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Scambi gassosi e risposte fisiologiche di viti di Sauvignon blanc durante deficit idrici in pre- e post-invaiatura

Gas exchange and physiological responses of cv. Sauvignon blanc grapevines during pre- and post-veraison water deficit

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Abstract

As global climate change intensifies, adapting viticulture to shifting conditions will be crucial. In regions experiencing higher temperatures and increased water scarcity, effective management of water stress will emerge as a vital component of vineyard management. Understanding the physiological responses of grapevine to water limitations can inform targeted interventions, ensuring that vine health and grape quality are maintained. The study aimed to evaluate the effects of two consecutive drought stress cycles, one before and one after veraison, on Sauvignon Blanc grapevines through physiological approaches. The trial was conducted in a semi-controlled tunnel at the "L. Toniolo" Experimental Farm of the University of Padua, in Legnaro, northeast Italy, from mid-June to mid-July 2024. Fifty vines of Sauvignon Blanc clone 108, grafted onto Kober 5 BB rootstock, were grown in 30-liter pots and divided into two groups: well-watered control plants and water-stressed plants. The control group was maintained at 85-90% of field capacity throughout the experiment, which started just before veraison (June 18th) and lasted until ripening (July 22nd). In the water-stressed group, irrigation was halted until a stem water potential (SWP) of -1.3 MPa was reached (First stress), followed by a six-day recovery period with well-watered conditions. After recovery, a second drought stress cycle was imposed: half of the stressed plants experienced similar conditions to the First stress (Second short-stress), while the other half underwent a more prolonged stress, with an SWP of -2.3 MPa (Second long-stress). Both groups then went through another six-day recovery period at well-watered conditions. Throughout the experiment, daily measurements of stomatal conductance were taken using a porometer (LI-COR 600), while chlorophyll fluorescence and leaf gas exchange were assessed during peak stress and recovery phases using a LI-COR 6800 system. *A/Ci* measurements were conducted at 30°C and 400 ppm CO_2, followed by 300, 200, 100, 50, 400, 400, 600, 800, 1000, 12000 and 1400 ppm CO_2 under 1500 µmol m^2 s^{-1} of photosynthetic photon flux density (PPFD). The Farquhar, von Caemmerer, and Berry photosynthesis model ('FvCB model') was fitted to the experimental data to

estimate the maximum carboxylation (Vc_{max}) and maximum electron transport (J_{max}) rates. Plant water status was assessed by stem and leaf water potential (Ψleaf, MPa) using a PMS-600 pressure chamber. Malic acid levels were monitored starting just before the onset of veraison. The results confirmed the detrimental effects of water deficit on photosynthesis and stomatal conductance. Significant differences emerged between the second long-stress and both the first and second short-stress cycles, highlighting the grapevines' ability to recover from water stress. However, following the extended second-stress cycle, this recovery capacity weakened. This decline was reflected in the photosynthesis and fluorescence data, indicating a reduction in photosynthetic efficiency. This research contribute to a deeper understanding of the effects of climate change on viticultural practices, adding knowledge for the study of water management strategies in the viticulture sector.

Chapter 1. Introduction

1.1. Climate change impacts on global viticulture

Global climate change represents one of the most significant challenges facing humanity today. The cause of climate change is considered to be the greenhouse effect, but it actually is a natural process that makes possible the life in the Earth. Anthropogenic activities have modified the greenhouse effect as they have led to the increase in the global atmospheric concentration of CO_2, NH_4, N_2O. The concept of climate encompasses both average atmospheric conditions—such as temperature, rainfall, and wind patterns—and the inherent variability of these factors over time. Currently, the most worrying feature of climate change is that is becoming less predictable due to its rapid evolution, in extent and variation (Sodini et al., 2016). During the second half of the 20th century average, Northern Hemisphere temperatures were very likely higher than during any other 50-year period in the last 500 years (Pereira et al., 2011). Over the last 50 years some extreme weather events have changed in frequency and intensity, because of the rise of the average annual temperatures (Tombesi et al., 2018). These extreme events are mainly: heatwaves, intense rainfall, wildfires, smoke, frost risk and increased intensity of drought due to the augmentation of the evaporative atmospheric demand. For the cultivation of agricultural crops climate is a decisive factor because it determines the geographical suitability and the effects on yield and quality. These strong connections have defined, over human history, the economic and cultural development of regions and have its most expression in grape and wine production (Schultz and Jones, 2010; Santos et al., 2020). Climate parameters have ever been used to bound wine regions. The main grape growing and producing areas have traditionally been situated between 30° to 50°N and 30° to 40°S. To date, grapevines are cultivated in 90 different countries, across a large diversity of climates with the majority occurring in temperate climate regions. Due to the need to ensure quantitatively satisfactory productions and to achieve high quality levels to compete effectively in national and international markets, in a global context where the quality of wine is increasingly

appreciated, the grapevine is in a delicate position. On the short-term, weather considerably governs the whole grapevine development process, as it requires suitable temperatures, radiation intensities, and duration, as well as specific levels of water availability. Alterations in the spatial patterns and temporal regimes of temperature and precipitation may significantly modify current viticultural bioclimatic zones (Fraga et al., 2016). The climatic characteristics of a given viticultural region are key factors in understanding its varietal suitability (Carbonneau, 2003). Then, climate combined with the soil type and human activity contribute to identifying the *terroir*, a key aspect in determining the wine quality related to the typicity of a given region (Santos et al., 2020). Since very often the image of a wine growing region is determined by one or a limited number of cultivars, changing these cultivars may not be the immediate appropriate solution. Additionally, within Europe, the use of specific varieties is often regulated by law, which may become problematic considering future climatic developments. On a global scale, climate change could cause current wine-growing regions to lose ground and other regions to gain. Higher elevations might become increasingly suitable for viticulture (Santos et al., 2020), but this upslope expansion could encroach on wild habitats in mountain regions (Van Leeuwen et al., 2024). Early analysis of the impacts of climate change on global viticulture were conducted by Kenny and Harrison (1992). They predicted parts of Southern Europe to become too hot and northern regions to become suitable for grape cultivation. To date, these predictions have been largely confirmed. INRAE, Bordeaux Sciences Agro, the CNRS, the University of Bordeaux and the University of Burgundy analyzed future developments in current and emerging wine regions around the world to adapt wine production to climate change. The results, published in Nature Reviews Earth and Environment, show that around 90 per cent of coastal and low-altitude wine-growing regions in southern Europe such as Spain, Italy and Greece and southern California risk losing their ability to produce quality wine at economically sustainable yields by the end of the century if global warming exceeds +2°C because of excessive drought and more frequent heatwaves (Van Leeuwen et al., 2024). The most important climate factors playing a role in yield formation, grape development and grape composition are temperature and water supply. Higher temperatures primarly impair phenology. In most

winegrowing regions around the globe, grape harvests have advanced by 2–3 weeks over the past 40 years. Earlier phenology means that ripening will occur in a warmer period. It is likely that different varieties respond differently to warming, despite that red varieties appear to tolerate warm conditions better than white varieties (Schultz and Jones, 2010). High temperatures during the veraison to maturity period can negatively affect sugar accumulation, often leading to elevated alcohol levels, while also disrupting organic acid metabolism and reducing the biosynthesis of secondary metabolites such as phenolics and sensory volatile compounds (Schultz a Jones, 2010). Both the formation of malic acid before veraison and its degradation after veraison are highly sensitive to temperature (Kliewer, 1973; Ruffner et al., 1984). Reduced acidity lowers microbiological stability, which may result in the development of off-flavors. It is crucial to acknowledge climate change as a key driver of intensified water scarcity. Rising temperatures increase the amount of moisture the air can hold, leading to a higher vapor pressure deficit (VPD) and greater evapotranspiration (ET). Consequently, even if precipitation levels remain relatively stable, plant water usage will rise with increasing temperatures, heightening the risk of drought (Van Leeuwen et al., 2024). Plant photosynthetic activity is influenced by both temperature and sunlight, and the light saturation threshold for photosynthesis increases with air temperature, peaking at an optimal range between 25 and 30°C. However, in hot and dry conditions, photosynthesis can be impaired even when light is abundant (Van Leeuwen et al., 2024). Water fluxes in the Soil-Plant-Atmosphere Continuum (SPAC) are regulated by leaf stomata. To prevent excessive water loss, plants respond to drought conditions by inducing stomatal closure. However, since CO_2 enters the leaf mesophyll through these same stomata, water deficits also reduce photosynthesis, ultimately leading to a decline in crop productivity (Van Leeuwen et al., 2024). Water stress significantly impacts various stages of grapevine development, including vegetative growth, inflorescence formation, berry set, berry development, and fruit quality. The magnitude of these effects depends on factors such as the plant's phenological stage, the severity and duration of the water deficit, and environmental conditions (Hardie et al., 1976). Addressing these climate-related challenges requires robust, science-driven adaptation strategies. As a result, both national and international research

efforts have intensified to deepen our understanding of the complex physiological responses of grapevines to shifting environmental conditions.

1.2. Stress phenomena

1.2.1. Water deficit

The effects of climate change are essentially expressed by amplifying and increasing episodes of stress (water, heat, radiative...). It is therefore necessary to better study stress syndromes in order to – possibly – prevent or manage them. Historically, the study of stresses began by considering water scarcity and its effects on growth and productivity. The first studies about water deficit date back to 1970s (Begg and Turner, 1976) and emphasized the importance of plant water deficit rather than soil water deficit, but after a decade of steady progresses in this field researchers shifted focus on plant growth regulators (such as abscisic acid) and how they are linked to leaf turgor. Leaf turgor was considered the transducer of plant water deficit and osmotic adjustment was just recognized as an adaptive mechanism that plants use to maintain growth (Turner, 1986).

Turgor pressures (P) can be estimated from the difference between measured values of water potential (Ψ) and osmotic pressure (π), which depends on the concentration of solutes. The hypothesis that turgor pressure is the transducer of water deficit in the cell raised after the recognition that plants accumulate solutes such as sugars, proline, and potassium in response to water deficit to lower their osmotic potential, allowing them to retain water and maintain cell turgor to sustain cellular functions even when external water availability is low (Tombesi et al., 2018). This mechanism is called osmotic adjustment and is a key survival mechanism (Beg and Turner, 1976; Gambetta et al., 2020). Ultimately, after the discovery that endogenous levels of abscisic acid increase severalfold when plants were subjected to water stress and that abscisic acid close stomata and reduce transpiration, it was concluded that plant physiological processes are more closely correlated to soil water content / potential than with leaf turgor (Turner, 1986).

1.2.2. Water flow and transpiration

The development of water deficit is a consequence of water loss from the leaf as the stomata open to allow the uptake of carbon dioxide from the atmosphere for photosynthesis. This mechanism of water loss is called transpiration. Several environmental and plant factors have been shown to influence the transpiration efficiency. The main environmental factor is the Vapor Pressure Deficit (VPD) at leaf surface. VPD is basically the difference between the actual amount of moisture that is in the air at a certain point in time and the maximum amount of moisture that air could hold at that temperature (i.e. at saturation). It is used to estimate the evaporative demand of the atmosphere and can be easily calculated with simultaneous measurements of air temperature and relative humidity (Kirkham, 2014). When the plant transpires, transpiration from the leaf surface pulls water through the plant via negative pressure that is perpetuated through the plant because of water's cohesive quality (Gambetta, 2016). The water lost by transpiration must be replaced by water drawn from the soil through the root, stem, and leaf via the xylem. The xylem is one of the main two tissues of the plants' vascular system. The xylem is typically composed of cells that at maturity are dead and empty and resemble a series of tubes. These tubes are called xylem vessels and transport mainly water and other solutes.

1.2.3. Water potential

The pathways of water movement from the soil into the atmosphere is usually considered as a continuum, frequently referred to as soil-plant-atmosphere continuum (SPAC). This model is mainly based on the theory that water must be under tension to be transported through the plant's xylem (Turner, 1986), along a gradient of an appropriate force, related to the thermodynamic status of water. The chemical potential of water, known as Water Potential (Ψ), is a sensitive physiological indicator that integrates both soil and climatic conditions with plant water status. The conceptual development of water movement along the soil-plant-atmosphere continuum has led to crop growth processes being correlated with the total water potential, particularly leaf water potential.

Pressure chamber measurement (Scholander et al., 1965) is the most common method for measuring plant water potential in field studies because of its speed, reliability, and the lack of any need for careful temperature control. It can provide values of dawn leaf water potential (dawn Ψ), daily leaf water potential (leaf Ψ) and stem water potential (stem Ψ). Predawn leaf water potential measures plant water status at zero plant water flux, when the plant is close to equilibrium with the soil, so it provides information on the root zone soil water potential. The measure that reflects the combination of VPD at leaf surface, soil water availability, internal plant hydraulic conductivity and stomatal regulation is daily leaf Ψ and it's recorded on a single leaf. Stem Ψ is measured on a non-transpiring leaf. Daily stem Ψ is the result of whole plant transpiration, and soil and root/soil plant hydraulic conductivity (Choné et al., 2001).

1.2.4. Drought effects on water flow

Soil water availability strongly impacts physiological and growth processes of grapevines. Grapevines rely on an efficient water transport system (xylem) to distribute water from the roots to the leaves. Drought stress compromises hydraulic conductivity, sometimes causing embolism (air blockages) in xylem vessels, reducing water transport capacity through the grapevine (Gambetta et al., 2020). Evidence of the reduced ability of the plant to draw water from the soil into its tissue is also the decrease in leaf water potential (Escalona et al., 2020). Grapevines can exhibit either isohydric behavior or anisohydric behavior. This distinction is important for understanding varietal differences in drought tolerance. Isohydric grapevines tightly regulate water potential by closing stomata early during drought, limiting water loss and the possibility of xylem embolism to occur; anisohydric varieties maintain stomata open longer, allowing greater gas exchange to sustain photosynthesis longer but at the cost of higher water loss (Gambetta et al., 2020).

1.2.5. Drought effects on stomatal regulation

Moreover, another first response of grapevine to drought is stomatal closure (Escalona et al., 2002). Stomata are like hydraulic valves. They are mainly found on the underside of leaves and are formed by two specialized epidermal

cells side by side, called guard cells, which delimit an opening between them, called the stomatal aperture or stomatal rhyme. Stomata role is to regulate the exchange of H_2O, O_2 and CO_2 at leaf level. Stomatal regulation is the key of the balance between CO_2 uptake and water loss, which is essential for their survival. Stomatal functioning is a highly regulated mechanism. The plant hormone abscisic acid (ABA) plays a crucial role in stomatal regulation under drought. As soil moisture decreases, ABA accumulates and signals the stomata to close, conserving water (Gambetta et al., 2020). This restricts both water loss (transpiration) and carbon dioxide intake, which is necessary for photosynthesis. Generally stomatal closure enhances grapevine Water Use Efficiency (WUE) because it reduces water loss more than it reduces CO2 uptake (Escalona et al., 2002). However, when CO2 becomes limiting, net carbon assimilation (An) drops significantly (Escalona et al., 2002).

1.3. Photosynthesis

Photosynthesis is the physiological process by which carbon dioxide and water are converted into carbohydrates through the transformation of solar energy into chemical energy. In eukaryotic organisms such as plants and algae, this process occurs in the chloroplasts. Inside the chloroplasts, a system of membranes forms stacks of flattened sacs called thylakoids, where chlorophyll molecules are located. These chlorophyll molecules are organized into complex pigment-protein systems known as reaction centers, comprising Photosystem II (PSII) and Photosystem I (PSI). During the first stage of photosynthesis, known as the light-dependent reactions, a series of oxidation-reduction reactions pass electrons through a chain of cofactors. The movement of these electrons is initiated by light energy, which is captured by photosynthetic pigments and transferred to the chlorophyll molecules. In PSII, electrons are passed through free electron carriers (plastoquinones) and subsequently to other membrane protein complexes. A similar process occurs in PSI, where the electrons are ultimately transferred to NADP+, resulting in the production of NADPH. Concurrently, a proton gradient is established across the thylakoid membrane, which drives the synthesis of ATP. The electrons required for these reactions come from the splitting of water molecules, leading to the release of

oxygen (O2) and the production of both ATP and NADPH. The ATP and NADPH generated during the light-dependent reactions are then used in the second stage of photosynthesis, known as the Calvin-Benson cycle, or the light-independent reactions, as they do not require light. In C3 plants, the initial fixation of carbon dioxide (CO2) occurs via ribulose bisphosphate (RuBP) and the enzyme ribulose bisphosphate carboxylase-oxygenase (Rubisco), resulting in the formation of 3-phosphoglyceric acid (PGA). This compound is subsequently converted into triose phosphate using ATP and NADPH. Additional ATP is required to regenerate RuBP, the substrate for the carboxylation reaction, ensuring the cycle can continue. Through this two-stage process, photosynthesis efficiently converts light energy into chemical energy, enabling the production of carbohydrates essential for plant growth and survival (Sharkey, 1985).

1.3.1. Ecophysiological studies as an essential characterization tool

The study of stresses and their effect on the photosynthetic efficiency of plants has benefited greatly from a series of techniques inherent to ecophysiology: the definition and measurement of water potential, the monitoring of gas exchange, fluorescence, etc. The monitoring of gaseous exchanges (transpiration, photosynthesis), which began in the late 1950's (Gaastra, 1959), and the characterization of stomatal regulation have made it possible to determine the homeostatic control capabilities of plants. The paper by P. Gaastra (1959) is an influential early work that investigates the various factors affecting the rate of photosynthesis in crop plants. He focused on light intensity, CO_2 concentration, temperature, and stomatal diffusion resistance and highlighted the interdependence of these factors, showing that they do not act in isolation but in combination to determine the overall efficiency of photosynthesis. A significant contribution of this paper is Gaastra's development of mathematical models that takes into account all these factors to predict photosynthesis under varying environmental conditions. These models have been valuable for agricultural research, enabling predictions of crop performance under different climatic conditions. They also laid the groundwork for further research into crop physiology and the environmental regulation of photosynthesis (Gaastra,

1959). Today, the equipment is extremely sophisticated. Gas exchange techniques to measure photosynthesis (through CO_2 uptake) and respiration (through CO_2 release) directly assess plant gas exchange with the environment. Gas exchange data provide valuable insights into photosynthetic efficiency, the potential limiting steps in the photosynthesis process and how plants dynamically adjust photosynthesis in response to changing environmental conditions. These dynamic responses often require high-resolution, time-sensitive measurements to capture accurately (Sharkey, 2016; Hunt, 2003; Long & Bernacchi, 2003; Bush et al., 2024). Many studies rely on Infrared Gas Analyzer (IRGA) for gas exchange measurements, a highly sensitive method for tracking CO_2 flux. While IRGA is reliable, it requires careful calibration and control over environmental conditions to ensure reliability (Hunt, 2003; Busch et al., 2024). Fluctuations in light, temperature, and humidity can complicate the measurement of stable photosynthetic rates. Accurate measurements require standardized conditions and strict protocols, as this ensures comparability across studies and improves the reproducibility of findings. (Long & Bernacchi, 2003; Millan-Almaraz et al., 2009). Recent technological advancements, such as automated gas exchange systems with better environmental control, are improving data reliability (Busch et al., 2024). To provide a fuller picture of photosynthetic (PSII) efficiency or, for example, differentiating limitations to photosynthesis caused by stomatal conductance (the CO_2 diffusion barrier at the leaf surface) from those caused by internal biochemical factors (such as Rubisco activity), complementary techniques like chlorophyll fluorescence are frequently used (Millan-Almaraz et al., 2009; Sharkey, 2016).

1.3.2. Modeling of photosynthesis and gas exchange

The availability of appropriate techniques and the general advancement of knowledge on the biophysical and biochemical aspects of photosynthesis and gas exchange has also enabled the development of sophisticated mathematical models of the process. The development of such models is important for many reasons: i) the development of a model allows knowledge to be ordered, highlighting what is not yet adequately known; ii) if the model is mechanistic

(i.e. it mathematically represents cause-effect relationships, based on laws of physics and/or chemistry and/or biochemistry), the parameters of the model (derived by 'fitting' the model to the data that have been collected), have 'further' significance, condensing and revealing important properties of the system; iii) if the model is mechanistic (and not merely statistical), it can also be used to predict the behaviour of the system under conditions not explored by experimentation.

The 'strongest' model for C_3 plant photosynthesis was developed in Australia, by Graham Farquhar, Susanne von Caemmer and Joe Berry. Farquhar, Caemmerer, and Berry's 1980 paper introduced a pioneering biochemical model for CO_2 assimilation in C_3 plants, providing a quantitative framework to predict photosynthesis rates based on key environmental and physiological factors. This model broke new ground by describing photosynthesis through two primary limiting processes: i) Rubisco-limited photosynthesis (Vc_{max}): this part of the model is driven by the rate of Rubisco enzyme activity, which depends on CO_2 availability at the enzyme's active site; ii) Electron transport-limited photosynthesis (J_{max}): this component reflects limitations imposed by the rate of electron transport in the chloroplast, which is closely tied to light conditions and affects the regeneration of Ribulose-1,5-bisphosphate (RuBP).

This model makes it possible to describe the photosynthetic activity of plants with relatively simple equations built on parameters such as changes in light, CO_2 concentration, and temperature that can be estimated from the analysis of experimental gas exchange data. In particular, the model describes the photosynthetic assimilation under steady-state conditions of all environmental parameters as the internal CO2 concentration changes. The net photosynthetic assimilation (An) can be expressed by means of the following equation:

$$A_n = V_c - V_o - R_{day}$$

Where:

- **Vc** is the rate of carboxylation by the Rubisco enzyme,
- **Vo** is the rate of oxygenation by Rubisco, and
- **Rday** represents the rate of mitochondrial respiration in the leaf tissue during daylight.

In their paper, Farquhar, von Caemmerer, and Berry (Farquhar et al., 2001) revisited and refined their initial model by integrating regulatory processes and feedback mechanisms. The authors recognized that photosynthesis is not static but is dynamically regulated by internal and external signals, therefore they created more complex and adaptive model that better simulates real-world plant responses to fluctuating environmental conditions. Although the paper is primarily focused on the biochemical modeling of photosynthesis, it provides foundational knowledge for understanding drought responses, especially in terms of how these limitations affect photosynthetic efficiency in C_3 plants. They emphasized 3 main regulatory layers:

1) Stomatal conductance regulation: this regulation adjusts the aperture of stomata in response to environmental changes such as light, CO_2 levels, humidity, and water availability. By fine-tuning CO_2 uptake and water vapor release, stomatal conductance plays a key role in balancing the plant's needs with environmental conditions. This feedback is crucial, particularly under drought, where the plant needs to optimize photosynthesis while minimizing water loss. The closure of stomata restricts the influx of CO_2 into the leaf mesophyll, which lowers the internal CO_2 concentration (Ci) and limits the substrate availability for Rubisco, the primary enzyme for CO_2 fixation. This reduction in Ci directly limits the photosynthetic rate since Rubisco activity depends on a steady CO_2 supply.
2) Biochemical adjustments in the Calvin Cycle: the Calvin cycle, particularly RuBP regeneration, is modulated by the plant's internal conditions and environmental stress. The regeneration of Ribulose-1,5-bisphosphate (RuBP), which is essential for the Calvin cycle, is dependent on ATP and NADPH production that in turn is dependent on electron transport. Drought can impair electron transport, thus even if CO_2 is available, the Calvin cycle cannot proceed at its normal rate, further slowing photosynthesis.
3) Thermal dissipation: the 2001 model includes also advances about how plants manage a low CO2 intake and energy excess. Plants dissipate energy via non-photochemical quenching, a protective mechanism

crucial for preventing photodamage to PSII when excess light energy cannot be used in carbon fixation.

1.3.3. *A/Ci* curves

(from *Susanne von Caemmerer,* Case Study 1.1 - Development of $A:C_i$ curves) Particularly useful is the analysis of *A:Ci* curves (e.g., CO_2 response curves), a plot of photosynthetic CO_2 assimilation rate (*A*) against the intercellular CO_2 concentration (*Ci*). To generate this curve, the CO_2 concentration around a leaf is progressively changed while measuring the photosynthetic rate.

These curves are fitted to the FvCB model, which describes how photosynthesis responds under two key limiting conditions: i) at low *Ci* (CO_2 concentration), photosynthesis is limited by the availability of CO_2 for Rubisco to fix (the curve is Rubisco-limited (driven by Vc_{max})); ii) at higher *Ci*, photosynthesis is limited by the plant's ability to regenerate RuBP, which depends on ATP and NADPH produced during the light reactions of photosynthesis (the curve becomes electron transport-limited (driven by J_{max})). By fitting the *A/Ci* curve, we can estimate Vc_{max} and J_{max}, which provide insight into how the biochemical machinery of photosynthesis is functioning.

Kinetic properties of Rubisco and electron transport rates change with temperature, therefore the estimates of Vc_{max} and J_{max} can be temperature-dependent. Adjustments are often made to account for the leaf temperature at the time of measurement.

1.4. Fluorescence

Chlorophyll fluorescence has become one of the most powerful and widely used techniques for investigating plant photosynthetic performance (Murchie and Lawson, 2013), largely due to the development of user-friendly and portable fluorometers (Maxwell et al., 2000). The correlation between fluorescence parameters and standard indicators of plant water status (e.g., stomatal conductance [g_s] and water potential [Ψ]) is frequently utilized in studies evaluating the effects of increasing water stress on grapevines (Medrano et al., 2002). The basis of chlorophyll fluorescence lies in the fact that light energy

absorbed by chlorophyll molecules in the leaves can be: i) used to drive photosynthesis, ii) dissipated as heat, or iii) re-emitted as light in the form of chlorophyll fluorescence (Maxwell et al., 2000). These three processes are competitive, meaning that an increase in the efficiency of one will result in a decrease in the yield of the other two (Maxwell et al., 2000). Fluorescence emission occurs at a longer wavelength than absorption, and measurements are taken by exposing the leaf to light of a specific wavelength and detecting the re-emitted light at longer wavelengths (Maxwell et al., 2000). Early observations on changes in fluorescence yield were made by Kautsky and colleagues (Kautsky et al., 1960). When photosynthetic material is transferred from darkness to light, fluorescence yield increases over a period of approximately one second. This initial increase is attributed to the reduction of electron acceptors in Photosystem II (PSII). Once the first acceptor, plastoquinone A (Qa), receives an electron, it cannot accept another until it passes the electron to the subsequent carrier, plastoquinone B (Qb), which causes a temporary increase in fluorescence yield. Over the course of a few minutes, fluorescence levels decline due to two processes: i) photochemical quenching (qP), resulting from the accelerated rate of electron transport away from PSII, and ii) non-photochemical quenching (NPQ), where excess energy is dissipated as heat. To assess photosynthetic performance, it is essential to differentiate between photochemical and non-photochemical contributions to fluorescence quenching. Commonly used fluorescence values include:

- **Fm**: Maximum fluorescence in the absence of photochemical quenching, measured by applying a saturating flash that transiently closes all PSII reaction centers.
- **F'm**: Maximum fluorescence yield under light conditions.
- **Ft**: Steady-state fluorescence yield under light conditions.
- **F°**: Fluorescence yield in the absence of photosynthetic light.

The estimation of F° is achieved by dark-adapting the leaf, followed by the application of far-red light (>680 nm) for a few seconds before and immediately after the end of illumination, ensuring that all PSII reaction centers are open.
Commonly used fluorescence parameters:

1. Photochemical quenching parameters:

- **ΦPSII**: Quantum yield of PSII photochemistry.
- **qP**: Proportion of open PSII reaction centers.
- **Fv/Fm**: Maximum quantum yield of PSII.

2. Non-photochemical quenching parameters:
   - **NPQ**: Non-photochemical quenching, representing the amount of energy converted to heat.

The quantum yield of PSII photochemistry (ΦPSII), defined by Genty et al. (1989), is calculated as:

$$\Phi PSII = (F'm - Ft) / F'm$$

This parameter can be used to calculate the linear electron transport rate (J) as follows:

$$J = \Phi PSII * PFDa * 0.5$$

Where **PFDa** is the absorbed photon flux density (μmol photons m^{-2} s^{-1}), and 0.5 accounts for the partitioning of energy between PSII and Photosystem I (PSI).

The photochemical quenching parameter **qP**, which indicates the proportion of open PSII reaction centers, is calculated as:

$$qP = (F'm - Ft) / (F'm - F'°)$$

The relationship between **ΦPSII** and **qP** yields another important parameter, **Fv/Fm**, which represents the maximum efficiency of PSII when all PSII centers are open. This is a key indicator of PSII photoinhibition due to stress (Krause and Weis, 1991):

$$Fv/Fm = \Phi PSII / qP = (Fm / F°) / Fm$$

For calculating non-photochemical quenching (NPQ), the measurement of a "dark-adapted" value of **Fm** is necessary, where photochemical efficiency is maximal, and heat dissipation is minimal. NPQ is quantified as the ratio of the change in **Fm** to the final value of **F'm**:

$$NPQ = (Fm - F'm) / F'm$$

This comprehensive approach to chlorophyll fluorescence analysis provides valuable insights into the photosynthetic efficiency and stress responses of plants.

1.5. Grape technological maturity – malic acid

Organic acids are a key component of the organoleptic quality of fruit. If acid content is too low, the wine's body becomes unbalanced. Temperature and soil water availability affect grapevine vegetative growth and berry composition. Tartaric and malic acids typically account for 69 to 92% of the organic acids in grape berries and leaves (Kliewer et al., 1966). Malate in the berry is synthesized from phosphoenolpyruvate (PEP) via phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase (MDH). Cytosolic malate is transported and sequestered in the vacuole (Schulze et al., 2002). Malate can be degraded through the reversible reaction of MDH and nicotinamide adenine dinucleotide phosphate-malic enzyme (NADP-ME) (Sweetman et al., 2009). Malic acid content decreases as light intensity increases and water supply decreases. Grapes accumulate malate until berries undergo a metabolic shift at veraison, making it available as a potential carbon source for respiration (Ruffner et al., 1982). Heating during veraison and ripening stages induces a higher rate of respiration, reducing malate content due to increased degradation into glucose (Sweetman et al., 2014; Pandell, 1999). Malic enzyme activity increases with rising temperatures (between 10°C and 46°C), indicating high thermal stability (Lakso and Kliewer, 1975). Grape malic acid levels are typically higher in cooler regions than in warmer ones (Blouin and Guimberteau, 2003). However, Sweetman et al., 2014 noted that differences in malate response to heating between pre-veraison, veraison, and ripening stages could not be explained by changes in enzyme activity alone. Instead, it may be related to differences in the regulation of malate compartmentalization across these developmental stages. Water stress can affect grape composition both directly and indirectly, either positively or negatively depending on the severity and duration of the stress. López et al., 2007 observed an increase in malic acid concentrations under certain conditions. In contrast, De Souza et al., 2005, Matthews and Anderson, 1989, and Kizildeniz et al., 2015 reported a decrease in malic acid concentration, while Esteban et al., 1999 found no significant changes. Geng et al., 2022 demonstrated that heavy pre-veraison water stress inhibits organic acid synthesis by reducing the activity of malic acid anion

channels and proteins on the vacuole membrane, blocking malate release into the cytoplasm and further affecting MDH expression.

1.6. Berry calcium content

In grapevine, cellular structure, physiological processes as well as berry development and ripening are strongly related to the water and nutrients entering the berry (Duan et al., 2022; Porro et al., 2010). One of the main elements found in berries is calcium. Calcium (Ca) plays a crucial role in berry skin structure, as it is involved as a cell wall component, specifically bound as pectate in the middle lamella, which reinforces the cell wall structure and helps plants maintain rigidity and resistance to mechanical stress, pathogen attacks, fungal diseases, and insect pests.

Calcium movement occurs in the xylem, so its availability depends on water flow through the soil-root-shoot pathway, as well as on nutrient mobility in the soil. Xylem-mobile minerals like Ca accumulate mostly prior to veraison. At veraison, a rupture of the xylem vessels occurs in the pericarp, which is likely responsible for the halt in calcium accumulation in this compartment (Düring et al., 1987). After veraison, changes occur in the mechanical properties and cell wall components of grape skin. In particular, during the loosening of the skin, the cell walls continuously lose structural polysaccharides and Ca (Huang et al., 2005). Cabanne and Donèche (2003) reported that calcium accumulation ceases at the onset of ripening in the pericarp but persists in the seeds. At the same time, calcium is likely translocated from the flesh to the skin and seeds. Water stress may influence growth during the berry softening and ripening processes (Porro et al., 2010). When water stress conditions are imposed, the content of Ca and other nutrients in berries change significantly because water stress creates competition for nutrient uptake, affecting the structural properties of the berry epidermis, particularly skin thickness. Porro et al. (2010) recorded high Ca levels in berries from water-stressed vines, indicating the important role of Ca in enhancing berry skin thickness.

Moreover, calcium's involvement in cellular signaling is essential for regulating various physiological responses in grapevine growth, particularly under stress

conditions, including water scarcity. Calcium ions (Ca^{2+}) act as secondary messengers in signal transduction pathways that mediate responses to environmental stimuli. When grapevines encounter stress - such as drought or nutrient deficiencies - Ca^{2+} concentrations within specific cellular compartments increase transiently. These calcium “waves” act as signals that trigger downstream responses within the plant. In grapevines, this activation impacts cellular responses like gene expression, enzymatic activities, and cellular stress responses (Duan et al., 2022). An impairment in calcium accumulation was found by Duan et al., 2022 to result in weakened growth and lower resistance to environmental stresses.

Chapter 2. Materials and Methods

2.1. Plant material and experimental setup

Figure 1. Experimental setup.

Experiments were carried out on potted grapevines, *Vitis vinifera* L. cv. Sauvignon Blanc (clone 108) grafted onto Kober 5 BB (K5BB) (*V. berlandieri* × *V. riparia*) rootstock. The experiment was conducted in semi-controlled tunnel at the Experimental Farm of the University of Padua "L. Toniolo" in Legnaro (North-East of Italy) during summer 2024 from mid-June to mid-July. In January, during the winter season, fifty plants of Sauvignon Blanc were transplanted from 10 ℓ pots into 30 ℓ containers. The substrate used for this process had a medium-coarse texture, high porosity, and moderate water retention capacity. It was composed of blonde and brown peat with a small percentage of pumice (commercially known as Terflor – Vulcan Estivo PF substrate). A drip system of irrigation was installed in all fifty pots.

2.2. Water treatments

Within the experimental setup, 2 treatment groups were established, consisting in 20 control plants and 30 water stressed plants. The Control group was kept at well-watered conditions (85-90% of field capacity) during all experiment period, that started before véraison (18/06/2024) until maturation (22/07/2024). On the other hand, plants subjected to water stress were kept without water until reaching a stem water potential (SWP) of -1.3 MPa (called here as First stress). Afterward, the stressed plants underwent a six-day recovery period, during which they were maintained under control conditions. After the recovery phase, a second cycle of water stress was initiated. To implement this, the 30 stressed plants were divided into two groups: 15 plants experienced a similar stress level to the first (SWP of -1.3 MPa), referred to as the Second short

stress, while the other 15 plants were exposed to a more prolonged stress (SWP of -2.3 MPa), referred to as the second long stress. After both the second short and second long stresses, the plants were again given a six-day recovery period. In order to mitigate the fluctuations in soil water content, all pots were weighed at 6 pm daily and the amount of water needed to maintain the desired soil field capacity was then added.

Figure 2. Second-Short Stress (left) vs. Second-Long Stress (right).

2.3. Leaf gas exchange and chlorophyll fluorescence

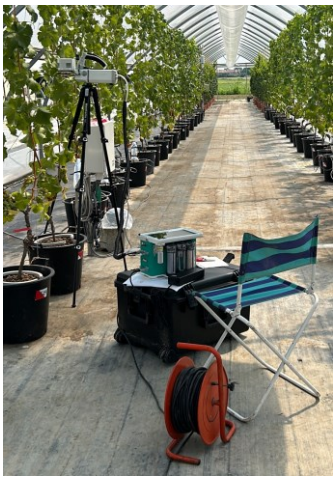

Figure 3. LI-COR-6800, LI-COR Inc., Lincoln, NE, USA.

Single-leaf gas exchanges and chlorophyll fluorescence were measured with an open system apparatus (LI-COR-6800, LI-COR Inc., Lincoln, NE, USA) when plants reached the maximum stresses and when they were fully recovered from the stress. Measurements were conducted using a 6 cm^2 leaf cuvette equipped with Multiphase Flash Fluorometer (6800- 01A) that is a combined light source and chamber for simultaneous measurements of gas exchange and chlorophyll fluorescence from the same leaf area. Before *A/Ci* curves measurements, which depicts the net CO_2 assimilation rate (*A*) in relation to the calculated internal CO_2 concentrations, leaves were dark-adapted for 2 hours to determinate Fv/Fm parameter. After the dark flash, leaves were subjected to at least 20-min of acclimation at a constant saturating photosynthetic photon flux density (PPFD) of 1500 µmol of photons m^{-2} s^{-1} before starting the measurements at CO_2 concentration at 400 µmol CO_2 mol^{-1} (followed by 300, 200, 100, 50, 400, 400, 600, 800, 1000, 1200 and 1400 µmol CO_2 mol^{-1}). Leaf temperature was maintained at 30 °C. Relative humidity (RH) was ranging between 50 and 70 % allowing ~1.5 kPa of vapor pressure deficit (VPD) inside the chamber.

Measurements were performed on at least three fully expanded leaves per treatment, between 11:00 and 14:00 solar time. The photosynthesis model proposed by Farquhar, von Caemmerer, and Berry (Farquhar et al., 1980), commonly referred to as the 'FvCB model' was calculated. Vc_{max}, J_{max} and the intercellular CO_2 concentration at which the transition from Rubisco to RuBP regeneration limitation occurs were calculated for the *A/Ci* curves by fitting the FvCB model using the 'Plantecophys' R package 'fitaci' function (Duursma 2015). In addition, in order to have daily control of the stomatal conductance, measurements using LI-COR-600 (LI-COR Inc., Lincoln, NE, USA) were taken. The LI-600 is an advanced, compact porometer designed for quick and precise measurements of stomatal conductance and chlorophyll a fluorescence that minimally disturbs light, CO_2, and H_2O during the recordings. The apparatus takes measurements over the same leaf area simultaneously.

Figure 4. LI-COR-600, LI-COR Inc., Lincoln, NE, USA.

2.4. Vine water potential

Figure 5. Scholander pressure chamber.

The leaf water potential (Ψleaf, MPa) was measured at pre-dawn and midday, as well as stem water potential (SWP) using a Scholander-type pressure chamber (model PMS-600, PMS Instruments, Corvallis, OR, USA). For SWP six randomly chosen sun-exposed, and fully expanded leaves per treatment, which had been enclosed in an opaque plastic bag for more than 1h to prevent transpiration and allow them to reach equilibrium with the water potential in the stems, were measured from solar noon until the early afternoon. Each leaf was excised from the shoot with a scalpel blade and placed in the pressure chamber with the petiole protruding from the chamber lid. The chamber was pressurized using an air pressure tank, and SWP was recorded

as soon as the xylem sap was observed emerging from the cut end of the petiole.

2.5. Malic acid

For the analysis of malic acid content, some berries were collected at irregular intervals and not at the same time as the other stress cycle surveys. The objective was to observe the development of malic acid content before (synthesis) and after (consumption) veraison and make the comparison between the two treatments (well-watered/water stressed). The berries were divided into three parts and each became a replicate. The analysis was performed using R-Biopharm's I-Magic M9 enzyme analyzer with its Enzytec Liquid L-Malic specific kit (code E8280).

2.6. Calcium

Calcium in the samples was determined using the Enzytec Calcium kit (R-Biopharm) based on the colorimetric test with Arsenazo III. The test was performed on undiluted must samples using the I-Magic enzyme analyser.

2.7. Statistical analysis

Statistical analysis was performed using R software. ANOVA was performed to identify the differences between the time points and the treatments, and then, when significant, Tukey's test was applied. Differences were considered significant at the $P \leq 0.05$ level.

Chapter 3. Results and Discussion

3.1. Vine water status

Pre-dawn water potential (Ψpre-dawn), a proxy for soil water availability accessible to the plant, for the water-stressed groups reflected the progressive increase in water limitation over the stress periods. In contrast, control group plants maintained stable Ψpre-dawn values close to 0 MPa. Plants subjected to water stress displayed a pronounced decrease in Ψpre-dawn, dropping to approximately -1 MPa at the end of both the First stress and the Second short stress, and further declining to around -2 MPa at the conclusion of the Second long stress. During recovery periods, stressed plants exhibited a return of Ψpre-dawn values close to those of the control group, although they remained slightly lower (Figure 1).

Midday stem water potential (Ψstem or SWP), which reflects the water availability within the plant's conducting tissue, also showed consistent patterns. Control group plants maintained steady midday Ψstem values around -0.5 MPa. Similar midday Ψstem values were recorded at the end of both the First stress and the Second short stress, reaching -1.3 MPa, while the Second long stress induced a more significant decline in SWP to around -3 MPa. Recovery periods allowed SWP values in stressed plants to return close to those of control plants, although significant differences persisted.

The water potential measured at midday generally corresponds to the time of peak water stress, as transpiration reaches its highest rate. Control plants maintained a slightly negative midday water potential, indicating minimal water loss despite maximal evaporative demand. Similar values around -1.5 MPa were observed at the end of the First and Second short stress periods, whereas the Second long stress prompted a much stronger response, with Ψmidday reaching -3 MPa.

3.1.1. SWP-g_s correlation

Figure 7 explores the relationship between stomatal conductance (g_s) and stem water potential (SWP) under varying treatment conditions and across different

time points. The graph reveals a moderate positive correlation between g_s and SWP, with an R^2 value of 0.552. This suggests that stomatal conductance responds positively to higher stem water potential, although the correlation is not particularly strong.

Stomatal conductance is known to be highly sensitive to water availability, as plants modulate g_s to control water loss under stress conditions (Lavoie-Lamoureux et al., 2017; Farquhar & Sharkey, 1982). The data (Figure 2) show that, under stress conditions (purple points), g_s values are generally lower than those observed in the control group, particularly during initial and extended stress periods. This aligns with previous findings by Lavoie-Lamoureux et al. (2017), demonstrating that reduced water availability, as reflected in more negative SWP values, leads to decreased g_s as part of the plant's adaptive water conservation mechanism. Moreover, these results are consistent with Choné et al. (2001), who identified SWP as a reliable indicator of plant hydration status.

Although the g_s-SWP relationship is positive, the correlation remains moderate, likely due to the influence of additional variables. Studies by Farquhar and Sharkey (1982) and Lavoie-Lamoureux et al. (2017) indicate that factors such as experimental design, plant developmental stage, and environmental conditions (e.g., light intensity, humidity, and soil moisture levels) can influence plant water status and introduce variability in g_s responses to water stress.

Throughout the transition from stress to recovery phases, plants adjust stomatal conductance in response to changing water availability. Recovery from water stress is typically accompanied by a gradual increase in g_s, although the extent and timing of this recovery may depend on the severity and duration of the prior stress (Lavoie-Lamoureux et al., 2017). Indeed, plants previously exposed to stress, such as those in the "Recovery-Short Stress" and "Recovery-Long Stress" phases, exhibit SWP-g_s relationships that differ from those of the control group. This observation is further supported by Tombesi et al. (2018), who found that repeated or prolonged stress can alter a plant's capacity to maintain specific SWP values at given levels of g_s, indicating potential adjustments in water regulation mechanisms in response to sustained water stress.

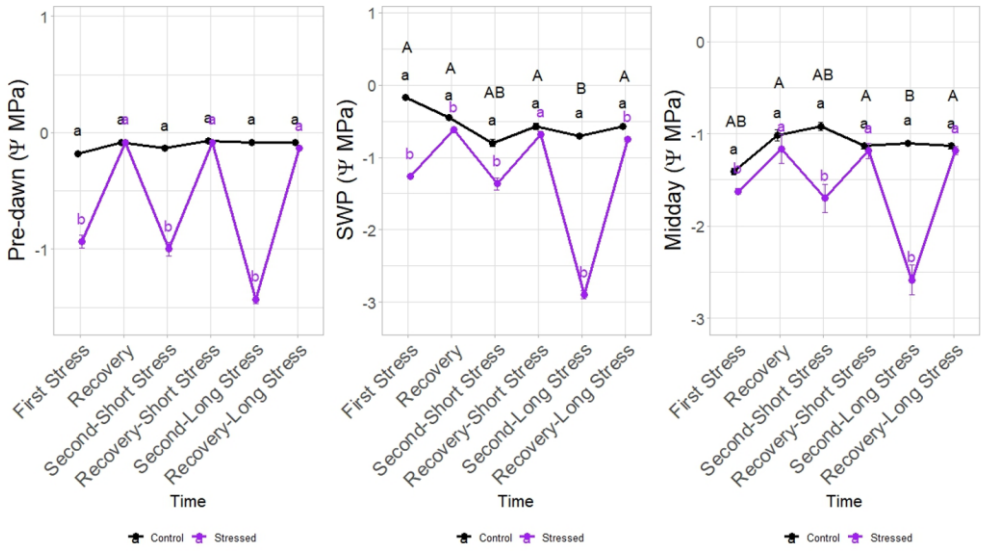

Figure 6. Water potential measurements of grapevine leaves under control (black) and drought-stressed (purple) conditions at pre-dawn, stem water potential (SWP), and midday across multiple stress-recovery cycles. Measurements include the first stress, recovery, subsequent short and long stress events, and their corresponding recovery phases. Significant differences between control and stressed groups at each time point are indicated by different lowercase letters, while significant differences between phases are indicated by uppercase letters.

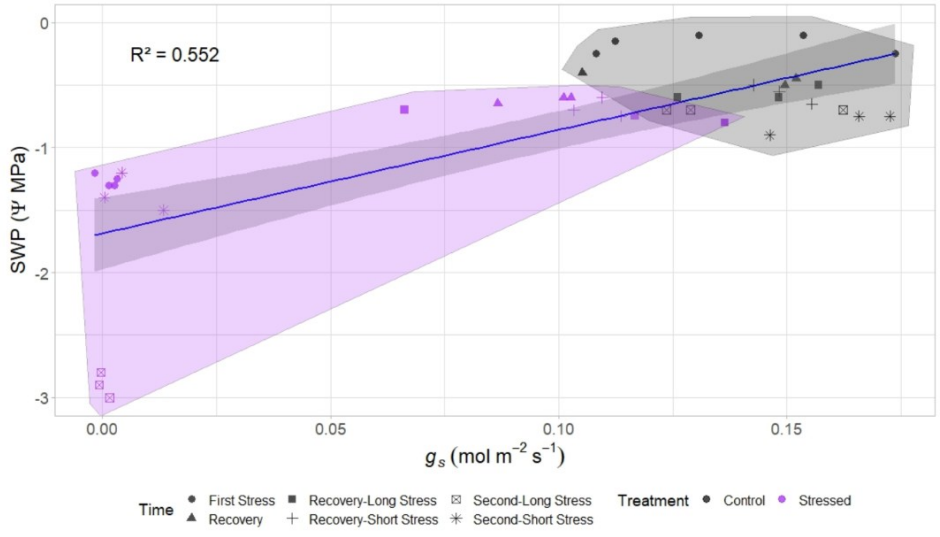

Figure 7. Relationship between stomatal conductance (g_s) and stem water potential (SWP) across different treatment conditions and time points.

3.2 Leaf gas exchange and transpiration

The three graphs illustrate the coordinated responses of plants to fluctuations in water availability, showing adjustments in stomatal aperture, transpiration, and photosynthesis during cycles of stress and recovery. To evaluate plant gas exchange rates, three key physiological parameters were monitored: *A* (net photosynthesis or assimilation), *E* (transpiration), and g_s (stomatal conductance). These parameters are closely interconnected; *A* and *E* reflect changes in g_s over time (Medrano et al., 2002). Stomatal closure is an early plant response to water deficit, aimed at minimizing water loss through reduced transpiration, but it also limits CO_2 uptake. During recovery, stomatal reopening allows for the restoration of gas exchange and water flow, normalizing *E* and *A* levels.

Cifre et al. (2005) established g_s ranges that correspond to different stress intensities: mild (0.40–0.15 mol H_2O m^2 s^{-1}), moderate (0.15–0.05 mol H_2O m^2 s^{-1}), and severe (<0.05 mol H_2O m^2 s^{-1}). Under moderate water stress, reduced photosynthesis results from stomatal closure (Medrano et al., 2003). When stress intensifies, non-stomatal mechanisms further constrain photosynthesis (Flexas et al., 2002).

In well-watered plants, g_s remained stable, ranging from 0.12 to 0.15 mol m^2 s^{-1}. By contrast, water-stressed plants exhibited near-zero g_s during stress periods, indicating that grapevines experienced severe drought conditions leading to full stomatal closure. During the First Stress, stomatal closure occurred sharply and rapidly, a response repeated in the Second Stress, suggesting that the initial stress may have primed the plants for faster stomatal closure in subsequent cycles. In recovery phases, g_s returned to baseline levels.

Control group plants maintained steady net photosynthesis (*A*) values between 9 and 11 μmol m^{-2} s^{-1} throughout the experiment. However, water-stressed plants exhibited a marked reduction in A during stress, dropping to approximately 0–2 μmol m^2 s^{-1} at peak stress. Notably, stressed plants demonstrated an ability to recover between stress periods, with A values returning to near control levels upon rewatering. This contrasts with observations from severe stress studies, where metabolic impairments may

prevent full photosynthetic recovery (Flexas et al., 2004). Here, only the *A* value after the First Recovery phase remained significantly different from the control. Transpiration rate (*E*), representing water lost through stomata, increased gradually through the Second-Short Stress, peaking at around 3 mmol m^2 s^{-1} before stabilizing at 2.5 mmol m^2 s^{-1}. Conversely, stressed plants exhibited a sharp decrease in E during water deprivation, with values approaching 0 mmol m^2 s^{-1}. In recovery phases, E rose again, though it remained significantly below control levels, except after the Second-Long Stress.

Overall, stressed grapevines displayed adaptive, water-conserving strategies-such as stomatal closure and reduced transpiration-that resulted in significant reductions in gas exchange during stress. These adjustments suggest that the plants may have developed enhanced water retention and the capacity to sustain minimal photosynthesis during recovery, indicators of physiological adaptation (Tombesi et al., 2018). This response implies that the initial stress did not permanently damage the stomatal or photosynthetic apparatus, hinting at a form of “drought memory” that strengthens plant resilience in recurring stress scenarios (Tombesi et al., 2018).

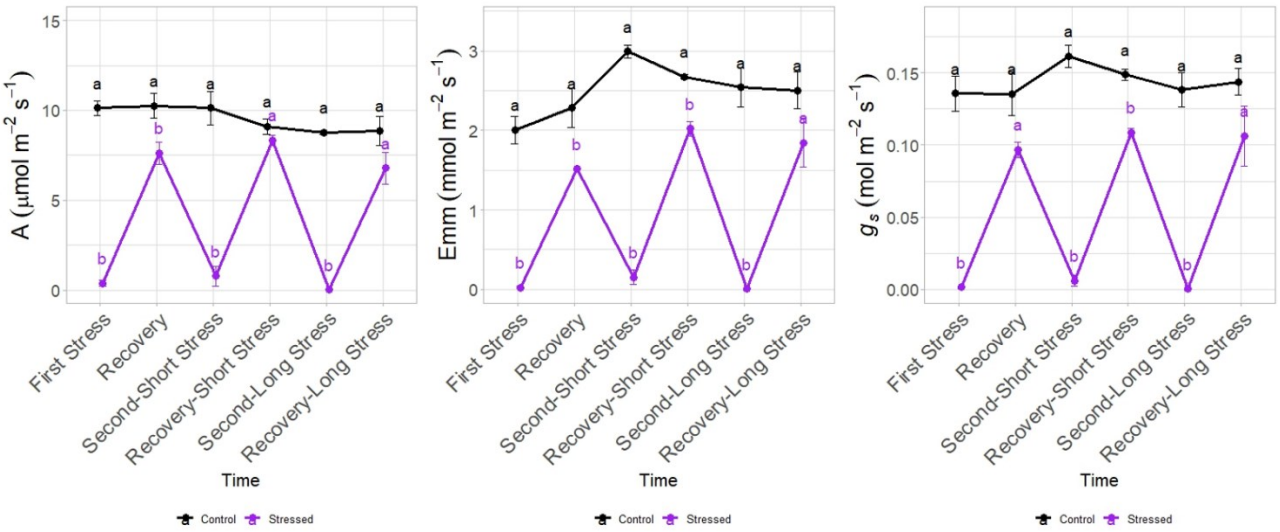

Figure 8. Gas exchange measurements in grapevine leaves under control (black) and drought-stressed (purple) conditions across multiple stress-recovery cycles. Parameters measured include photosynthetic rate (*A*, μmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}), and stomatal conductance (g_s, mol m^{-2} s^{-1}). Different letters indicate significant differences between control and stressed groups at each time point.

3.1.2 *A*- g_s correlation

It is widely recognized that in condition of drought stress stomatal conductance is a central parameter in the regulation of photosynthesis. Drought causes stomata to close, reducing g_s, which consequently decreases A due to limited CO_2 uptake (Escalona et al., 2002; Medrano et al., 2002). In the graph, it is possible to see a strong positive correlation between g_s and A ($R^2 = 0.923$), where both parameters decrease under stressed conditions, as shown by the purple (stressed) data points. The clustering of control points along the trend line suggests that under well-watered conditions, photosynthetic rate is more predictable compared to stressed conditions, where responses vary based on the level and duration of drought (Medrano et al., 2002). Tough in the early to moderate stages of drought stress, photosynthesis is primarily limited by stomatal closure, under severe and prolonged drought non-stomatal factors such as biochemical limitations in the chloroplasts, reduced activity of photosynthetic enzymes, or damage to PSII can become prominent (Flexas and Medrano, 2002; Medrano et al., 2002). This is evident in the variability within the stressed group in the graph (lower purple shaded points). Some data points in the stressed group fall below the trend line, indicating that even when g_s is moderately high, photosynthetic rates are lower than expected. The graph also shows that during recovery, some data points in the stressed group still remain below the control trend line, suggesting that full recovery of photosynthetic activity did not occur likely due to residual non-stomatal limitations caused by the stress severity (Escalona et al., 2002; Medrano et al., 2002).

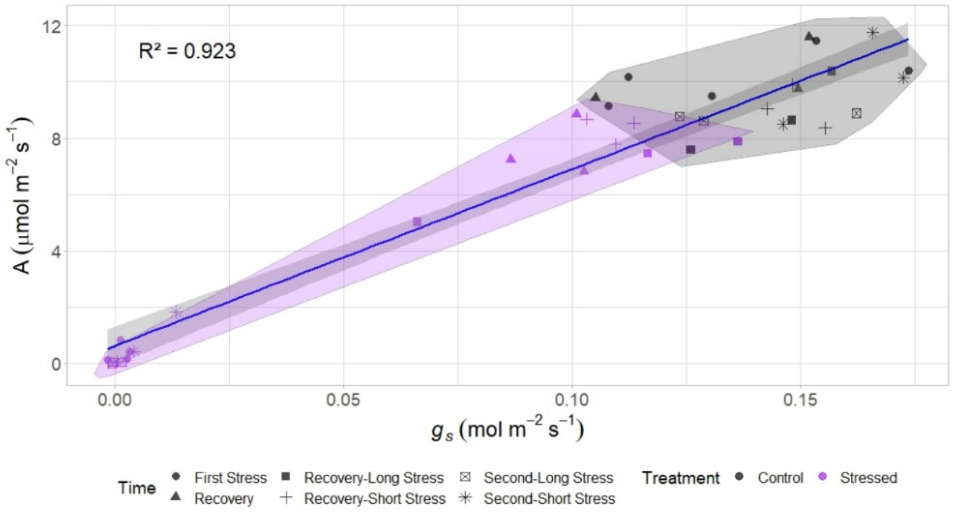

Figure 9. The graph shows the strong positive relationship between g_s (stomatal conductance) and A (photosynthetic rate), as indicated by the R^2 value of 0.923. Control data points are shaded in gray, while stressed data points are in purple.

3.2. Fluorescence

The progression of Fv/Fm, ΦPSII, ETR, and qP demonstrates how water stress significantly reduces photosynthetic efficiency in plants. A decline in these parameters reflects impaired photochemical activity under stress conditions. In contrast, the increase in NPQ (non-photochemical quenching) indicates the plant's protective response to excess light energy, dissipating it as heat to prevent photooxidative damage to the photosystems. This increase in NPQ helps protect the photosynthetic apparatus by safely managing the surplus energy that cannot be utilized in photochemistry under limited water availability. However, this strategy leads to a reduction in photosynthetic efficiency. Maximum quantum efficiency (Fv/Fm) and effective quantum efficiency of PSII (ΦPSII) are well representative of photosynthetic stress and directly correlated to ETR. Well-watered plants were able to maintain likely steady and high values of Fv/Fm. Water stressed plants displayed different responses to the drought cycles. The First Stress had no effect on the maximum quantum efficiency and plants recovered to same values as the Control plants. Second-Short and Long stress determined a considerably reduction of Fv/Fm, stricter during the Second-Long, that did not completely recover indicating that water stress has led the plant to no longer using light energy effectively due to damage to the

PSII. The reduction of ΦPSII during stress correlates with a decrease in the phototransduction capacity of plants. ΦPSII and ETR of Control group plants were relatively stable if not for a slight initial reduction. In contrast, water stressed plants showed a significant decrease in both ΦPSII and ETR during each drought cycle and a limited recovery capability. Water stress prevents the normal functioning of electron transport, slowing down the flow of energy in photosynthesis. This is evident also in the development of qP, a measure of the proportion of open reaction centers in PSII available for light use in photosynthesis. Fewer open reaction centres mean less efficiency in the use of light energy. When qP decreases, an overall reduction in photosynthetic efficiency is observed, as shown by the Fv/Fm and ΦPSII parameters. Stressed plants showed strong fluctuations in qP with a substantial drop during all drought cycles, particularly during the Secon-Long Stress suggesting a worsening of the plant's ability to keep PSII reaction centers open under prolonged stress.

The NPQ values in the bottom right graph represent the non-photochemical quenching levels. Under drought conditions, NPQ tends to increase as an adaptive response to protect against photodamage by dissipating excess energy that cannot be used in photosynthesis due to drought-induced limitations. The NPQ value of the stressed treatment during the Second-Short Stress phase being similar to that of the control could be explained by changes in the efficiency and resilience of the photosynthetic machinery, particularly PSII (Photosystem II), as reflected in the Fv/Fm and ΦPSII graphs. Second-Short Stress represents a relatively brief stress period. Shorter stress likely causes a transient reduction in photosynthetic efficiency without severely impairing PSII efficiency. It is also possible to assess that prior stress and recovery cycles may have “primed” the plants and that PSII might have improved repair mechanisms, with the photoprotective response (NPQ) being minimized to a level similar to control plants. The contrasting NPQ values between Second-Long Stress (low NPQ) and Recovery-Long Stress (high NPQ) in grapevine leaves under drought stress could indicate a shift in the plant’s protective strategy caused by the exposure to a prolonged stress. Low NPQ during Second-Long Stress likely indicates a downregulated or exhausted photoprotective response under sustained stress conditions. Chronic stress

could have led to partial deactivation of PSII. When photosynthesis slows, there's less need for photoprotection because the plant is absorbing less light overall. This could manifest as a reduction in NPQ, as there is less excess light energy to dissipate. High NPQ during Recovery-Long Stress reflects a reactivated photoprotective response as the plant adjusts to improved water availability. The plant may increase NPQ in anticipation of increased light absorption as photosynthesis begins to recover, even if its efficiency remains temporarily low. Plants sometimes exhibit "stress memory" where responses to prolonged drought, such as hormonal signaling (e.g., abscisic acid, ABA), linger even after re-watering (Tombesi et al., 2018). This could maintain a high NPQ as a protective buffer, in case of a sudden return to drought conditions.

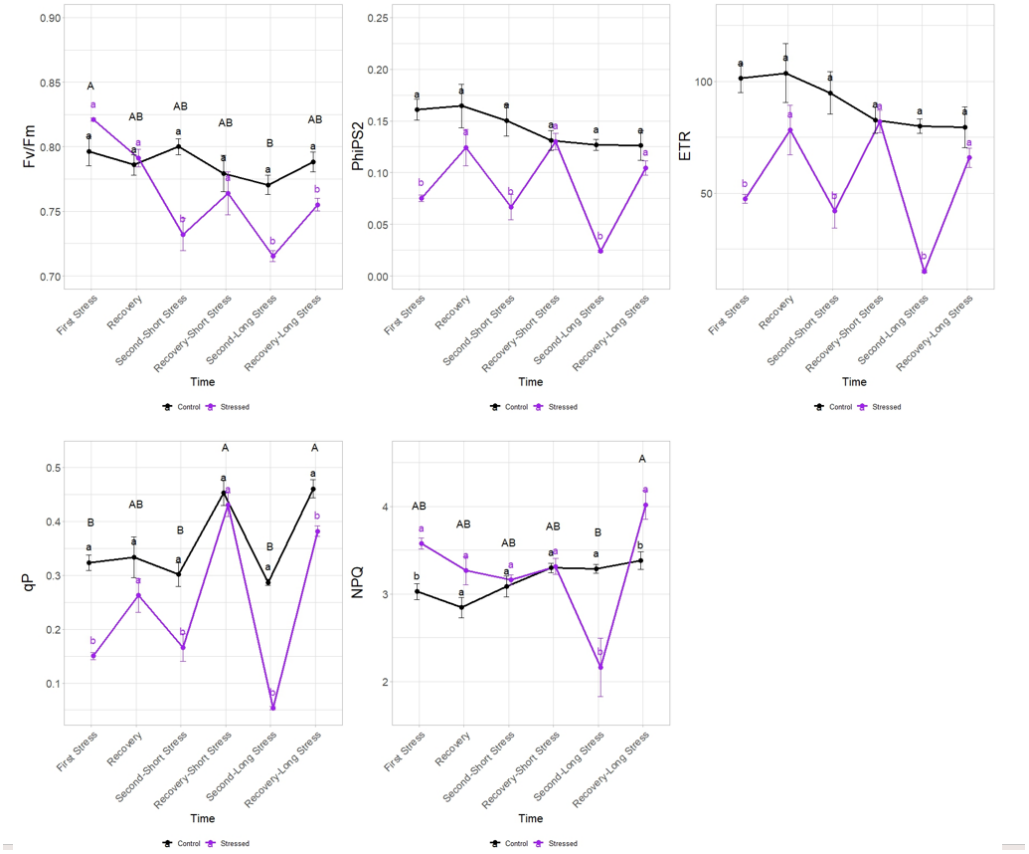

Figure 10. Photosynthetic performance parameters under control (black) and stressed (purple) conditions. Each time point represents a different stage of stress or recovery. The graphs show changes in: maximum photochemical efficiency of PSII (Fv/Fm), effective quantum yield of PSII (ΦPSII), electron transport rate (ETR), photochemical quenching (qP), and non-photochemical quenching (NPQ).

3.3. Vc_{max} and J_{max}

Vc_{max} and J_{max} ($\mu mol\ m^{-2}\ s^{-1}$) provide insight into how the biochemical machinery of photosynthesis is functioning. These parameters describe the maximum rate of carboxylation (Vc_{max}) and the maximum rate of electron transport (J_{max}) and offer key insights in how plants respond to stresses, such as water stress, and how they recover. They are derived from fitting CO_2 response curves using the Farquhar, von Caemmerer, and Berry (FvCB) model for photosynthesis.

The stressed plants show a more substantial drop in Vc_{max}, suggesting that stress impairs Rubisco activity or decreases Rubisco concentration.

In recovery periods, stressed plants seem to recover Vc_{max} close to control levels, as indicated by the statistical analysis. A significant drop in Recovery-Long Stress implies that prolonged stress has a lasting impact on the Rubisco capacity.

Jmax reflects the plant's ability to regenerate RuBP, which is linked to the energy (ATP and NADPH) produced in the light reactions of photosynthesis. Similar to Vc_{max}, J_{max} decreases significantly in the stressed plants, indicating a disruption in electron transport and RuBP regeneration capacity. It is possible to see partial recovery in each stress cycle but longer periods of stress (like Recovery-Long Stress) seem to have a prolonged effect on Jmax.

The correlation between A and J_{max} is well-documented in photosynthetic research. Flexas et al. (1999) highlighted that the A-J_{max} relationship can be stress-dependent, in particular the correlation strengthens under severe stress, which is consistent with the patterns in the graphs.

Both A and J_{max} show reduced values in response to water stress, particularly in stressed plants, indicating that declines in J_{max} might limit A. This aligns with the physiological role of J_{max} as it affects the availability of ATP and NADPH needed for carbon fixation (Flexas et al., 1999).

Again, both A and J_{max} exhibit partial recovery following stress events, with stressed plants maintaining lower values on average compared to control plants.

The graphs suggest that short-term stresses (such as Second-Short Stress) allow for quicker recovery of photosynthetic machinery, as indicated by the non-

significant differences. In contrast, long-term stress (like Second-Long Stress) severely impairs the plant's photosynthetic potential, likely due to damage to Rubisco or the photosynthetic electron transport system.

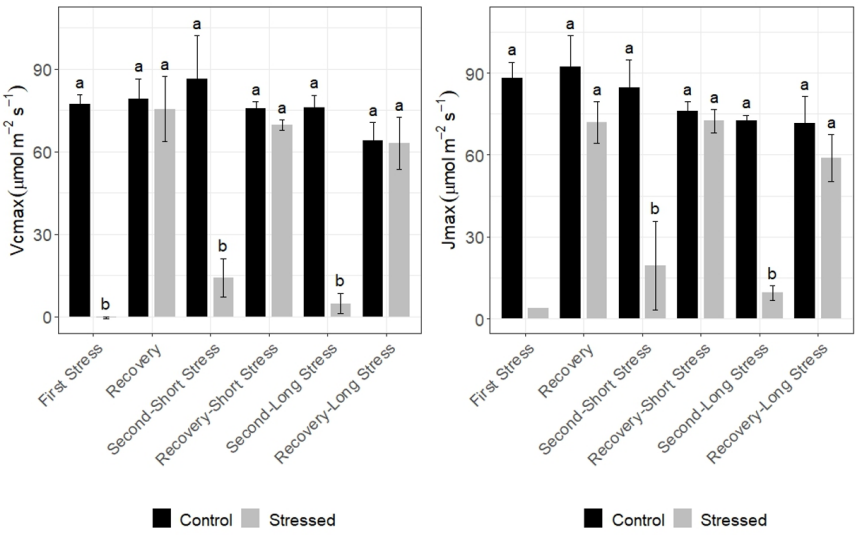

Figure 11. Comparison of the maximum rate of carboxylation (Vc_{max}) and the maximum electron transport rate supporting RuBP regeneration (J_{max}) between control (black) and stressed (gray) conditions across stress and recovery phases. The stressed treatment shows significantly reduced Vc_{max} and J_{max} in particular during the First and Second-Long Stress phases, highlighting the impact of drought on photosynthetic capacity and electron transport efficiency.

3.4. Malic acid concentration

Malic Acid is a key organic acid in grapevine physiology, crucial for respiration, taste, and overall metabolism. Its levels can be influenced by environmental factors, such as drought. Before véraison grape berries accumulate malic acid to make it available as a potential source of carbon for respiration (Ruffner et al., 1982). Malic acid levels start high across all treatments. First stress, that occurred before véraison, did not significantly reduce malic acid synthesis content in water stressed plants but the onset of drought conditions likely disrupted malic acid synthesis. Indeed, after Recovery the Control and First stress showed statistically different levels of malic acid. Geng K. et al., 2022 reported that the synthesis of organic acids is inhibited by heavy pre-veraison water stress. Moreover, malic acid content is usually disrupted by heating at

véraison and ripening stages because it induces higher rates of respiration and increases malic enzyme activity. Indeed, after véraison (when the other two drought cycles took place) malic acid content began to decrease across all treatments. Curiously, the Second-Short Stress caused slightly higher levels of malic acid content in stressed vines possibly due to some form of acclimation or metabolic adjustment to prolonged stress. Malic acid levels then continued to decrease for all treatments. Long-stressed plants exhibited a different pattern, with slightly higher levels of malic acid. The green bars (long stress) show a somewhat different response compared to short stress. This could suggest that long-term stressed plants undergo metabolic adjustments, allowing them to conserve or accumulate malic acid slightly better than short-term stressed plants. The overall malic acid levels seem to stabilize at lower levels across all treatments, suggesting continued stress has in the end depleted malic acid reserves. The differences between treatments and time points are statistically significant, showing how drought affects malic acid metabolism in different ways depending on the type and duration of stress.

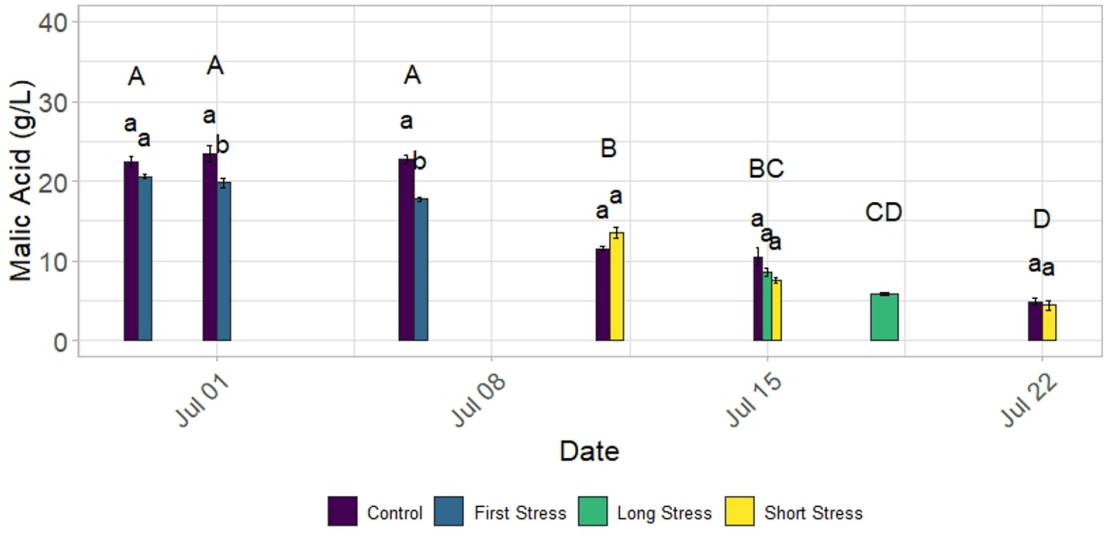

Figure 12. Malic acid concentration (g/L) in grapevine berries over time (June 18 to July 22) under various water stress treatments. Capital letters (A, B, C, D) denote statistically significant differences in malic acid levels between dates, while lowercase letters (a, b) indicate significant differences between treatments on the same date. Initial malic acid levels (pre-veraison) are high across all groups, remaining stable through the beginning of July but declining significantly by the middle of July and continuing to drop by July 22, with variations based on stress treatment, illustrating the impact of water stress on malic acid levels over time.

3.5. Berry calcium concentration

Before veraison, calcium levels are high across all treatment groups, with values around 150 mg/L. The First Stress (July 1) determined a decrease in calcium content but then it restores to levels similar to the control.

Then, after veraion, calcium levels in all treatment groups decrease significantly, averaging around 100 mg/L or less. The trend continues through July 22, with lower calcium levels compared to the initial readings.

Calcium deficiency is exacerbated by water stress because Ca uptake and transport are water-dependent, as calcium moves through the xylem along with water flow (Duan et al., 2022). The drop in calcium levels in stressed grapevines reflects the challenges in calcium transport under limited water conditions.

The calcium concentration in the Long Stress and Short Stress groups generally shows a lower or comparable calcium concentration to the control group, with slight variations.

Notably, on July 15, the Short Stress group has lower calcium levels than the Long Stress group, thus significantly lower than the control. The different responses between Long and Short Stress treatments suggest that the duration and intensity of water stress impact calcium availability in the berries differently. A short but acute water stress might have led to more immediate reductions in calcium concentration in berries compared to longer, sustained stress.

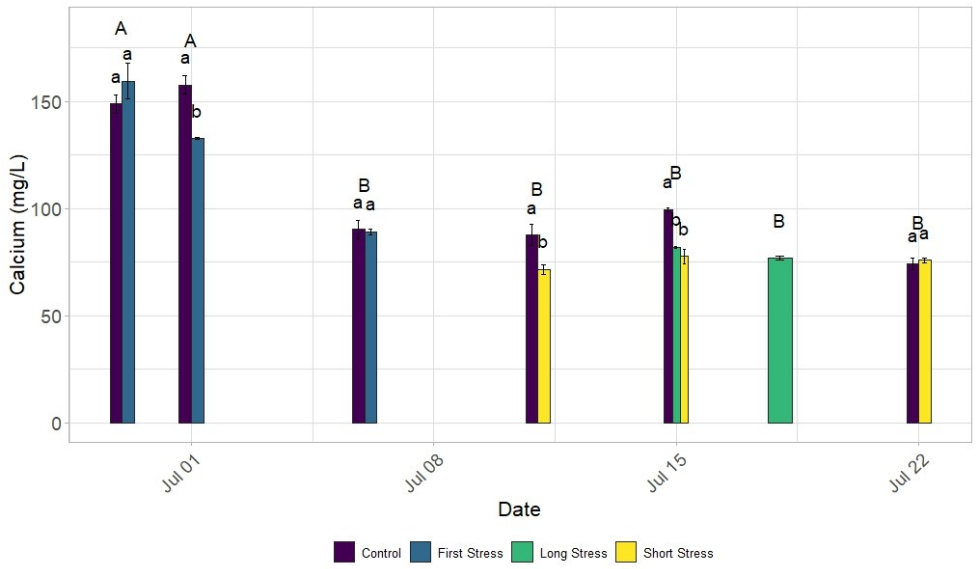

Figure 13. Calcium concentration (mg/L) in grapevine berries measured over time (June 18 to July 22) under varying water stress conditions. Capital letters (A, B) indicate significant differences in calcium levels between dates, while lowercase letters (a, b) denote significant differences between treatments on the same date.

Chapter 4. Conclusion

This study highlights the intricate physiological responses of grapevines to consecutive drought cycles, revealing critical insights into water relations, gas exchange, and biochemical processes. Our findings underscore that while grapevines exhibit an impressive capacity for resilience in the face of short-term drought stress, prolonged exposure leads to significant and potentially lasting impairments in photosynthesis and overall plant health. Key observations include the drastic declines in pre-dawn leaf water potential and midday stem water potential during drought, coupled with substantial reductions in stomatal conductance and photosynthetic rates. The pronounced decrease in fluorescence parameters during the second stress cycle suggests that chronic water deficit significantly hampers photochemical efficiency and energy flow through photosystem II (PSII), potentially leading to photodamage and impaired recovery mechanisms. Notably, the study demonstrates that initial drought episodes may prime grapevines to cope more effectively with subsequent stress through adaptive mechanisms, such as rapid stomatal closure. However, it is crucial to acknowledge that these adaptations are insufficient to sustain normal growth and productivity under extended drought conditions. The study emphasizes the necessity of strategic water management in vineyards, particularly in regions facing water scarcity. By understanding the specific limitations imposed on photosynthesis through the detailed analysis of gas exchange, fluorescence, and biochemical parameters, growers can better inform crop breeding strategies aimed at enhancing photosynthetic performance under stress conditions. Moreover, the observed shifts in malic acid concentration and calcium content further illustrate the metabolic adjustments that occur in response to water stress, providing additional avenues for optimizing nutrient management in drought-affected vines. By leveraging these insights, vineyard managers can implement more effective irrigation practices and cultivar selection to mitigate the adverse effects of drought, ultimately ensuring sustainable grape production and vineyard viability in an increasingly variable climate.

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