-tolerant genotype and requires lower sugar contents to achieve maturation (e.g., sugar-less trait). Nevertheless, not much is known about the responses of this variety to a multi-abiotic stress environment. This study focuses on understanding the ecophysiological responses of sugar-less VDQA-G5 variety under two canopy height treatments combined with three soil cover practices (non-irrigated spontaneous vegetation, irrigated bare soil, and irrigated sown soil). It also aims to compare these responses with another fungus-tolerant but non-sugar-less genotype, the 3159B. Ecophysiological parameters, including stomatal conductance, intrinsic water use efficiency, and photosynthesis, are measured to assess carbon and water use dynamics at the leaf level. The results effect of canopy height treatments over the variables was strongly seen. The G5 genotype on spontaneous soil treatment showed a significant decrease in gsw over time, while G5 on bare and sown treatments had higher gsw values. Furthermore, Amax of G5 on high canopy plants was 25% greater than the same treatment in 3159B. Equations were produced to estimate Amax and Asat values using stomatal conductance as a proxy.

Keywords

climate change; resistant varieties; VDQA; reduced leaf height; soil cover; water use efficiency; carbon metabolism; water consumption limitation

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RESUME

Titre : Analyse de l'impact de la hauteur de la canopée, de la couverture du sol et de l'irrigation sur le fonctionnement écophysiological d'une variété sugar-less (VDQA-G5)

Le changement climatique représente une menace importante pour la viticulture, en particulier dans des régions vulnérables comme la Méditerranée. L'augmentation des températures et la multiplication des événements de sécheresse ont des répercussions sur la qualité des raisins et la production de vin. Les baies mûrissent plus tôt et ont tendance à accumuler plus de sucre, produisant des vins à forte teneur en alcool et faible acidité. L'utilisation excessive de produits phytosanitaires, principalement des fongicides, suscite des préoccupations environnementales. Pour faire face à ces défis, des stratégies telles que des modifications de la gestion des vignobles et la sélection de nouvelles variétés de raisins sont explorées. La variété VDQA-G5 est un génotype tolérant aux champignons et nécessite des teneurs en sucre plus faibles pour atteindre la maturation (e.g. sugar-less). Néanmoins, on en sait peu sur les réponses de cette variété à un environnement de stress multi-abiotique. Cette étude vise à comprendre les réponses écophysiologicals de la variété sans sucre VDQA-G5 sous deux traitements de hauteur de feuillage combinés à trois pratiques de couverture du sol (végétation spontanée non irriguée, sol nu irrigué et sol semé irrigué). Elle vise également à comparer ces réponses avec un autre génotype tolérant aux champignons mais non sans sucre, le 3159B. Les paramètres écophysiologicals, notamment la conductance stomatique, l'efficacité intrinsèque de l'utilisation de l'eau et la photosynthèse, sont mesurés pour évaluer les dynamiques d'utilisation du carbone et de l'eau au niveau de la feuille. Les résultats montrent l'effet des traitements de hauteur de feuillage sur les variables. Le génotype G5 en traitement de sol spontané montre une diminution significative de la conductance stomatique au fil du temps, tandis que G5 en traitements de sol nu et semé présente des valeurs cohérentes de conductance stomatique. De plus, Amax de G5 sur des plantes à feuillage élevé est de 25 % supérieur à celui du même traitement chez 3159B, ce qui indique une moindre force source/puits dans le génotype sans sucre. Des équations ont été produites pour estimer les valeurs de Amax et Asat en utilisant la conductance stomatique comme proxy.

Mots clés

Changement climatique ; variétés résistantes ; VDQA ; hauteur réduite de la canopée ; couverture du sol ; efficacité de l'utilisation de l'eau ; métabolisme du carbone ; limitation de la consommation d'eau

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ABBREVIATIONS AND ACRONYMS

ABsys: Agrosystèmes Biodiversifiés
Amax: photosynthetic rate under light-saturating conditions
An: net CO₂ assimilation
Asat: photosynthetic rate under light and CO₂-saturated conditions
BARE: bare soil cover
CBGP: Centre de Biologie pour la Gestion des Populations
CC: Climate change
CIRAD: Centre de coopération Internationale en Recherche Agronomique pour le Développement
ETR: electron transport rate
G5: 3197-81-B genotype
gsw: stomatal conductance
HIGH: high canopy height
IFV: Institut Francais de la Vigne et du Vin
INRAE: Institut national de la recherche agronomique
IPCC: Intergovernmental Panel on Climate Change
L600: Li-cor600
L6800: Li-cor6800
LEPSE: Laboratoire d'Écophysiologie des Plantes Sous Stress Environnementaux
LOW: low canopy height
Nmin: mineral nitrogen
Ntot: Total nitrogen
PET: Potential evapotranspiration
PhiPS2: quantum efficiency of photosystem II
PPP: Plant Protection Products
RUBISCO: Ribulose-1,5-bisphosphate carboxylase/oxygenase
SOWN: sown soil cover
SPO: spontaneous soil cover
Tleaf: leaf temperature
Tref: reference temperature
VDQA: Vin De Qualité à teneur réduite en Alcool
VSP: Vertical shoot positioning
WD: Water deficit
WUEi: intrinsic water use efficiency
 Ψ_{plwp} : pre-dawn leaf water potential

1. INTRODUCTION

Climate change endangers natural and agronomical systems. Projections show an increase in temperature of 1.5 to 2°C by the end of this century and more extreme drought events. The Mediterranean region is a very vulnerable region and is classified as a climate change hotspot (IPCC, 2022).

Viticulture, the first agricultural activity in this region, is also impacted by climate change due to the increased temperatures and drought conditions. Both the productivity of the vineyards and the grape quality are affected by it. One of the consequences of high temperatures is the advance in grapevine phenology. With earlier bud-break and flowering, berries ripening is anticipated and shifts into warmer and drier periods. On the one hand, this trend increases berry soluble sugar content, and on the other hand, it reduces malic acid content (Sweetman et al., 2014). These conditions lead to the production of unbalanced wines with very high alcohol content and insufficient acidity. In addition to high temperatures, the intensification of drought events is also a problem for grapevine production. Severe water deficit limits photosynthesis and the production of assimilates by the vines (Pellegrino et al., 2005). Therefore, the decreased carbon accumulation can impact the yield, reducing berry size and number (van Leeuwen et Darriet, 2016), thus harming wine quality.

Besides climate change, viticulture also faces the challenge of decreasing the use of phytosanitary products. The grapevine-growing sector in France accounts for 20% of the annual usage of plant protection products while covering only 3% of the arable land in the country (INRA, 2018a). According to (AGRESTE, 2021), more than $\frac{3}{4}$ of the phytosanitary used in viticulture products are fungicides used to control the two central cryptogamic diseases of the grapevines: downy mildew and powdery mildew.

Different strategies are studied to reduce viticulture's environmental impact and adapt the vineyard to extreme climatic conditions. Some involve changes in vineyard management, including modifications in the training systems, canopy height, soil cover, and irrigation management. Other strategies involve the selection of new varieties and rootstocks that could be more adapted to high temperature and water stress but also less vulnerable to fungal diseases. (Abad et al., 2019).

The varieties called "sugarless," or VDQA (Vin De Qualité à teneur réduite en Alcool, in French) were selected by researchers of INRAE in the late 60s (INRA, 2018b). They have drawn attention because of their low sugar requirement to reach ripening. Compared to classic varieties, the VDQA accumulates between 20 to 30% fewer hexoses, producing wines with lower alcohol content, between 9.5% v/v and 11.5% (Bigard et al., 2022). Furthermore, these varieties have an environmental interest. Indeed, they contain tolerant genes to downy mildew and powdery mildew, which are proven to reduce the use of phytosanitary products in vineyards (INRA, 2018a). Nevertheless, almost nothing is known about the ability of these varieties to be adapted to the climate change context.

This present study aims to understand the ecophysiological responses of the sugar-less VDQA-G5 variety under different canopy heights (75 and 50cm) when combined with three

irrigation and soil cover practices. In this experiment, VDQA-G5 was compared with another fungus-tolerant but non-sugar-less variety, the 3159-B.

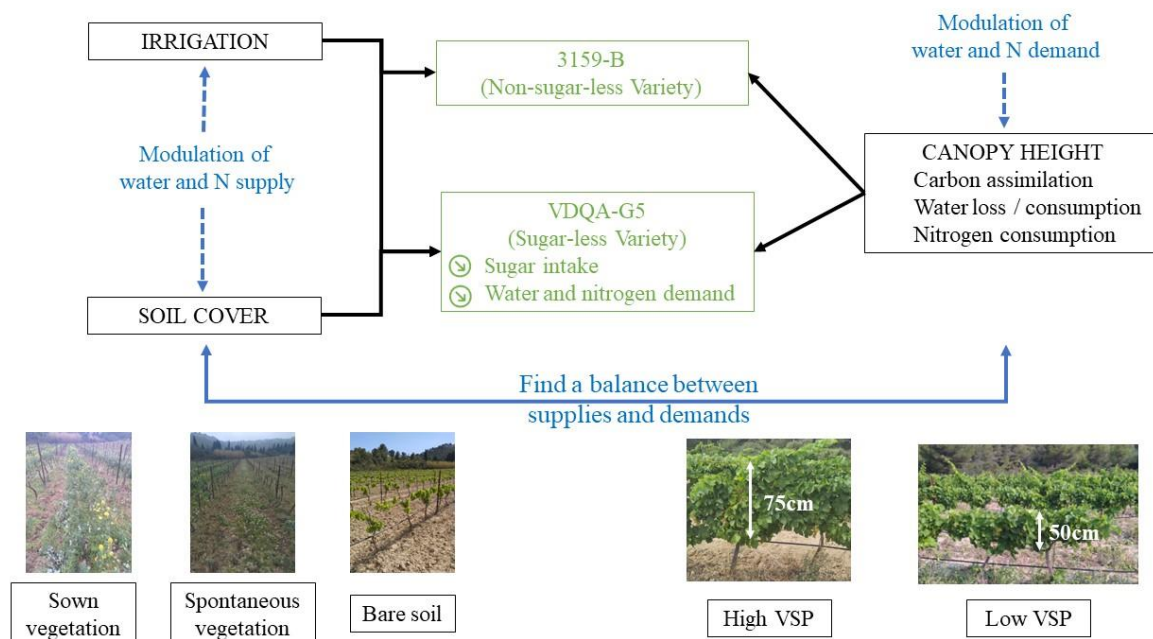


Figure 1: Experiment overview. Legend: black boxes = treatments; blue dotted lines = role of the treatments in the experiment; green boxes = studied varieties

Figure 1: Experiment overview. **Legend:** black boxes = treatments; blue dotted lines = role of the treatments in the experiment; green boxes = studied varieties

The reduction of canopy height was used to reduce plant water loss through foliage transpiration and nitrogen demands (Figure 1). Meanwhile, the soil cover and irrigation were used to modulate the disponibility of water and nitrogen to the grapevines.

In the long run, it is sought to find a balance between a foliage reduction that allows reducing water losses and, simultaneously, does not significantly impair plants' performance (vegetative and reproductive growth, accumulation of reserves, and yield) (Figure 1). When testing the reduction of canopy foliage in a sugar-less variety such as VDQA-G5, it is hypothesized that it would suffer a lower physiological impact to this reduction than non-sugar-less varieties because of its lower sugar needs to reach fruit maturity (less carbon assimilates).

Ecophysiological measurements were used to assess carbon and water use at the organ level and carbon allocation dynamics. Photosynthetic activity, intrinsic water use efficiency, stomatal conductance, pre-dawn leaf water potential, and SPAD-based leaf nitrogen estimation were measured. The field experiment was carried out at the experimental unit of INRAe Pech Rouge.

2. CONTEXT AND BIBLIOGRAPHY

2.1 Descriptions of leaf physiological parameters closely dependent on environmental variables

2.1.1 Stomatal conductance

Stomatal conductance (g_{sw}) can be defined as the reverse of the resistance to the CO₂ influx and water vapor efflux through the stomata of a leaf (Gowdy et al., 2022). It is measured in $\text{mmol m}^{-2}\text{s}^{-1}$. This variable can be measured using a porometer or a gas exchange meter equipment (measured on individual leaves).

Environmental factors such as net radiation, temperature, water vapor pressure deficit (VPD), available soil water, and ambient CO₂ concentrations affect stomatal conductance. (Tuzet, 2011). Because of its relation with leaf water potential, g_s can also be used as an indicator of plant water status Giménez, Gallardo, and Thompson, (2005) show that reductions in leaf water potential can induce stomatal closure. This happens through a mechanism involving the abscisic acid (ABA) produced on the roots and translocated to the leaves, resulting in lowered g_s . (Murchie et Lawson, 2013)

2.1.2 Transpiration

In plants, transpiration can be defined as water taken up from the soil and lost through the stomata in the leaves. (Tuzet, 2011). The regulation of transpiration is a response to the environmental conditions plants are exposed to, such as atmospheric conditions and water availability. An increase or decrease in transpiration rate is done by regulating stomatal conductance. Control of stomatal opening and hydraulic conductivity of the conductive vessels are some of the physiological mechanisms to adjust the water conductance. This conductance varies according to the days and the seasons (Gowdy et al., 2022). Xylem cavitation and loss of hydraulic conductivity are some important survival mechanisms plants use face to protect their physiological process during extreme drought conditions. (Aroca, 2013)

2.1.3 Photosynthesis

Photosynthesis is a plant process that converts atmospheric carbon dioxide into organic compounds, especially sugars, using energy from sunlight. It is divided into Light-dependent and light-independent reactions (or Calvin Cycle). In light-dependent reactions, radiation is absorbed by chlorophyll and converted into chemical energy in the form of electron carrier molecules (ATP and NADPH). Radiation is absorbed by light harvest photosystems I and II, both located on chloroplasts' thylakoid membranes. In light-independent reactions, carbohydrate molecules are assembled from carbon dioxide using the chemical energy harvested during the light-dependent reactions.

2.1.4 Chlorophyll fluorescence

Chlorophyll fluorescence is employed as a precise and non-destructive technique to measure photosynthetic efficiency. It directly or indirectly indicates the repercussions of environmental factors and changes in plants physiological state on the photosynthetic process. (Guidi, Lo Piccolo, et Landi, 2019).

Principle of chlorophyll fluorescence: light energy absorbed by chlorophyll molecules in a leaf can follow three pathways: it can be used to drive photosynthesis (photochemical quenching), be dissipated as heat (non-photochemical quenching; NPQ) or be re-emitted as light (chlorophyll fluorescence). These three processes occur in competition, such that any increase in the efficiency of one will result in a decrease in the yield of the others. Therefore, by measuring the yield of chlorophyll fluorescence, it is possible to obtain information about the leaf's quantum efficiency of photosystem II (Φ PSII), electron transport rate (ETR), and non-photochemical quenching (NPQ) (Zaman et al., 2018) (Licor, [s.d.]

2.2 Challenges in viticulture

2.2.1 Climate change

Climate change (CC) is primarily characterized by the rise in air temperature coupled with shifts in rainfall patterns (IPCC, 2022). Climate models predict a temperature increase from 1 to 4 degrees by the end of this century as an increase in extreme weather events, such as severe droughts. These climatic trends lead to negative effects on the grapevine, as changes in plant growth, physiology, and berry ripening, which can cause severe losses regarding yield and the quality of vines (Bernardo et al., 2018).

The most perceptible effect of climate change is the increase in average temperatures during the growing season of crops (Bernardo et al., 2018). This increase can already be observed today. As the air temperature increases, the evapotranspiration of the plants (PET) increases too. With this rise in PET, the water balances of the crops become more negative, and plants are more exposed to water deficit conditions. (van Leeuwen et Darriet, 2016).

The Mediterranean region, renowned as a significant wine-producing zone, has been classified by the IPCC as a climate change hotspot, indicating its high vulnerability to these changes. New vineyard management strategies to mitigate the impacts of CC on grape production are thus needed. Several consequences of CC can be mentioned, such as reduced plant growth, alterations in phenological stages, decreased yields, and grape quality.

2.2.1.1 Consequences of climate change for grapevine

A) Advancement of phenological stages

The increase in average temperatures in the past few years has already led to an advancement in key phenological stages of grapevines, such as bud break, flowering, veraison, and ripening (IFV, INRAE). Nowadays, the harvest periods have moved backward nearly two weeks compared to 1980 (Figure 2).

This temperature rise impacts the berry quality of the resulting berries, which mature under hotter and drier climate conditions. These early-matured berries tend to accumulate more sugars and degrade, accelerating the malic acid breakdown (Sweetman et al., 2014). This

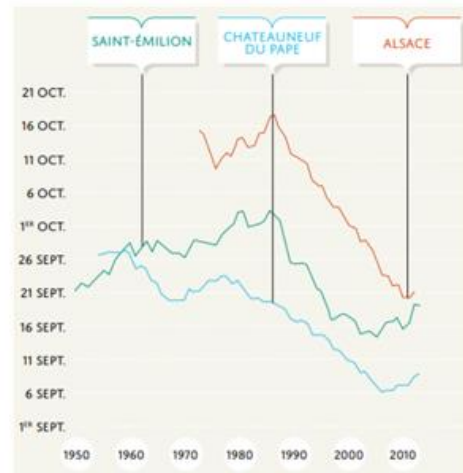
leads to the production of unbalanced wines with very high alcohol content and insufficient acidity.

B) Impact of water deficit on grapevine function

In the Mediterranean area, water deficits are usually observed during summer when temperatures and solar radiation are maximal. Stomatal closure is one of the first plant responses to drought. In mild water stress conditions, plants reduce their stomatal conductance (g_{sw}) to limit water losses by transpiration (E). It leads to a reduction in the plant's net CO_2 assimilation (A_n). (Scholasch et Rienth, 2019) and increase water-use efficiency (A_n/E and A_n/g_s). Regarding vegetative growth, one of the first physiological responses to mild water deficit is a reduction in shoot growth.

Under more severe water stress, non-stomatal regulation of photosynthesis is observed due to a decrease in the RUBISCO activity and an increase in mesophyll conductance. (Medrano et al., 2003). Besides leading to a decline in photosynthetic parameters, strong water deficits also decrease the overall quality of the berries. It negatively affects berry size by impeding cell division processes.

Levels of water stress based on pre-dawn leaf water potential measures were proposed to evaluate the degree of water deficits faced by the grapevines (Table 1) (Deloire, Pellegrino, et Rogiers, 2020).



Source: IFV, INRAE

Figura 2: Changes in harvest dates from 1950 to 2010 in three viticultural areas in France

Tabela 1: Threshold values of pre-dawn leaf water potentials (Ψ_{plwp} , MPa) and possible consequences for grapevine water status and physiological functioning Source: Adapted from Deloire, Pellegrino, et Rogiers (2020)

Predawn leaf water potential (Ψ_p , bar)	Level of water constraint or stress	Possible consequences for photosynthesis
$0 \text{ Bar} \geq \Psi_p \geq 3 \text{ bar}$	No water deficit	none
$3 > \Psi_p \geq 5$	Mild to moderate water deficit	none to reduced
$5 > \Psi_p \geq 8$	Moderate to severe water deficit	reduced to inhibited
$\Psi_p > 8$	severe to high water deficit	inhibited

2.2.2 Sustainability issues

2.2.2.1 Soil protection

Healthy soils are the foundation of sustainable viticulture. Besides providing water and nutrients for the vines, soils also host a huge diversity of organisms that perform important ecological functions and ecosystem services (Garcia et al., 2018). However, nowadays, vineyard management practices represent a threat to soil quality and soil biodiversity. In water-limited systems, the intensive weed control strategy is applied as traditional management to avoid competition between grapevines and weeds for water and nutrients (Giffard et al., 2022). Tillage and herbicides are also very common techniques in water-limited environments. These practices impact negatively the soil quality, increasing soil erosion, soil compaction, and loss of organic matter. Besides, using products like herbicides also damages soil-associated biodiversity through direct effects on the metabolism of the organisms and groundwater quality.

2.2.2.1 Use of fungicides

Besides caring for soil health, sustainable viticulture focuses on reducing the use of Plant Protection Products (PPP). This is a current issue as these products are still widely used in viticulture. According to (AGRESTE, 2021), more than $\frac{3}{4}$ of the treatments in French vineyards are fungicides. They are used against downy mildew (DM; causal agent *Plasmopara viticola*) and powdery mildew (PM; causal agent *Erysiphe necator*), known as grapevine's most important fungus diseases. The commonly used grape cultivars show high susceptibility to these fungal diseases (Schneider et al., 2019), and the main strategy used to control these fungi still relies on chemical treatments. Nevertheless, relying on chemical-based controls as the main option is not a sustainable long-term strategy and endangers local biodiversity, soil, and water quality. Therefore, there is an increasing need to explore alternatives to these methods.

2.3 Levers to respond to the challenges

Today's viticulture faces multiple challenges, such as decreasing the use of fungicides, adapting to climate change conditions, and preserving soil healthy. Some levers to respond to these challenges such as variety selection, canopy management, and soil management practices, will be discussed.

2.3.1 Variety selection

This use of resistant varieties has a major impact on the sustainability of the vineyards. They represent an economy of 80 to 90% in the use of fungicides. (INRA, 2018a) The French Institut INRAe (Institut National de la Recherche Agronomique) coordinates important research into resistant varieties, such as the research that led to the discovery of bouquet varieties and the ResDur breeding program.

2.3.1.1 Bouquet varieties

The varieties resistant to mildew and powdery mildew were discovered in the late 60s by INRAe. They were obtained from 4 or 5 generations of "backcrossing" between *Muscadinia rotundifolia* and *Vitis vinifera*. These varieties were called "Bouquet varieties" in honor of the researcher, Alain Bouquet, who contributed to this discovery. Some examples of Bouquet's varieties are the VDQA-G5 and 3159-B (shown in red rectangles Figure 3). These two varieties have not yet been registered in the French catalog of varieties, and they are still under evaluation for their long-term resistance and agronomic performances.

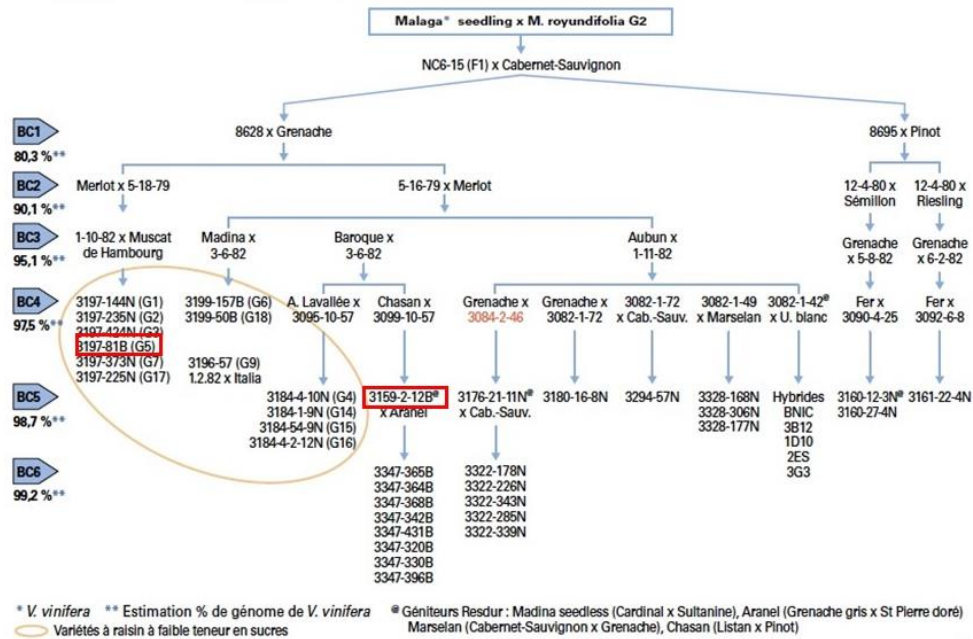


Figura 3: Scheme for the development of tolerant genetic material to powdery mildew and downy mildew through interspecific crossbreeding of *V. Vinifera* x *Muscadinia Rotundifolia* by Alain Bouquet..

The 3159-12-B is a white grape variety obtained by crossing the Chasan B and Mtp 3099-10-57. Its yield varies between 6 to 8t/ha in non-irrigated conditions. 3159 presents two resistance genes to downy mildew (genes Rpv1+Rpv3) and powdery mildew (Run1+Ren3). The 3197-81-B (G5), on its turn, is also a white grape variety with two resistance genes, but it was obtained by crossing 1-10-82 and Muscat de Hambourg. Although these varieties showed a high ability to strongly limit the development of powdery and downy mildew, one fungicide treatment for these pathogens at the pre-flowering stage is still recommended.

The G5 variety is part of a group of varieties called "sugar-less" or VDQA (Vin De Qualité à teneur réduite en Alcool, in French) (red circle in Figure 3). These varieties have the ability to produce grapes with low sugar content. The VDQA-G5, for example, reaches fruit maturity with 20-30% fewer hexoses than the usual varieties (such as Merlot and Grenache) without strong modification in berry size, total acidity, and secondary metabolite contents (Salmon, Ojeda, et Escudier, 2018). This characteristic allows the production of wines with a lower alcohol content, which is positive, regarding the increment of alcohol content in classic varieties due to climate change.

A study about the acclimatization of some tolerant varieties to different water statuses showed that G5 is a sensible genotype for water deficit. Regarding its physiological and reproductive parameters in water deficit conditions, G5 showed the highest reduction in berry weight and photosynthesis. However, it showed the highest WUE_i compared to the other studies genotypes (3159B, 3176N, G15, Artaban, and Floreal) (Almeida et al., 2023).

The G5 and 3159B adopt different strategies of stomata closure: 3159B is less conservative, adopting a strategy of late closure called tolerance. G5, on the other hand, adopts a more conservative strategy, closing its stomatal early (avoidance). These varieties present a WUE of 82 and 107 μmol of CO₂ per mol of H₂O, for 3159B and G5, respectively. (Almeida et al., 2023)

2.3.2.2 ResDur project

ResDur is an important INRAe breeding program launched in 2000. It aimed to create cultivars with durable resistance to downy and powdery mildews and with berry quality suitable for the production of high-quality wines. An accelerated selection procedure was applied based on MAS (marker-assisted selection) and multilocation field trials. This methodology helped reduce the time from seed germination to catalog entry to 15 years. In 2018, this project led to the registration of 4 resistant grape varieties on the French variety catalog: Artaban, Vidoc, Floréal, and Voltis. (Schneider et al., 2019)

2.3.1 Canopy management

The modification of canopy structures and size in viticulture is one of the main factors used to manage water plant consumption (de la Fuente, Linares, et Lissarrague, 2015). Several plant processes, such as transpiration and photosynthesis, are modulated depending on the spatial positions of leaves, branches, and clusters within the canopy. An adequate balance among crop load, training system, and irrigation water management should be required in warm climates as a key point in order to assess the expected quality of grapes and wine. (de la Fuente, Linares, et Lissarrague, 2015). Several viticultural practices (thinning, pruning, topping, flower removal) are used to optimize the ratio between leaf area and cluster number and light penetration within the canopy.

Abad et al. (2019) tested the consequences of different trimming intensities on grapevine cv. Tempranillo and cv. Graciano at the pea-size phenological stage in Spanish vineyards. Two intensities of trimming were imposed: 40% and 60% of shoot length (reduction from 15-30% of total leaf area). These treatments were compared to a control group of plants that were not trimmed. It was found that the severe trimming only improved grapevine water status when the year's climate conditions were drier (high evaporative demand).

Another experiment with cv. Tempranillo, in the northeast of Spain (under Mediterranean conditions), tested three canopy management strategies. A repeated hedgerow trimming at 1 m height was tested among the treatments. The trimming occurred after the berry set. It was concluded that the trimming could be beneficial in reducing the extraction of water in the soil under severe water stress conditions, helping to preserve this resource during the season. (Pascual et al., 2015)

2.3.2 Soil management

Cover crops have multiple benefits to the vineyards, such as increasing soil organic carbon, improving water infiltration and aggregate stability, and reducing soil erosion. However, as vines and cover crops coexist in the same space, they compete for water and nutrients during different periods throughout the growing season (Celette, Findeling, et Gary, 2009). When not limited, this competition can impact shoot fertility, berry set, yield, and grape composition.

Cover crops can be sown or spontaneous. When sown, Fabacea and Poaceae species mixtures are commonly used (Abad et al., 2021). These legumes contribute to increasing total nitrogen (N_{tot} = organic + mineral N) and mineral nitrogen (N_{min}) in the soil. This happens because legumes have the ability to capture atmospheric nitrogen through their roots with the help of Rhizobium bacteria (Peoples et al., 2009). On the other hand, plants from the group of grasses are known to significantly deplete soil nitrogen levels compared to other plant families, as they actively absorb nitrogen from the soil. (Abad et al., 2021).

The use of a sown cover crop containing grasses and legumes can enhance total soil nitrogen content by 43% when compared to tillage management (Table 2). Meanwhile, the use of grasses is shown to reduce the N levels in the soil by 25% (Table 2). The spontaneous vegetation cover slightly decreased the N_{tot} content compared to tillage management.

Tabela 2: Total nitrogen (N_{tot}) and mineral nitrogen (N_{min}) average soil content, grouped according to cover crop type/soil management. (prepared from 14 articles). Source: Abad et al. (2021)

Soil management	N_{tot} (g/kg)	N data	N_{min} (mg/kg)	N data
Grass	0.873	4	5.475	4
Grass + legumes	1.716	7	-	-
Legumes	1.555	2	17.458	5
Spontaneous vegetation	1.062	5	7.43	1
Tillage control	1.193	4	7.515	2
Herbicide control	1.053	3	1.9	1
Herbicide + tillage control	0.935	2	-	-

According to IFV, the Faba beans (*Vicia faba*) - one of the species used in the mix sown cover crop in this experiment - is a moderate-need plant that has a potential for nitrogen restitution of 47kg/ha when the crop achieves a biomass of 2t of dry matter per hectare. (IFV)

Regarding the competition for water, a study on a Mediterranean vineyard showed that the upper soil layers dry more rapidly with treatments with a cover crop in the inter-row than when there was only evaporation from bare soil. (Celette, Findeling, et Gary, 2009). Other studies showed that the water potential of the soil in a grassed treatment decreases more rapidly in the row and in the inter-row than in a bare soil treatment. Consequently, cover crops could be a strong competitor for water, particularly in dry years.

2.4 Context of the internship

The VITIFUTURE project, funded by the Occitanie Région, brings together a multidisciplinary group of INRAe's research unities to study innovative viticultural systems that can mitigate the impact of climate change and reduce the biodiversity erosion in the vineyards of the Mediterranean area. This project focuses on the study of the interactions between genotype (G), environment (E), and cultural practices (P) on grapevines. The main genotypes studied in VITIFUTURE are fungus-tolerant genotypes and sugar-less genotypes.

This project is divided into two parts carried out by Ph.D. students, one from UMR LEPSE and another from UMR CBGP. The research performed by UMR CBGP aims to evaluate how the implementation of agroecological practices impacts the ecosystem services related to soil functioning, water cycle, agronomic performance, and biological regulation of pests. Its experimental plots are placed at the Domaine du Chapitre (Institut Agro, Villeneuve-lès-Maguelone), and the study is performed in collaboration with UMR ABSYS.

Meanwhile, the research carried out at UMR LEPSE in the framework of the Ph.D. of M. Violet aims to understand how water and nitrogen resources, modified by cultural practices and vineyard management techniques, impact the functioning of disease-resistant grapevine varieties. Different soil cover practices (bare soil, spontaneous and sown vegetation) and irrigation treatments were used to manage the availability of water and mineral elements for the plants. In addition, three canopy managements (minimal pruning, high VSP, and low VSP) were applied to these modalities to modify plant water and nitrogen demand and source-sink balance.

The experimental plots of this project are located at INRAE experimental unit of Pech Rouge (Gruissan, France). Among the varieties studied, there are 3 Bouquet's varieties (3159B, 3176N, and VDQA-G5), ResDur variety (Artaban), and one conventional variety (Syrah). Phenotyping methods were used to describe organ functioning and resource allocation dynamics (water, carbon, nitrogen) between the organs. Meanwhile, leaf water and plant nitrogen status, canopy growth, photosynthesis, transpiration, berry sugar, and berry nitrogen loading will be monitored. This project should extend our knowledge about the carbon, nitrogen, and water functioning of fungal disease-resistant varieties in multi-factorial environments.

The present internship is a part of the thesis developed by UMR LEPSE. With the same objectives, my work was focused on the response of VDQA-G5 and 3159-B varieties subjected to different canopy heights (high and low VSP), soil management, and irrigation practices. It's important to note that this internship occurred during the project's initial phase when the irrigation schedule and water deficit gradients between soil treatments were still being implemented.

3 PROBLEM STATEMENT AND OBJECTIVES

Problem statement:

- How the physiological parameters, vegetative growth, and water status of the sugar-less variety VDQA-G5 are impacted by different soil management practices, irrigation, and thinning heights?
- Is the VDQA-G5 less sensitive to severe reduction in canopy high and water stress conditions than the non-sugar-less variety 3159-B?

General objective: Analyze the response of ecophysiological parameters hydric status of the VDQA-G5 variety under different soil/irrigation management and two canopy height treatments. Compare the results of this variety with variety 3159-B, which is fungal-resistant but not sugar-less.

Specific objectives: We aim to analyze the impact of the treatments on plant water consumption (stomatal conductance, WUE), carbon metabolism, and allocation (photosynthesis).

Hypothesis: We make the hypothesis that, as the G5 requires less sugar accumulation to reach berry maturity, the sink strength of this variety will be inferior to that of 3159-B, that do not present this sugar-less trait. We also make the hypothesis that the reduction in canopy height will increase plants' photosynthesis rate due to the increase in the efficiency of the remaining leaves. It is also hypothesized that the treatment with cover crop soil management will increase the nitrogen available for the plants. For the spontaneous vegetation, the hypothesis is that it will consume water and nitrogen without increasing nitrogen content in the soil and, consequently on the plant leaves.

General approach: The study was done on an experimental vineyard with the varieties VDQA-G5 and 3159-B. This experiment used an ecophysiological approach at the plant and organ scale. Measurements for evaluating the vegetative development, carbon assimilation, photosynthetic parameters, water status, and nitrogen content of the vines were collected from 30/05 to 08/08.

4 MATERIAL AND METHODS

4.1 Climatic conditions

Over the season of 2023, the minimum and maximum monthly average temperatures for 2023 (from April to August) were 10.6 °C/31.4 °C according to data from the closest meteorological stations. The 2023 growing season was characterized as drier, showing the lowest rainfall amounts, totalizing 107,5 mm (Figure 4). Average global radiation ranged from 2039 to 2617 during the same period.

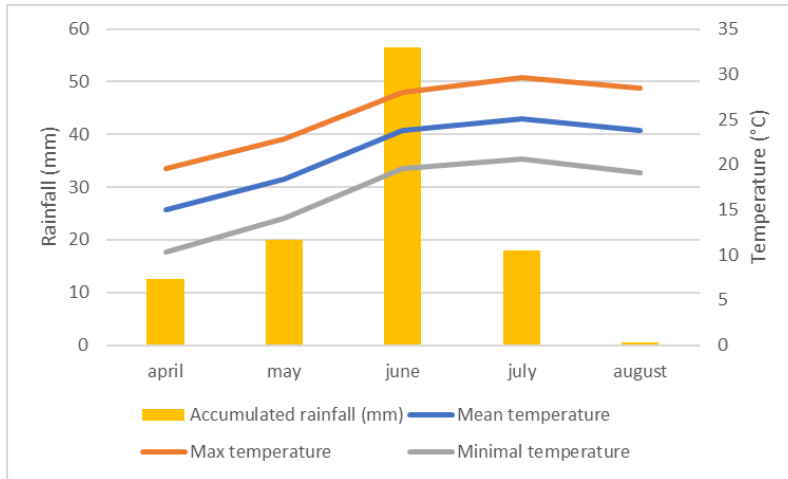


Figura 4: Ombrothermic diagram from April to August, Gruissan. Maximal, mean, and minimal temperatures refer to daily means of the maximal, mean, and minimal temperatures, respectively. Source: Meteorological station of Gruissan, agroclim/inrae

4.2 Experimental design

The experimental plot was located at the INRAE unit of Pech Rouge, Gruissan, France (43.14° North | 3.14° East). The genotypes used for this experiment were the VDQA-G5 and the 3159-B obtained by INRAE and known to be fungus-tolerant genotypes (*V. vinifera* L. × *M. rotundifolia* hybrids). All the plants were grafted onto 140Ru, a rootstock known to perform well under dry conditions (PI@antGrape, 2009). The 3159-B plot was planted in 2012, while the G5 plot was in 2015. The density of the plantation is 4400 plants/ha (2.6 × 0.9 m) in both plots, and the row orientation was SW–NE. All the selected plants were managed in VSP (vertical shoot positioning) through two pruning methods: simple cordon for the G5 plot and a double cordon for the 3159-B. The soil of the G5 plot is clayey-calcareous, with marl-type rocks. There was no available knowledge of these soils' soil water storage capacity.

In the years before the experiment, the soil was ploughed every two interrows, and kept with spontaneous vegetation in the other rows. The cultivated and plowed inter-rows have not been alternated over the years.



Figura 5: VDQA-G5 and 3159-B experimental plots plan at Gruissan (INRAe Pech Rouge). The measurement zones were represented in blue for the G5 plot and orange for the 3158-B plot.

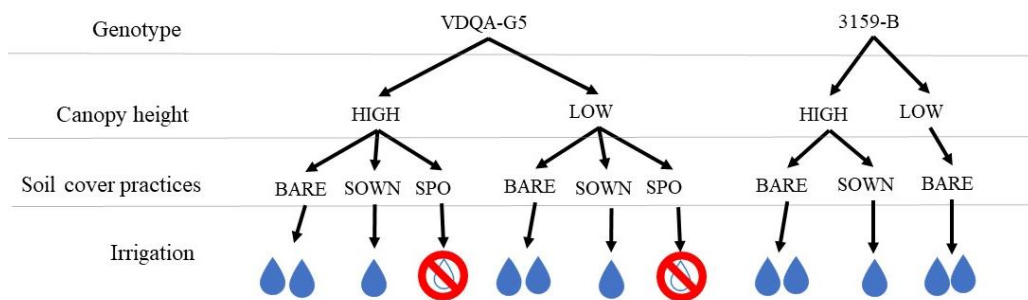


Figura 6: Schematic representation of the treatments of the experiment. LOW = low VSP; HIGH = high VSP; BARE = bare soil; SOWN = sown vegetation; SPO = spontaneous vegetation.

To create the treatments, soil covers were coupled with irrigation practices (Figure 6). In the G5 plot, six treatments were established by combining three types of soil cover/ irrigation and two canopy heights. They were called:

- **HIGH-BARE:** plants with a high canopy on bare soil (irrigated twice a week);
- **HIGH-SOWN:** plants with a high canopy on sown soil cover (irrigated once a week);
- **HIGH-SPO:** plants with high canopy heights on spontaneous soil cover (non-irrigated);
- **LOW-BARE:** plants with low canopy height on bare soil cover (irrigated twice a week);
- **LOW-SOWN:** plants with low canopy on sown soil cover (irrigated once a week);
- **LOW-SPO:** plants with high canopy heights on spontaneous soil cover (non-irrigated).

Three treatments were established in the 3159-B plot (Figure 5, B): **HIGH-SOWN**, **HIGH-BARE**, and **LOW-BARE**, which have the same description as presented for the G5 plot. For each treatment, twenty plants were chosen based on the soil resistivity (area with the same soil resistivity), pruning type (all simple cordon for G5 and all double cordon for 3159-B), and trunk circumference (the most homogeneous as possible). The same irrigation, soil, and canopy management patterns described in the G5 were applied to the 3159-B plot.

A) Soil cover management

Regarding soil management, the bare soil treatment was obtained with a plow at 15 to 20 cm depth. The spontaneous cover was obtained by letting the soil's seed sprout naturally and the sown soil by sowing a cover crop mix of fava beans (*Vicia faba*), oats (*Avena sativa*), barley (*Hordeum vulgare L*), grass pea (*Lathyrus sativus*), mustard (*Sinapis arvensis*). These species were sown in late September (20/09/2021) and destroyed in late April. Their residues were incorporated into the soil. All the treatments were fertilized with organic nitrogen (Angibaud SOF MB®, 800kg/ha) in one of two rows.

B) Watering management

The irrigation material chosen was drip irrigation. No irrigation was performed during the cover crop development. The amount of water was not the amount of water received by the two plots was not the same, with plot G5 receiving approximately 50 L/m² distributed during the experimental period, while plot 3159 received about 27 L/m². The soil water status was estimated using leaf predawn water potential, and the irrigation schedule was adjusted to create a gradient of WD between soil cover treatments.

The initial irrigation schedule was to irrigate the bare soil twice a week, the soil with sown cover once a week, and not to irrigate the spontaneous vegetation. This irrigation schedule's objective was to create a water gradient in the plot, together with the help of the soil covers in place. The plants on spontaneous soil treatments were expected to be in the highest water deficit conditions, the plants from bare soil treatments would not be subjected to any water deficit, and the plants on sown soil treatments would have a medium WD.

As there was no prior information about the soil water storage capacity of the plots and also due to the unavailability of water for irrigation during some periods, the irrigation schedule underwent adjustments and was not followed as the initial schedule theory during the whole time of this experimentation.

At the middle of May, three irrigation sessions with equal water input (10 mm) were performed for all experimental treatments in plot G5. This was done because the grapevines of plot G5 had their growth strongly slowed due to the drought in winter and spring in 2023 (climatic description in section 4.1). These initial water inputs helped plants resume growth. Two irrigations in the same week were performed on 27 July for the bare soil treatments to increase the contrast with the spontaneous treatments.

C) Canopy height management

When it comes to canopy management, in the high treatment, the grapevines were manually pruned on 26/06, at 75cm, while in the low treatment, they were pruned on 27/06 at approximately 50cm of canopy height, which represents a reduction of 33% in the canopy height.

4.3 Measured variables

4.3.1 Leaf chlorophyll content

SPAD (SPAD-502, Konica- Minolta , Osaka, Japan) is a portable, non-destructive measuring device that gives information on the chlorophyll concentration in leaves. Due to its strong correlation with leaf nitrogen content, this device can be used as an early diagnosis of plant nitrogen status (indirect estimation), showing whether the plant is under or oversupplied with nitrogen. (YARA, 2023). For the white grapevine variety, Chasselas blanc, SPAD values < 31 corresponded to a very low nitrogen supply, from 31-34 refers to low values, 34-38 normal values, and higher than 40 very high values. (Verdenal et al., 2023).

SPAD measurements were made in two leaves for each plant and ten plants for each treatment (which means 20 leaves for each treatment). Five measurements were taken at different locations on the same leaf, and an average value was calculated from each leaf. The measured leaves were fully expanded, with no rips or diseases. These measurements were repeated 4 times during the season (Table 3)

Tabela 3: Dates of SPAD measurements for VDQA-G5 plot and 3159-B plot

	SPAD	
	VDQA-G5	3159-B
session end may/ beginning june	30/05/2023	07/06/2023
session june	27/06/2023	27/06/2023
session july	17 and 20/07/2023	17/07/2023
session august	07/08/2023	02/08/2023

4.3.2 Leaf water potential

The predawn leaf water potential (Ψ_p) was measured with a Scholander pressure chamber. The measurement was taken from 2 a.m. until dawn. At this time of day, the sap tension of the plant is equal to the soil water potential. This measurement thus provides reliable information on the soil water status. (Dufourcq et Caboulet, [s.d.]). This method presents the advantage of being stable, regardless of climatic conditions. Six plants for each treatment had one leaf measured in the chamber (6 leaves for each treatment). These measures were repeated five times during the season: 06/06, 22/06, 29/06, 19/07 and 07/08.

4.3.3 Physiological parameters

Leaf photosynthesis capacities (A_{max} and A_{sat} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_{sw} , $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), transpiration (E) and leaf temperature (T_{leaf} , $^{\circ}\text{C}$) were measured. They were assessed using a portable photosynthetic system (Li-Cor 6800; Li-Cor Inc.). Another portable device, the Li-cor 600, which measures stomatal conductance (g_{sw}) and chlorophyll fluorescence, was also incorporated for data collection. It enables to estimate

quickly leaf fluorescence, and these measures can be used as a proxy to evaluate the leaf photosynthetic activity.

The physiological measurements were performed between 09h and 13h on sunny, cloudless days to ensure light-saturated stomatal conductance. They took place from May to August, once a month, on days close to the days of predawn water potential measures to relate the hydric status of the plants to their photosynthetic activity. (Medrano et al., 2002)

The leaf gas exchange measurements were performed under two different conditions within the leaf chamber of the Li-cor6800: current air CO₂ concentration (400 ppm) and saturated CO₂ (1800 ppm). These two conditions aimed at assessing A_{max} (carbon assimilation rate at saturated light) and A_{sat} (light and CO₂-saturated photosynthetic capacity), respectively. The temperature in the chamber was fixed at 27.5 °C and VPD at 2 kPa for all the measurements. The measurements were done on three plants and one leaf per plant for each treatment (indicated in Figure 7 by the “#” symbol).

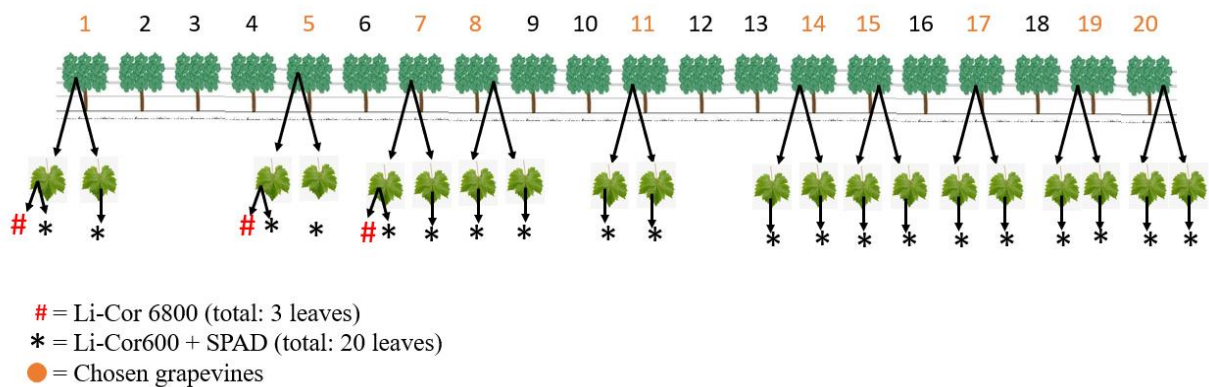


Figura 7: Sampling scheme of SPAD, Li-cor 600, and Li-cor6800 measurements along a row for a given treatment

Two leaves per plant were measured with the Licor-600 and with the SPAD. The chosen leaves were fully expanded, exposed to direct solar radiation, and on the same row side. from three different plants. These measurements were repeated on ten plants for each treatment, totaling twenty leaves measured for each treatment. Three of these twenty leaves were also measured by the Li-cor 6800 (Figure 7).

Intrinsic leaf water use efficiency (WUE_i) was calculated as the ratio of A_{max} to g_{sw}, both variables measured by the Licor6800. It represents the gain of carbon per unity of water loss.

Tabela 4: Measurement dates and respective session for Li-cor600 (g_{sw}) and Li-cor6800 (g_{sw}, A_{max}, A_{sat}, E) on each plot (VDQA-G5 plot and 3159-B plot)

	L600		L6800	
	VDQA-G5	3159-B	VDQA-G5	3159-B
session end May/early June	30/05/2023	15/06/2023	-	15/06/2023
session June	26 and 27/06/2023	27 and 29/06	26 and 27/06	27/06/2023
session July	17 and 20/07	17/07/2023	17/07/2023	17/07/2023
session August	07 and 08/08	02/08/2023	02 and 03/08	07 and 08/08

4.3.4 Soil cover variables

On 12/04, a species survey was carried out using the quadrat vegetation sampling method (CIRAD, 2002). On G5 plots spontaneous vegetation and cover crops vegetation (in G5 and 3159 plot) were sampled in the inter-rows. The quadrat vegetation is a simple method and allows obtaining data on plant biodiversity directly on the field. A wooden square of dimensions 50x50cm (0.25m²) was used to perform it. This square was positioned on the inter-rows (at an equal distance for the two adjacent rows) at 3 points distributed along the length of the row. After positioning the quadrats, the following procedure was performed: identification of the species present in the delimitation of the quadrat; visual estimation of the coverage of the main species in % of the soil area; estimation of the rate of bare soil; collection of all species (with their roots) in the quadrat and storage in paper bags. The species were identified through botanical knowledge and with the help of the application for plant recognition software, Plant Net (Copyright © 2023 Pl@ntNet™). After all the samples were left to dry in an oven at 60°C for 72h. Once completely dried, the total dry weight of the samples was measured.

4.4 Data analysis

All the plots and statistical tests were performed using R studio software (version 4.2.1). The effect of soil cover and canopy height treatments on plant variables was assessed.

a) SPAD and pre-dawn leaf water potential

A linear model with soil cover, canopy height, and the interaction between them was used to analyze the variability in leaf water potential during each measurement session. The same model structure was used for SPAD as well.

$$\begin{aligned} \text{leaf_potential} &\sim \text{soil_cover} + \text{canopy_height} + \text{soil_cover} \times \text{canopy_height} \\ \text{SPAD} &\sim \text{soil_cover} + \text{canopy-height} + \text{soil_cover} \times \text{canopy height} \end{aligned}$$

For the measurements sessions that happened before the 26/06, the term “canopy_height” was not included in the model because this treatment was not wet in place. The data of each genotype was analyzed separately for each measurement session.

b) Ecophysiological variables (gsw, Asat, Amax, WUEi)

Each measurement session of the physiological variables were analyzed separately to avoid confusion that could result from the period of measurements. As the measures of ecophysiological variables occurred from 9 to 13 am and, sometimes, during more than one day, an effect of “time of measurement” and “day” was integrated into the model.

Two linear models were constructed to analyze the ecophysiological parameters (stomatal conductance, saturate photosynthesis, maximal photosynthesis, and intrinsic water use efficiency). The first one was used to make an analysis of each genotype separately. It included soil cover, canopy height, their interaction, time and day, and their interaction.

$$\text{Ecophysiological_variable} \sim \text{soil_cover} + \text{canopy_height} + \text{time} + \text{day} + \\ \text{soil_cover} * \text{canopy_height} + \text{time} \times \text{day}$$

A second linear model was constructed to analyze the effect of genotype (G5 versus 3159). It contained soil cover, canopy height, genotype, time, day, interactions between soil cover, canopy height, and genotype, and interactions between time and day to analyze the variability of the ecophysiological parameters in function of genotype.

$$\text{Ecophysiological_variable} \sim \text{genotype} + \text{soil_cover} + \text{canopy_height} + \text{time} + \text{day} + \\ \text{soil_cover} \times \text{canopy_height} + \text{soil_cover} \times \text{genotype} + \text{canopy_height} \times \text{genotype} + \text{time} \times \\ \text{day}$$

As these models include qualitative and quantitative variables, an ANCOVA (p-value <0,05) was performed. The normality of data was verified by the Shapiro-Wilk test, and the homoscedasticity by the Breusch–Pagan test. Tukey's HSD (honestly significant difference) was used to compare means. In the case of non-respect of the conditions for ANCOVA, a non-parametric test of Kruskal-Wallis was performed, and after, a post hoc of Dunn.

c) **Multiple linear regression**

A multiple linear regression was built to predict the photosynthesis rates measured by the L8600 (Asat and Amax) from the quantitative variables collected by the L600 (such as gsw = stomatal conductance; Tleaf = leaf temperature; Qamb = ambient light; PhiPS2 = efficiency of photosystem II, time of measurement). A backward stepwise regression was performed using the stepAIC function. One equation was obtained to estimate Amax and another to estimate Asat. The data used to obtain the coefficients of these equations contained only the leaves that received the L600 and L6800 measurements (three leaves from different plants per treatment). These regressions used data from the leaves measured in all the measurement sessions without distinguishing between treatment, genotype, or date. This was done in order to obtain as many observations as possible to calibrate the equation. The model's validity domain was studied through a graphical evaluation of the residuals and an ANOVA using genotype, date, treatment, and measurement time as variables.

$$\text{Residuals} \sim \text{genotype} * \text{soil_cover} * \text{canopy_height} + \text{time} * \text{session}$$

5 RESULTS

5.1 Characterization of the water deficit during the time

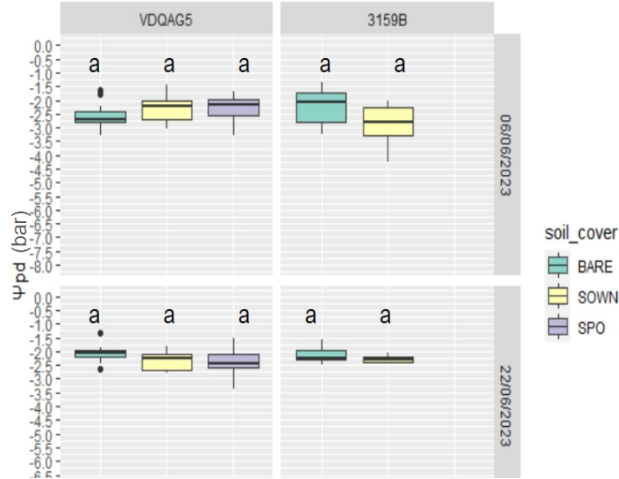


Figura 8: Comparison of pre-dawn leaf water potential (in Bars) of early and mid-June, for VDQA-G5 and 3159 genotypes. Different letters indicate statistical differences, at a 0.05 significance level, among treatments for each measurement date (Tukey adjustment)

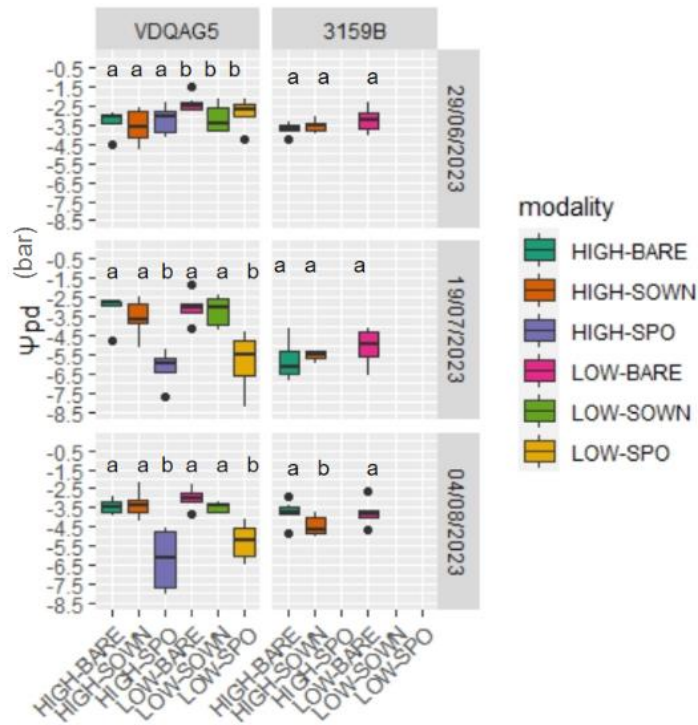


Figura 9: Comparison of pre-dawn leaf water potential (in Bars) of June, July, and August sessions for VDQA-G5 and 3159 genotypes. Different letters indicate statistical differences, at a 0.05 significance level, among treatments for each date of measurement (Tukey adjustment). (n=10). HIGH-BARE represents plants with high canopy on bare soil cover; HIGH-SOWN represents plants with high canopy on sown soil cover; HIGH-SPO represents plants with high canopy heights on spontaneous soil cover; LOW-BARE represents plants with low canopy height on bare soil cover; LOW-SOWN represents plants with low canopy on sown soil cover; LOW-SPO represents plants with high canopy heights on spontaneous soil cover.

Regarding the two first measurement sessions (06/06 e 22/06, Figure 8), the leaf potential between the genotypes were at the same level (Ψ_{pd} around -2,2 to -2,4 bar). Within each of the genotypes, there were no significant effects of the soil cover treatments on the plants' WD, so all the treatments were at the same level.

On June, 29 (Figure 9), genotype 3159 had slightly lower base potential levels than G5 (-3.5 vs. -3, respectively). For genotype G5, canopy height significantly affected Ψ_{pd} (p-value = 0.02), and so the plants in the low canopy treatments had lower base potentials than those in the high canopy treatment (-3.4 and -2.8 Bar, respectively), representing a difference of 21%. For 3159, there was no difference between the treatments. On July 19, the difference in leaf potential between the genotypes became stronger, with -4.1 for G5 and -5.5 for 3159.

Regarding the G5 genotype, the treatments differed due to a significant effect of the soil cover (p-value < 0.001). There were no statistical differences between sown and bare soil covering treatments (Ψ_{pd} around -3.2). Nevertheless, spontaneous soil treatment showed the lowest Ψ_{pd} , around -6.0 Bar, which made it statistically different from the other soil cover treatments. For 3159, no effects of soil cover treatment (p-value = 0.85) or canopy height treatment (p-value = 0.16) were observed. The treatments of 3159 showed an Ψ_{pd} around -5.5, which was not far from the range of values founded on spontaneous treatments of G5.

In August, the overall difference in leaf potential between the genotypes increased, and G5 had a lower leaf potential than 3159. However, when analyzing only the common treatments between the two genotypes, it was seen that 3159 had lower leaf potentials than G5.

For the genotype G5, there was an effect of soil cover treatments (p-value < 0.001) and, as shown in the previous session, no significant differences were found between the bare and sown groups, but the spontaneous treatment (Ψ_{pd} = -5.2 Bar) differed from bare (Ψ_{pd} = -3.2 Bar) and sown treatment (Ψ_{pd} = -3.4 Bar).

For the 3159, soil cover treatments also had an effect on plants' leaf potential (p-value = 0.04), which means, sown soil treatment (Ψ_{pd} = -4,5) presented lower leaf potential than bare soil treatment (Ψ_{pd} = -3,7).

5.2 Characterization of the soil cover: higher biomass dry weight for sown modalities of G5 plot

Tabela 5: Dry weight and estimated yield for sown and spontaneous treatment collected with the quadrat method on April 12. 'mean' was calculated from the values of the three replications of the method of squares made in each treatment. 'sd' represents the standard deviation. The values for spontaneous cover for the G5 variety were separated into 'HIGH' and 'LOW' to capture the variability of the vegetation in the different inter rows. The 'HIGH and LOW-SOWN' values for G5 are the same because the samples were collected in a common inter-row between the treatments.

Genotype	Modality	Biomass dry weight (kg/m ²)		Estimated yield (t/ha)
		mean	sd	mean
VDQAG5	HIGH and LOW -SOWN	0,33	0,05	3,25
	HIGH-SPO	0,08	0,01	0,84
	LOW-SPO	0,08	0,02	0,79
3159B	HIGH-SOWN	0,15	0,03	1,48

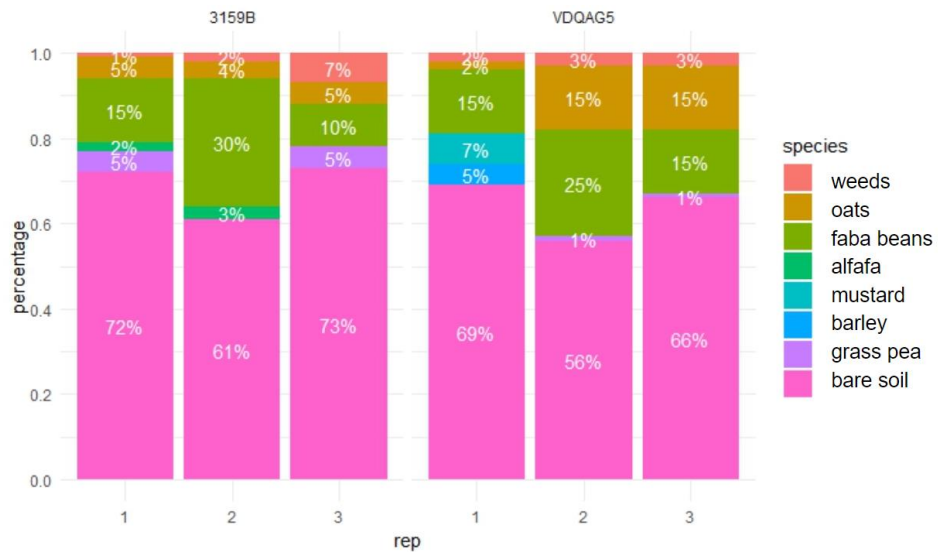


Figura 10: Comparison of the covered area (%) by species for sown soil cover treatments on VDQA-G5 plot and 3159-B plot. Repetition 1 represents the beginning of the interrow; Repetition 2, the middle and Repetition 3, the end.

The sown soil treatment on G5's plot presented a dry biomass weight twice as high as the same treatment on the 3159's plot (0,33 vs. 0,15 respectively), resulting in a higher estimated yield for G5 (Table 5).

When comparing the estimated cover area per species in the sown soil treatment (Figure 10), it can be seen that the percentage of bare soil in the 3159B plot was higher and ranged from 61% to 73%, while in G5, it was 56% to 69%. The dominant species in the sown soil for both genotypes was Faba beans. In the G5 plot, this species occupied 15 to 25% of the area, while in the 3159 plot, it was 10% to 30%. The highest percentages of Faba beans were found in repetition 2 in both plots.

Regarding the spontaneous cover treatment (graphic in annex 4), 30% of the soil was occupied by weeds, while 70% was bare. These results were the same for the two interrows (from 'HIGH-SPO' and 'LOW-SPO'). The most present species in spontaneous soil treatment, in terms of frequency, were Bromegrass (*Bromus spp.*), hawksbeard (*Crepis bursifolia L.*), white rocket (*Diploaxis eruroides L.*), and Field Milk-thistle (*Sonchus arvensis*).

5.3 Characterization of estimated leaf nitrogen content during the time

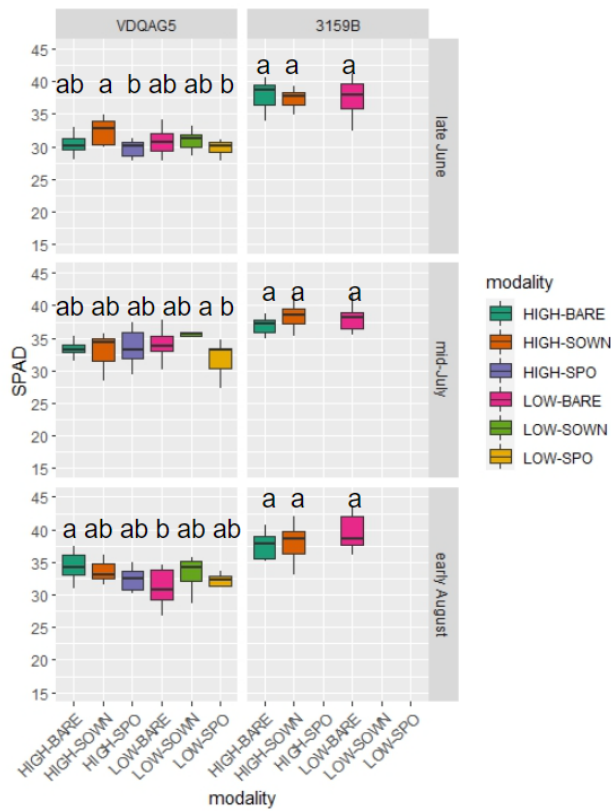


Figure 11: Stomatal conductance for VDQA-G5 and 3159 varieties, session of June, July, and August. Different letters indicate statistical differences, at 0.05 significance level, among treatments for each measurement date (Tukey adjustment). $n=10$. HIGH-BARE represents plants with high canopy on bare soil cover; HIGH-SOWN represents plants with high canopy on sown soil cover; HIGH-SPO represents plants with high canopy heights on spontaneous soil cover; LOW-BARE represents plants with low canopy height on bare soil cover; LOW-SOWN represents plants with low canopy on sown soil cover; LOW-SPO represents plants with high canopy heights on spontaneous soil cover.

5.3.1 For G5

A) Effect of soil cover

On the sessions of May and June, soil cover treatments had a significant effect on SPAD values. At the end of May (30/05), bare soil treatment had the lowest SPAD values (26,2), which made it statistically different from sown (30,2) and spontaneous soil covers (30.9). In June, sown soil treatment had significant greater values than spontaneous soil cover treatment (31.7 vs. 29.7). Bare and spontaneous soil were statistically different, but sown soil did not differ from those two.

B) Interaction of factor

In mid-July (17 and 20/07), SPAD values for all treatments were close, varying from 32 to 35,7. No significant effect of soil cover (p -value=0.06) or canopy height (p -value=0.34) were found, however, the interaction between the two factors was significant (p -value=0.02). For plants in bare and sown treatments, the high canopy treatment had the highest SPAD values. However, this is reversed for plants in spontaneous soil, and the low canopy treatment had the highest SPAD.(graphic in annex 3).

In sown soil, SPAD values for plants with low canopy height (35,7) were significantly higher than for those with high canopy height (33,3). For the spontaneous soil cover, low and high canopy treatments did not differ significantly between them (p -adjusted = 0.11).

At the beginning of August, SPAD values ranged from 31,1 to 34,4. There were no significant effects of soil cover, but there were significant effects of canopy height and of the interaction between soil cover and canopy height. In general, sown soil cover had the highest SPAD values and bare soil cover had the lowest. High canopy treatments had higher SPAD values than low. Low canopy height on bare soil showed the lowest SPAD, with 31,1.

5.3.2 For 3159

A) Effect of soil cover

There were no differences in SPAD values between bare, sown, and spontaneous soil covers. Thor SPAD was around 37.6 and 37.9.

B) Effect of canopy height

There were no differences in SPAD values between high and low treatments.

5.3.3 Differences between G5 and 3159

In the May session, there was no difference between genotypes. G5 had a SPAD of 29 while 3159, 27,5. During June, July, and August, SPAD values of 3159 were superior to those from G5. In June, the average SPAD for the 3159 was 37.6, significantly higher than the G5's 29.7. In July, the difference became smaller, 32.8 for the G5 and 37.9 for the 3159 (a difference of 15% between genotypes). In August, the 3159 continued to have higher values without an increase in the difference established in July.

5.4 Stomatal conductance (gsw)

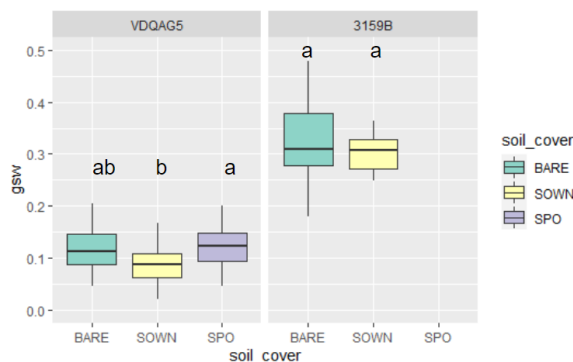


Figura 12: Stomatal conductance for VDQA-G5 and 3159-B varieties in late May/early June. BARE stands for bare soil cover treatment; SOWN stands for sown soil cover and SPO, spontaneous soil cover. Different letters indicate statistical differences, at 0.05 significance level, among treatments for each measurement date (Tukey adjustment). (n=20)

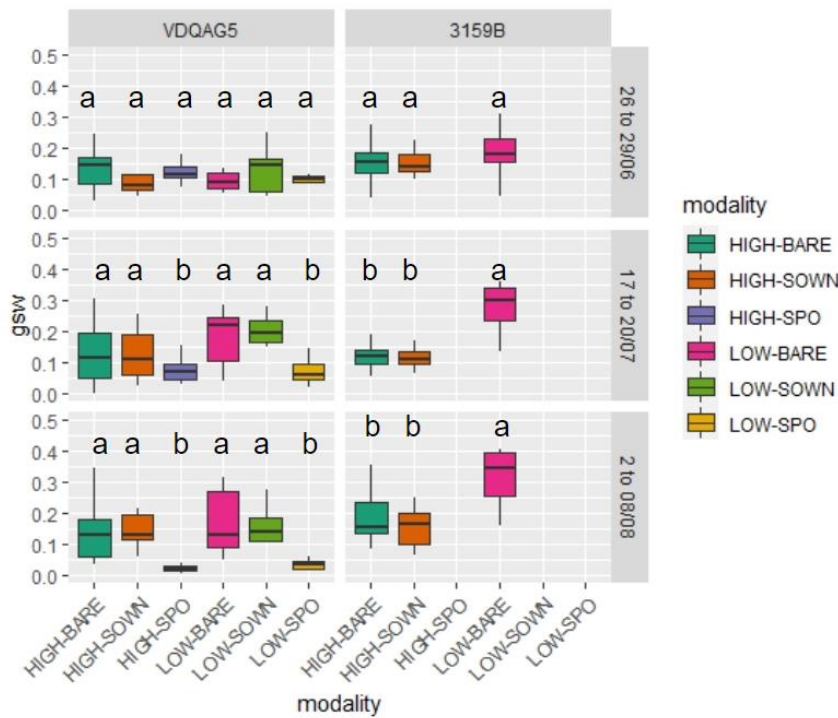


Figura 13: Stomatal conductance (mol H₂O m⁻² s⁻¹) for VDQA-G5 and 3159 varieties, session of June, July, and August. Different letters indicate statistical differences, at a 0.05 significance level, among treatments for each measurement date (Tukey adjustment). n=10. HIGH-BARE represents plants with high canopy on bare soil cover; HIGH-SOWN represents plants with high canopy on sown soil cover; HIGH-SPO represents plants with high canopy heights on spontaneous soil cover; LOW-BARE represents plants with low canopy height on bare soil cover; LOW-SOWN represents plants with low canopy on sown soil cover; LOW-SPO represents plants with high canopy heights on spontaneous soil cover.

5.4.1 For G5

A) Effect of soil cover

In the sessions of May, July, and August, soil cover had a significant effect on plants' stomatal conductance. In general, it can be seen that the plants on spontaneous treatment had their stomatal conductance decreased as the sessions went by. Initially, in May, plants on spontaneous soil had the same level of gsw as those on bare soil (around 0.12 mol H₂O m⁻² s⁻¹). However, in the sessions of June, the stomatal conductance of plants on the spontaneous treatment decreased to 0,08 mol H₂O m⁻² s⁻¹ and, in August, even more, to 0,04 mol H₂O m⁻² s⁻¹ being, in both sessions, statistically lower than the gsw from plants on bare and sown treatments.

Looking at the stomatal conductance of the plants in bare soil, it is possible to see that, in each of the sessions mentioned, the conductance values of this treatment are not significantly different from those of the plants in the 'sown soil'. For the 'bare soil,' these stomatal conductance values varied from 0.12 to 0.16 mol H₂O m⁻² s⁻¹, while for the sown soil, they were 0.12 to 0.17 mol H₂O m⁻² s⁻¹.

B) Effect of canopy height

The effect of canopy height on stomatal conductance was only significant in July's session. Plants with low canopy height had higher stomatal conductance than plants with high canopy height. This difference is more pronounced when comparing plants with high canopy on bare and sown soils (0,13 H₂O m⁻² s⁻¹) with plants with low canopy on the same soil treatments (0,19 H₂O m⁻² s⁻¹).

5.4.2 For 3159

A) Effect of soil cover

Soil cover treatment had a significant effect on plants' stomatal conductance in the sessions of July and August. Stomatal conductance on bare soil was higher than in sown soil (0,28 vs 0,12 H₂O m⁻² s⁻¹). However, when comparing the stomatal conductance of plants on bare and sown soil covers, they did not differ during the sessions of June, July, and August.

B) Effect of canopy height

Canopy height treatments had a significant effect on stomatal conductance in July and August sessions. Plants with low canopy heights on bare soil (0,28 mol H₂O m⁻² s⁻¹) have significantly higher levels of stomatal conductance than plants with high canopy heights also on bare soil (0,18 mol H₂O m⁻² s⁻¹).

5.4.3 Differences between G5 and 3159

In all the sessions, there was a significant effect of genotype on stomatal conductance. In the May session, the plants of the 3159-B genotypes (average gsw = 0,30 mol H₂O m⁻² s⁻¹) had around three times higher values of stomatal conductance than the G5 plants (average gsw = 0,11 mol H₂O m⁻² s⁻¹). In June' and July and August, stomatal conductance from 3159 plants kept being higher than G5 plants but with lower differences. The genotypic differences were more clearly observed when comparing the treatments low-bare between G5 and 3159: in July, the plants in this treatment at 3159 had a gsw 39% higher than in G5 (0.25 vs 0.18 mol H₂O m⁻² s⁻¹), and in August this difference was 88% (0.32 vs 0.17).

5.5 Maximal Photosynthesis (Amax)

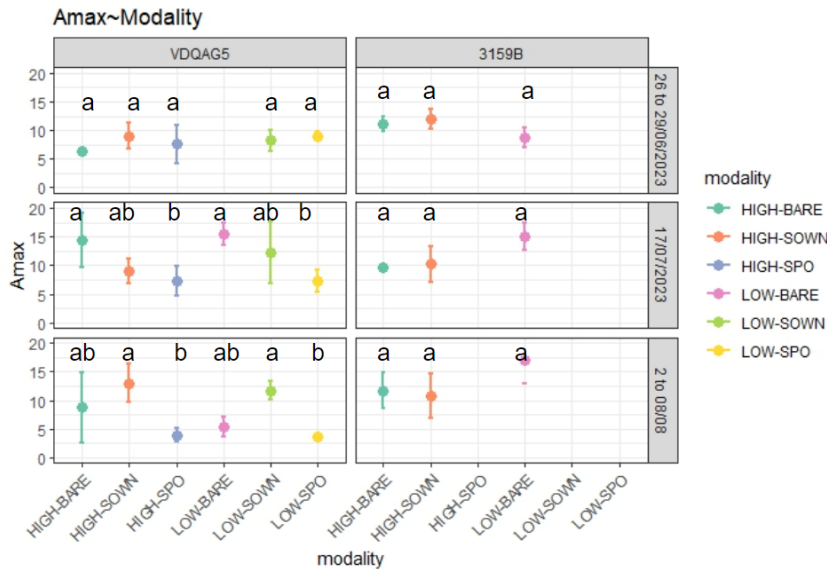


Figura 14: Amax for each genotype during the measurement sessions of June, July and August. The bars around the dots represent the standard deviation. Three replicates were considered for each session ($n=3$) and measurement dates, except for June's session of 3159 which only contains two repetitions due to difficulties in calibrating the gas exchange in high-temperature conditions. HIGH-BARE represents plants with high canopy on bare soil cover; HIGH-SOWN represents plants with high canopy on sown soil cover; HIGH-SPO represents plants with high canopy heights on spontaneous soil cover; LOW-BARE represents plants with low canopy height on bare soil cover; LOW-SOWN represents plants with low canopy on sown soil cover; LOW-SPO represents plants with high canopy heights on spontaneous soil cover

5.5.1 For G5

A) Effect of soil cover

During the June session, there was no significant effect of soil cover on Amax, but in July and August, this effect was present. In July, plants in spontaneous soil cover had the lowest values of Amax (around $7,2 \mu\text{mol m}^{-2} \text{s}^{-1}$) and were significantly different from the plants on bare soil, which had the highest Amax (around $15 \mu\text{mol m}^{-2} \text{s}^{-1}$). Plants on sown soil cover had intermediate values (around $10 \mu\text{mol m}^{-2} \text{s}^{-1}$), not being statistically different from plants on spontaneous and bare soil covers.

In August, plants of the sown treatments had the higher Amax, around $12,5 \mu\text{mol m}^{-2} \text{s}^{-1}$, which made them statistically superior to the other soil treatments. Plants of the spontaneous soil cover kept having the lowest Amax (around $3,7 \mu\text{mol m}^{-2} \text{s}^{-1}$) and differed from plants on the sown treatments. Meanwhile, plants on the bare soil treatment had intermediate values of Amax ($7 \mu\text{mol m}^{-2} \text{s}^{-1}$) and did not differ from spontaneous and sown treatment.

B) Effect of canopy height

There was no statistical difference between high and low canopy treatments on Amax values in any of the measurement dates.

5.5.2 For 3159

A) Effect of soil cover

For the 3159 variety, there were no significant effects of soil cover for any of the measurement sessions. Amax values ranged from 8,8 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (LOW-BARE) to 11,9 (HIGH-SOWN).

B) Effect of canopy height

For 3159, there was an effect of canopy height only on July's session, but the Amax values of 3159 treatments do not differ statistically. They range between 9,7 and 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

5.5.3 Differences between G5 and 3159

In June, the Amax of G5 was greater than the 3159 genotype (comparing only the common modalities between genotypes), while August was the opposite.

There was a significant effect of genotype on June and August sessions: more elevated WUEi for G5. In June's session, the Amax of HIGH-BARE treatment was 45% greater in G5 than in 3159 (11 $\mu\text{mol m}^{-2} \text{s}^{-1}$). In August, HIGH-BARE of G5 had lower values than the same treatment for 3159 (8,8 vs. 11,67, respectively).

5.6 Water Use Efficiency (WUE)

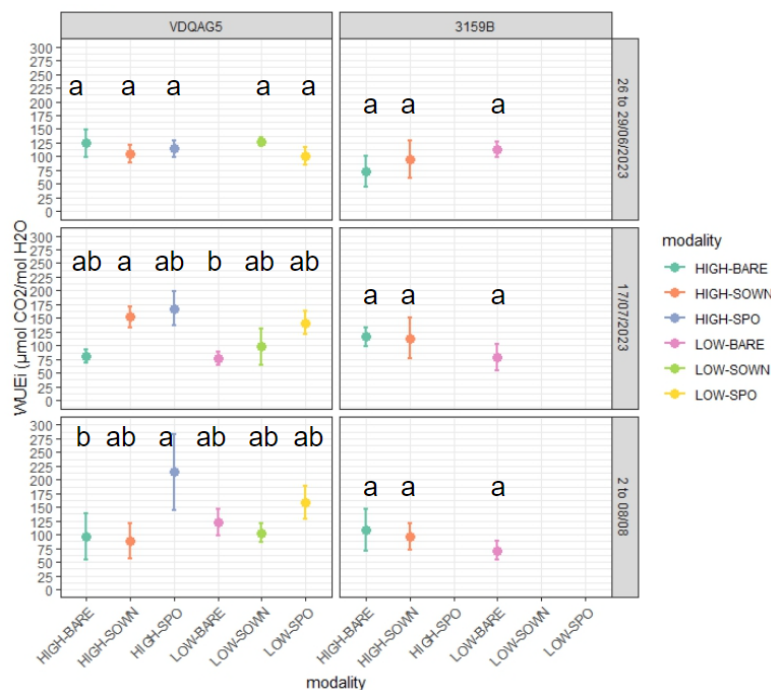


Figura 15: Intrinsic water use efficiency (WUEi) for each genotype during the measurement sessions of June, July, and August. Three replicates were considered for each treatment ($n=3$), except for June's session of 3159, which only contains two replicates due to difficulties in calibrating the gas exchange in high-temperature conditions. 'LOW-BARE' of G5 as well.

5.6.1 For VDQA-G5

A) Effect of soil cover

In the June session, WUEi values were not significantly different from bare to sown or spo soil treatments. The range of WUEi was 100 to 126 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$.

A significant effect of soil cover on WUEi was present in the sessions of July and August: sown soil treatments had the most elevated values of WUEi. In the July session, WUEi ranged from 75 to 167 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$. The lowest WUEi were found in plants on bare soil (around 77 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$), while the highest, on spontaneous cover soil (167 to 141 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$, which means around 77% higher WUEi than bare soil treatments). In the August session, G5's WUEi ranged from 88 to 213 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$, and as seen in the previous session, plants in the spontaneous soil cover continued with the highest WUEi (186 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$).

B) Effect of canopy height

A significant effect of canopy height was seen in the July and August sessions: plants with high canopy had higher values of WUEi than plants on the low canopy treatments (a difference of 21% in June and 3% in August).

5.6.2 For 3159-B

A) Effect of soil cover and canopy height

Soil cover treatments and canopy height did not affect significantly the WUEi of 3159 in any of the measurement dates. In June, the WUEi ranged from 72 to 112 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$, in July from 78 to 115 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$ and in August from 70 to 108 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$.

5.6.3 Differences between G5 and 3159

In general, variety G5 had a higher WUEi than var 3159. There was a significant genotype effect on WUEi in the August session. Taking into account only the treatments in common between the two genotypes (high-bare, high-sown, and low-bare), the WUEi averages of G5 in this session were 11% higher than 3159B (101 vs. 93 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$, respectively).

In June's session, plants with high canopy height on bare soil from G5 had 40% higher WUEi when compared to the same treatments in 3159

5.7 Multiple linear regression for Amax and Asat

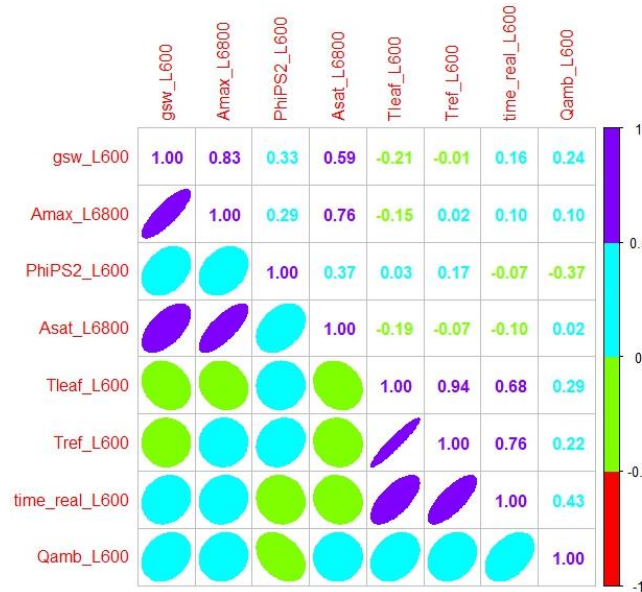


Figura 16: Correlation matrix between the variables selected to construct the multiple linear regression. The coefficient of variation between variables is represented in the upper right corner of the correlation matrix. All variables ending with 'L600' were obtained by using the Li-cor600 and all variables ending with 'L6800' were collected using the Li-cor6800. 'gsw' represents leaf stomatal conductance, 'PhiPS2' photosystem II efficiency, 'Asat' photosynthesis under saturated conditions, 'Amax' maximum photosynthesis, 'Tleaf' leaf temperature, 'Tref' reference temperature, 'time_real' measurement time and 'Qamb' environmental radiation.

There is a high correlation between stomatal conductance (gsw_L600) and Asat ($r = 0.59$) and between stomatal conductance (gsw_L600) and Amax ($r = 0.96$, figure 16). PhiPS2 shows an intermediate level of correlation with Asat (37%) and Amax (29%).

Regarding the correlation within L600 variables, a strong correlation was observed (68%) between leaf temperature (Tleaf) and the time of measurement (time real L600), as well as with the reference temperature (98%).

The equation obtained for Asat from the variables measured by Licor 600 was:

$$Asat = 21.67 + 21.671 \times gsw_L600 - 0.79 \times time_real_L600 + 8.13 \times PhiPS2_L600$$

This model has an adjusted R-squared of 39%. It contains as explanatory variables the stomatal conductance (gsw_L600), the time of measurement (time_real_L600), and the efficiency of photosystem II (PhiPS2_L600). Time has a negative relationship with Asat while gsw and PhiPS2 have a positive one.

The equation obtained for Amax was:

$$Amax = 6.12 + 35.95 \times gsw_L600 + 1.22 \times PhiPS2_L600$$

This model has an adjusted R-squared of 68%. It contains as explanatory variables the stomatal conductance (gsw_L600) and the efficiency of photosystem II (PhiPS2_L600), both of them related positively with the values of Amax.

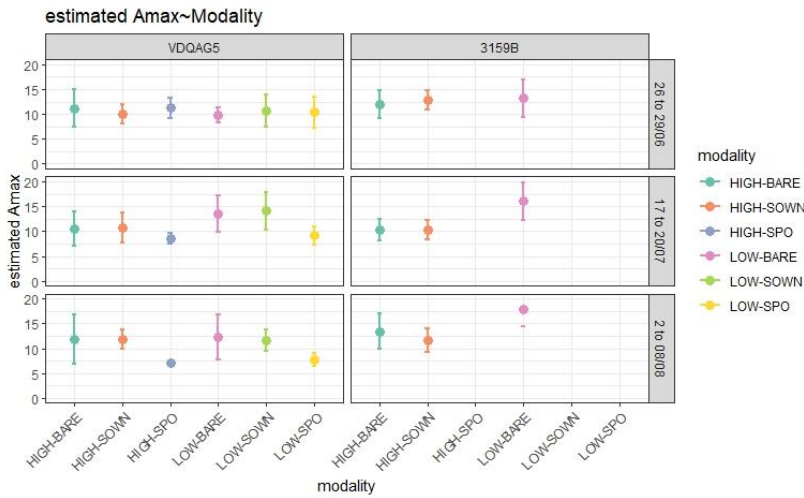


Figure 17: Estimated Amax for VDQA-G5 and 3159-B genotypes during the measurement sessions of June, July, and August. The bars around the dots represent the standard deviation. Three replicates were considered for each session (n=3) and measurement dates, except for June’s session of 3159, which only contains two repetitions due to difficulties in calibrating the gas exchange in high-temperature conditions.

A general comparison of the actual Amax values (shown in section 5.5) with the Amax values estimated using the above equation shows that the estimated Amax is, on average, 15% higher than the Amax measured in the field (Figure 14, section 5.5). More specifically, for the g5 variety, they were 18% higher than the values in the field, while for 3159-B they were 9% higher.

Analysis of Amax model residuals

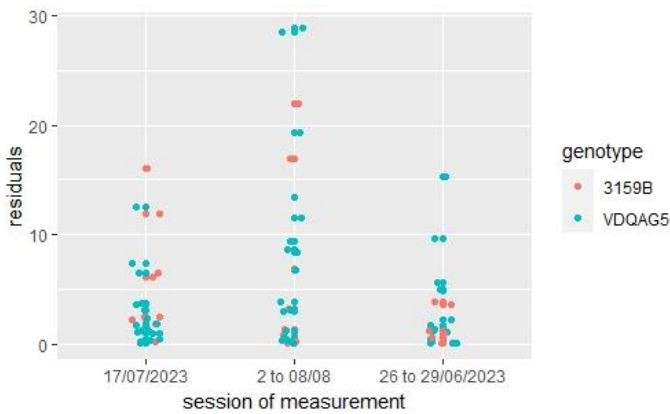


Figure 18: Representation of model residuals as a function of the measurement sessions. The model residuals were calculated by squaring the difference between the actual and estimated Amax values.

The result of the ANOVA of the model residuals showed a significant effect of the measurement sessions (p-value = 0.02). By graphically analyzing the distribution of the residuals as a function of each of the measurement sessions (Figure 18), it can be seen that the August session had higher residuals than the June and July sessions. This indicates that the equation proposed to estimate Amax had more difficulty predicting the values of the August session (which was also the session with the highest leaf temperatures).

6 DISCUSSION

6.1 Discussion of the methods

The study of plant physiological parameters based on field conditions encountered some main challenges. Factors such as climatic conditions or the spatial variability of soil characteristics can induce large variations in plant responses, making it more difficult to assess the real effects of the treatments.

The experimental design (specifically, the layout of measurements in the plot and the large distance between the G5 and 3159-B plots) made it impossible to collect the physiological parameters by alternating measurements between treatment and genotypes. This meant that some of the variations observed between treatments could result only from the variations in the time of the day at which the measurements were performed. More precisely, temperature strongly increased in the morning (the period of the day during which measurements were performed), and since temperature is correlated with leaf gas exchange, it can create confounding effects that make it difficult to analyze the effects of treatments.

When analyzing plant physiological variables, it is important to remember that gas exchange measurements provide instantaneous information located at the leaf level. This prevents the observations of water and carbon flows at the plant levels or at the level of the day (Wilhelm, Pellegrino, Hernan Ojeda, et al., 2021).

Concerning the statistical analysis methods, there are limitations in the models used to analyze the variables in this report. These models could be improved by creating mixed models and adding the date and time of measurements as random factors.

6.2 Installation of the treatments, differences in soil cover and biomass, irrigation: did the treatments reach the expectations?

In June sessions (06/06, 22/06, and 29/06), all the plants of both genotypes were under well-watered conditions (-2 and -3.5 bars for predawn water potential) (Deloire, Pellegrino, et Rogiers, 2020), and there was no different in water deficit intensity between soil treatments. However, these similar conditions changed during the growing season.

In July, for the G5 genotype, the predawn leaf potential of plants on spontaneous soil treatments decreased to approximately -5.8 bar, which indicates a moderate water deficit. Regarding the leaf potential of plants under bare and sown treatments, both were exposed to mild water deficits without a gradient of WD between them. These differences in WD levels were maintained during the August session.

For the 3159 genotypes, all the treatments reached mild to moderate WD levels (around -5.5 bars) in July. In August, the WD decreased and was close to mild WD (-4 bar), which also means it was closer to WD on the G5 plot.

The initial irrigation objectives were partially met at the end of the experiment. In G5's plot, it was possible to create a contrast between the spontaneous treatments and the other two soil treatments, but there was no clear WD gradient between the sown and bare soil treatments. This may have happened due to the initial phase of adjustment in the irrigation schedule between these soil treatments.

The least stressed treatment was the one with plants with a low canopy in bare soil, while the most stressed was with plants with a high canopy in spontaneous soil. It was expected for all the soil cover treatments that plants on low canopy height treatments were under lower water stress since they have lower leaf area and lower transpiration demands.

In the 3159 plot, the differences in WD of bare and sown soil were more pronounced than G5(-3,8 for the bare soil and -4,5 for sown) however, there was still no significant difference between their treatments.

Regarding the implementation of soil cover treatments, the sown soil presented differences between the plots: In G5's plot, it had a much higher biomass weight than the 3159 plot. This difference may have been caused by the differences in the soil of the plots. The 3159 plot has a higher proportion of stones in the soil compared to the G5 plot's soil, which may decrease its water storage capacity and make it difficult for the sown plants to grow and develop. However, this difference in inter-row cover did not cause more WD for G5 plot.

6.3 Evaluation of the effects of canopy height, soil cover, and irrigation on the leaf gas exchange variables

A) Effect of soil cover and irrigation

Looking only at the G5 treatments that were under mild water deficit conditions (notably bare and sown soil treatments), the values of gsw ranged from 0.10 to 0.21 all over the growing season, with an average of 0.14, which is similar to the values found by Medrano et al. (2002) in grapevines subjected to mild water deficit. In this water deficit condition, the treatments that showed higher gsw values (LOW-BARE, HIGH-BARE, LOW-SOWN, HIGH-SOWN) also exhibited higher Amax (maximum photosynthetic rate).

Amax, in turn, ranged from 6.2 to 15.5. This variation is substantial, and another factor that may have contributed to this significant variation is the levels of SPAD (e.g., leaf nitrogen levels). It is observed that the plants with the highest Amax values are those that also had higher SPAD, notably ranging from 33.2 to 35.7 (values considered slightly low to normal according to the literature). This relationship between Amax values and SPAD values occurs because when a plant is deficient in nitrogen, the content of the enzyme RUBISCO in their leaves also decreases, which reduces the efficiency of photosynthesis since RUBISCO is a key component responsible for incorporating CO₂ into plants during the photosynthesis process (Paul et Foyer, 2001).

In mild water deficit conditions, G5 plants had WUE_i (intrinsic water use efficiency) ranging from 75 to 126, with an average of 101.

The spontaneous soil treatments were under moderate to severe water deficit conditions, and their values of gsw ranged from 0.03 to 0.08 during the season, which correspond to the values found by Medrano et al. (2002) also for moderate water deficit. Under these water deficit conditions, Amax ranged from 3.55 to 7.27 $\mu\text{mol m}^{-2} \text{s}^{-1}$, averaging 5.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The plants in the spontaneous soil cover had WUE_i (intrinsic water use efficiency) values of 170.

B) Effect of Canopy Height

For G5, only in the July session, there was a significant effect of the canopy height treatment on plants' stomatal conductance: plants on low canopy height had higher gsw than high-canopy treatments. In this July session, the SPAD levels of the plants in both the low canopy and high canopy treatments were low (33.9 for low and 33.3 for high), and the water deficit level was around -4 bars for both. However, this canopy height effect needs to be taken into account cautiously because in this measurement session, the hour of measurement also had a significant effect on plant gsw. This may have contributed to an increased difference between treatments, as the same significant effect of canopy height was not repeated in August (when the time of measurement was not significant).

Hypothesis 1: Decreasing canopy height will increase photosynthetic efficiency.

The canopy reduction treatment also did not significantly affect the Amax values in any of the measurement sessions (June, July, and August), which makes sense since the stomatal conductance values were also not significantly affected.

6.4 Comparison between genotypes

The effects of genotype on the variables analyzed must be analyzed with caution because the plants of the two genotypes were not always under the same WD and SPAD conditions when compared in the measurement sessions.

In Late May sessions, both varieties had no water deficit and had very low leaf nitrogen content. At the end of June sessions, both genotypes with mild water deficit. However, the leaf nitrogen content for 3159 was at normal levels according to the literature 37.6 while G5 was at low levels. Moving on to July session, 3159B genotype was under moderate sweater deficit (-5.5 bar), while G5 was less stressed, under mild WD (-3.2). Regarding leaf nitrogen levels, G5 was with low levels (33.5) and 3159 with normal ones (37.9). Finally, in the August session, 3159 presented normal SPAD values (37.9) and G5 with low values (31.1)

Hypothesis 2: The Amax of genotype 3159 will be higher than in G5

The fundamental of this hypothesis is that by the fact that as G5 is a sugar-less variety, it requires fewer carbohydrates to reach fruit maturity and consequently, its source-sink rapport is lower compared to 3159, which is not a sugar-less variety. The article Paul et Foyer (2001) describes a process called down-regulation of photosynthesis, which happens when the source activity of leaves exceeds the sink strength and the assimilates start accumulating on the leaves

When comparing only the HIGH-BARE treatments between the two genotypes, it was seen that on June session, Amax from 31589 was 76% higher than G5 one. However, the same result was not observed during the July session; this time, 3159B amax was 33% lower than the G5 ones. For the last session performed in August, the 3159B rate of Amax returned to be superior to G5 by 33%. Looking at these results as an overview of the sessions, on average, 3159B Amax was 25% higher than G5 Amax. The results were obtained after analyzing three

repetitions of Amax for each genotype during each measurement session. To ensure the reliability of these findings, additional repetitions of Amax per genotype would be necessary.

Hypothesis 3: under the same level of water stress, the G5 will have a higher WUEi than the 3159

Comparing the WUEi values of the two genotypes only taking into account the same mild WD level (from -3 to -5 bar), the average WUEi of G5 was 106 while that of 3159 was 92 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$, a difference of 15%. This is consistent with the range of values described in the article of Wilhelm, Pellegrino, Hernán Ojeda, et al., (2021) on characterizing the drought tolerance of resistant varieties. The article found that, for WD levels from zero to -14bar, G5 had 106 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$, and 3159 had a WUEi of 82 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$.

6.5 Determination of a high-throughput phenotyping method to estimate photosynthesis

In this experiment, stomatal conductance (gsw_L600), time of measurement (time real L600) and Photosystem II efficiency (PhiPS2_L600) measurements were used as a proxy to estimate photosynthetic activity, more precisely one equation to estimate Amax and another to Asat. This method of using leaf fluorescence measurements as a proxy to estimate photosynthetic activity was also used in the study of (Coupel-Ledru et al., 2019), that was performed in a collection of apple genotypes. The results of Coupel-Ledru et al., (2019) showed that fluorescence measurements were suitable for estimating photosynthesis activity. Besides, it was shown that parameters related to PhiPSII, air temperature, and leaf temperature have a significant impact on the estimation of photosynthesis and helped to obtain robust calibrations with high R2 values.

Although Tleaf does not appear directly in the Amax equation obtained, it was possible to see from the correlation matrix that the time of measurement (time_real_L600) had a 98% correlation with leaf temperature (Tleaf) and with the reference temperature (Tref).

6.6 Proposition and perspectives

For the next years of the experiment, it may be better to use some environmental parameter instead of “time of measurement” in the statistics to compare the treatments and avoid bias due to variations in climatic conditions during the experimental periods. One parameter that can be used is Qamb (incident radiation in close vicinity of the leaf). This parameter was described by (Ye et al., 2020) as the most dynamic and significant environmental variable affecting photosynthesis. It would be necessary to build a response curve of the dependent variables, for example, stomatal conductance, as a function of Qamb, and then compare the treatment effects through their differences with the response curve.

It would be interesting to try to make new equations for Amax and Asat but segment the data by genotype or treatment (one equation for each genotype or one equation for each) to

try to increase the percentage of variability explained by the model. However, by segmenting the data by genotype, more replicates for genotype and for treatment would be required to obtain a good estimation of the equation's coefficients.

7 CONCLUSION

In conclusion, this study has provided insights into the impact of soil covers combined with water deficit (WD) on VDQA-G5 physiological variables.

A partial WD gradient was created and characterized to continue these experiments. By the end of the experiment, the grapevines in the spontaneous soil treatment exhibited moderate to severe water deficit conditions, which were reflected in the lowest values of g_{sw} and A_{max} , and the highest values of WUE_i . In contrast, the bare and sown soil treatments did not exhibit significant differences in WD, and these soil modalities showed higher g_{sw} and A_{max} values.

Furthermore, when considering an overview of the measurement sessions, G5 genotype exhibited lower A_{max} values compared to 3159B.

The modeling approach employed in this study allowed us to develop two equations, one for A_{max} and another for A_{sat} , with few input parameters. This emphasizes the importance of modeling approaches like these in expanding data collection, simplifying extensive data acquisition procedures in the field, and enhancing our comprehension of the studied treatments.

Projects like VITIFUTURE are very important in addressing the challenges posed by climate change in viticulture. contribute to an exchange of knowledge about the ecophysiological functioning of fungus tolerant and VDQA varieties.

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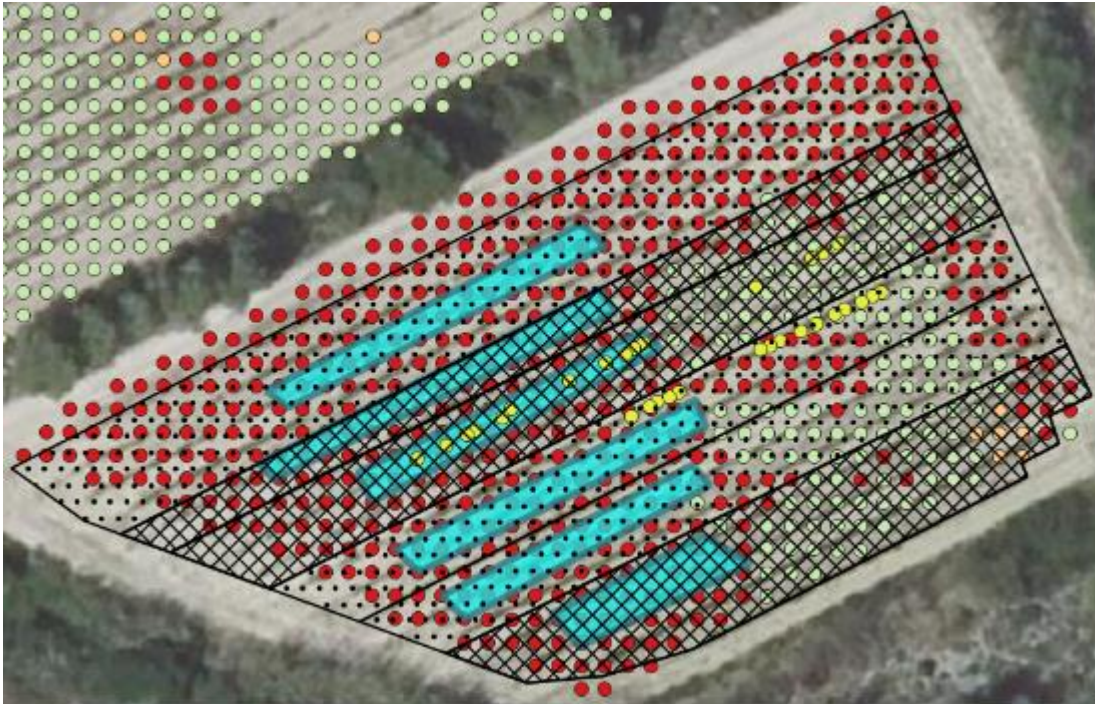
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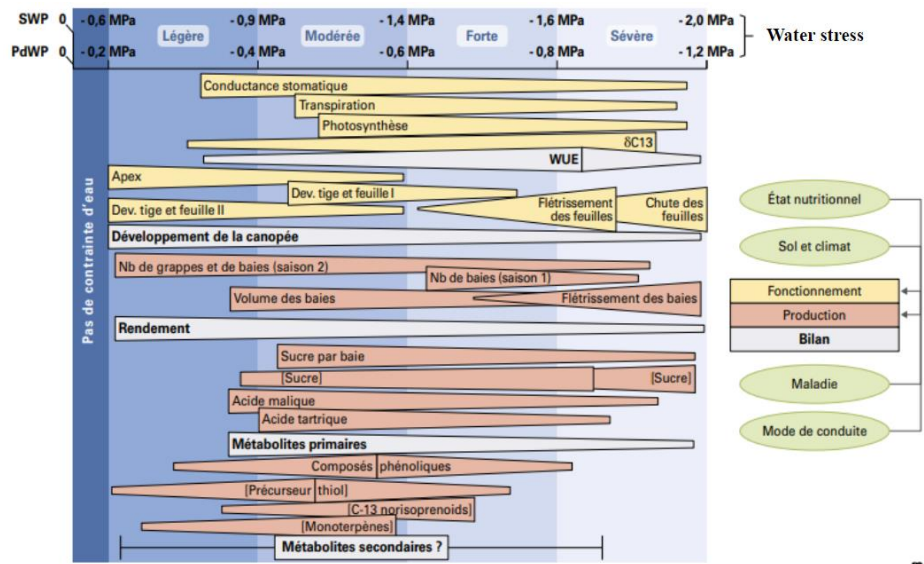
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ANNEXES

Annex 1: Map of soil resistivity - G5 plot

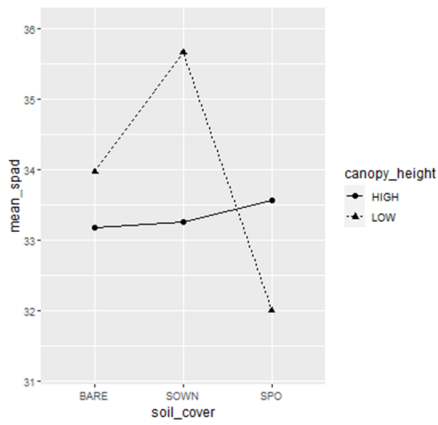


Annex 2 Sensitivity of different indicators to water deficit (increasing water deficit from left to right). The term '[]' indicates concentration. Functional and production variables are not specific to water stress and may be affected by other environmental factors (climate, soil, diseases), plant nutritional status, and management practices (pruning, training systems, leaf removal) (in green). source: revue des oenologues , (Wilhelm et al., 2021)

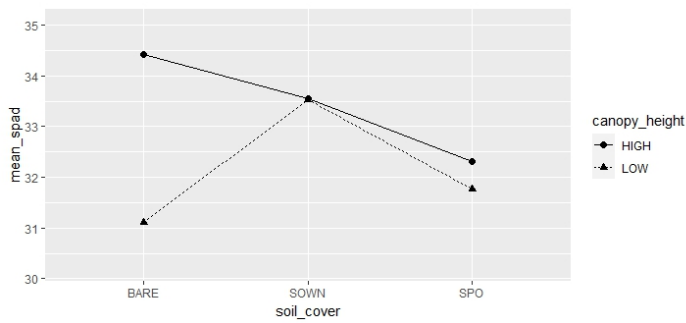


Annex 3: Interaction graphics of SPAD measurements

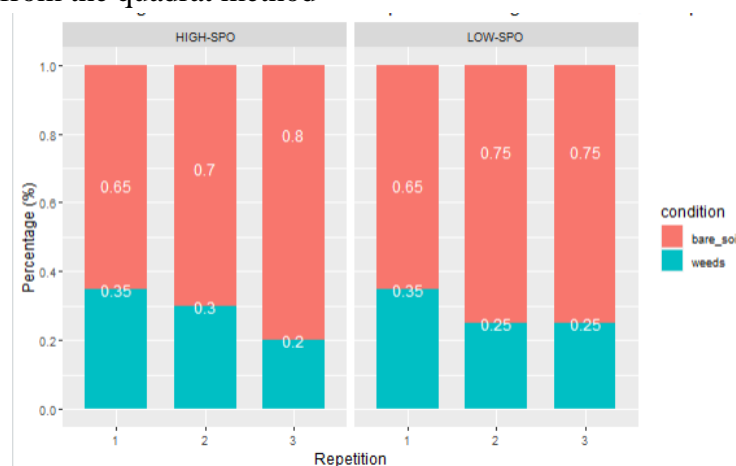
Correlation between soil cover and canopy height during mid-July session



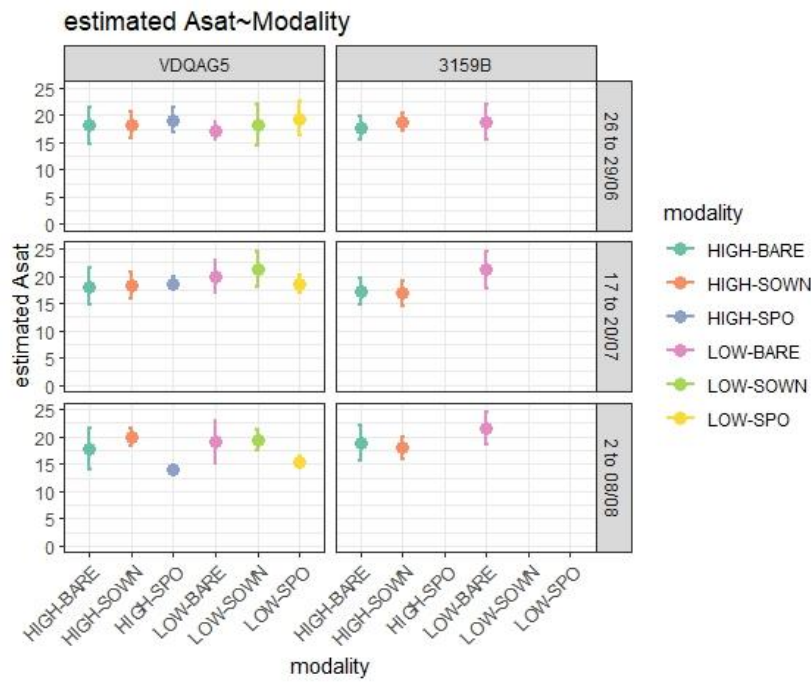
Correlation between soil cover and canopy height during August session



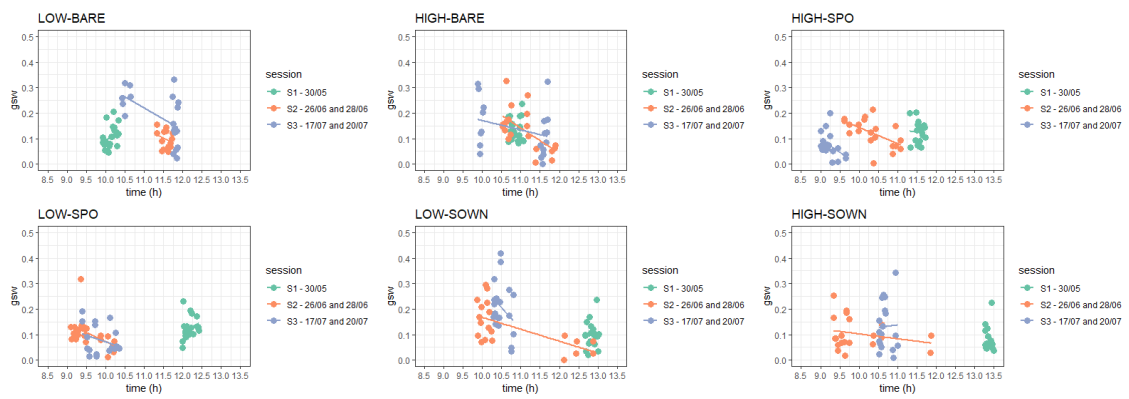
Annex 4: characterization of soil cover - Area covered by bare soil and weeds for the SPO modalities estimated from the quadrat method



Annex 5: estimated Asat for each modality of G5 and 3159B genotypes for the measurement sessions of June, July and August



Annex 6: Stomatal conductance values (gs_w) for the VDQA-G5 distributed per modality over the measurement hours.



Annex 7 : Table with p-values for each session of measurement for stomatal conductance

	Effects	may	june	july	august
within G5	Soil cover	0.004441 **	NS	0.000691 ***	1.424e-06
	Canopy height	-	NS	0.030847 *	ns
	time	NS	-	0.001796 **	
	day	-	-	NS	
	soil cover: canopy height	-	-	NS	ns
	time: day	-	-	NS	
within 3159- B	Soil cover	NS	NS	0.000191 ***	0.00183 **
	Canopy height	-	NS	3.47e-07 ***	0.00120 **
	Time	0.0238 *	0.0192 *	NS	ns
	Day	-	9.64e-05 ***	-	-
	soil cover: canopy height	-	NS	NS	ns
	time:day	-	-	-	-
Between G5 and 3159	Genotype	<2e-16 ***	1.776e-05 ***	0.00843 **	1.53e-08 ***
	Soil cover	0.0137 *	0.023341 1 *	4.08e-05 ***	2.30e-08 ***

	canopy height	-	0.031481 8 *	1.42e-06 ***	0.00126 **
	Genotype: soil cover	0.0100 *	0.000475 7 ***	NS	ns
	genotype: canopy_height	NS	NS	0.00708 **	0.01381 *
	time	NS	NS	3.54e-05 ***	ns
	date: time	NS	ns	NS	ns

Annex 8 : Table with p-values for each session of measurement WUEi

	Effects	may	june	july	august
within G5	Soil cover	-	ns	0.000492 ***	0.000492 ***
	Canopy height	-	ns	0.024480 *	0.024480 *
	time	-	ns	ns	ns
	day	-	ns	-	-
	soil cover: canopy height	-	ns	ns	ns
	time: day	-	ns	-	-
within 3159- B	Soil cover	-	ns	ns	ns
	Canopy height	-	ns	ns	ns
	Time	-	ns	ns	ns
	Day	-	ns	ns	ns
	soil cover: canopy height	-	ns	ns	ns
	time:day	-		-	
Between G5 and 3159	Genotype	-		ns	0.02592 *
	Soil cover	-		0.000261 ***	0.00269 **
	canopy height	-		0.016169 *	ns
	time	-		ns	ns
	day	-		-	ns
	Genotype: soil cover	-		ns	ns

	Genotype: canopy height	-		ns	ns
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Annex 7 : Table with p-values for each session of measurement for Amax

	Effects	may	june	july	august
within G5	Soil cover	-	ns	0.0106 *	0.00346 **
	Canopy height	-	ns	ns	ns
	time	-	ns	ns	ns
	day	-	ns	-	ns
	soil cover: canopy height	-	ns	ns	ns
	time: day	-	ns	-	ns
within 3159-B	Soil cover	-	ns	NS	ns
	Canopy height	-	ns	0.0468 *	ns
	Time	-	ns	NS	ns
	Day	-	ns	-	ns
	soil cover: canopy height	-	NS	NS	NS
	time:day	-	NS	-	NS
Between G5 and 3159	Genotype	-	0.0223 *	ns	0.000562 ***
	Soil cover	-	NS	0.00216 **	0.003864 **
	canopy height	-	NS	ns	ns
	Genotype: soil cover	-	NS	ns	0.025423 *

	genotype: canopy_height	-	NS	ns	0.031242 *
	time	-	NS	ns	ns
	date: time	-	NS	-	ns

Annex 8 : Table with p-values for each session of measurement for SPAD

		late May/ mid June	late June	mid-July	early August
within G5	soil cover	8.271e-06 ***	0.00769 **	NS	NS
	canopy_height	-	NS	NS	0.01975 *
	soil_cover*canopy height	-	NS	0.01997 *	0.03441 *
within 3159	soil cover	ns	NS	NS	NS
	canopy_height	-	NS	NS	NS
	soil_cover*canopy height	-	NS	NS	NS
within genotype	genotype	ns	3.088e-12 ***	5.233e-10 ***	8.845e-11 ***
	soil cover	NS	NS	NS	NS
	canopy_height	NS	NS	NS	NS
	soil_cover*canopy height	NS	NS	NS	NS

Annex 8: Photos of the soil cover treatments at G5 plot : A) sown soil cover, B) spontaneous soil cover and C) bare soil

A



A



B



C



Annex 9: Photos of the canopy height treatments at G5 plot : A) Plants with low-canopy

B) Plants with high-canopy

A



B



Abstract

Climate change poses a significant threat to viticulture, especially in vulnerable regions like the Mediterranean. Rising temperatures and increased drought events are affecting grape quality and wine production. Berries ripen earlier and tend to accumulate more sugars, producing wines with high alcohol content and low acidity. The excessive use of phytosanitary products, primarily fungicides, raises environmental concerns. To address these challenges, strategies such as vineyard management modifications and the selection of new grape varieties are being explored. The variety VDQA-G5 is a fungus-tolerant genotype and requires lower sugar contents to achieve maturation (e.g., sugar-less trait). Nevertheless, not much is known about the responses of this variety to a multi-abiotic stress environment. This study focuses on understanding the ecophysiological responses of sugar-less VDQA-G5 variety under two canopy height treatments combined with three soil cover practices (non-irrigated spontaneous vegetation, irrigated bare soil, and irrigated sown soil). It also aims to compare these responses with another fungus-tolerant but non-sugar-less genotype, the 3159B. Ecophysiological parameters, including stomatal conductance, intrinsic water use efficiency, and photosynthesis, are measured to assess carbon and water use dynamics at the leaf level. The results effect of canopy height treatments over the variables was strongly seen. The G5 genotype on spontaneous soil treatment showed a significant decrease in gsw over time, while G5 on bare and sown treatments had higher gsw values. Furthermore, Amax of G5 on high canopy plants was 25% greater than the same treatment in 3159B. Equations were produced to estimate Amax and Asat values using stomatal conductance as a proxy.

Keywords

climate change; resistant varieties; VDQA; reduced leaf height; soil cover; water use efficiency; carbon metabolism; water consumption limitation

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