EXTENDED ABSTRACT

# Grapevine gas exchange responses to combined variations of leaf water, nitrogen and carbon status – A case of study of fungi tolerant varieties

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## INTRODUCTION

In Mediterranean winegrowing regions, more frequent and intense periods of water (W) deficit and high temperatures due to climate change endanger production in terms of both quantity and quality, as well as the long-term sustainability of vineyards. Meanwhile, winegrowers are facing water scarcity and the pressure to limit inputs (such as nitrogen (N) and phytosanitary products) to reduce pollution and preserve biodiversity. In this context, swift adaptations, including the selection of genotypes and cultural practices, are required.

Rootstock and scion varieties exhibit a diversity of responses to W and N availability (soil, plant), which still need to be better explored (Bota et al., 2001). Additionally, new varieties tolerant to powdery mildew and downy mildew offer a reliable means to limit phytosanitary inputs. For a given scion/rootstock combination, several levers can be implemented to modulate the availability of W and N and/or improve their use efficiency. At the vineyard level, these levers notably rely on the rational supply of water and fertilizer, in conjunction with soil management practices, including the use of cover crops (Celette et al., 2013). At the plant level, practices such as pruning and/or canopy management, which modify the leaf-to-fruit ratio, plant architecture and organ microclimate, are key levers to optimize W and N efficiencies (Downtown et al., 1992). These practices also influence the balance between carbohydrate production through photosynthesis and its allocation for organ growth and metabolism (Zufferey, 2000).

At the leaf scale, the impact of W status on photosynthetic activity has been widely studied, showing that carbon (C) assimilation decreases under W constraint, due to limitations in stomatal conductance (gs). Intrinsic water use efficiency (WUEi), defined as the ratio of net photosynthesis rate to gs, is often estimated and linked to whole-plant WUE (Wilhelm de Almeida et al., 2024). The impact of leaf N status is also a key factor of photosynthetic activity (Prieto et al., 2012). High N status increases the chlorophyll content and enzymes involved in C fixation and reduction in leaves, leading to greater biomass accumulation at the plant level (Vrignon et al., 2022).

Finally the interactive effects of W and N on carbon metabolism both at the leaf and at the plant levels deserves attention. One of the difficulties in addressing the respective impacts of W and N status on C metabolism arises from their interconnections which are difficult to disentangle. Moreover, some feedback processes of C metabolism on W and N status (Verdenal et al., 2021) complicate their comprehension. The interactions between crop load and light environment on C and N metabolisms was studied on apple trees (Ngao, 2021). W deficit was added to the above factors in another study conducted on grapevine (Magali et al., 2024). However, there is still a lack of knowledge on the combined impacts of W, N and carbon status, notably at the leaf scale on photosynthetic activity.

## RESEARCH OBJECTIVES

The objective of this two-year study was to quantify the photosynthesis response of three tolerant grapevine varieties to powdery and downy mildews grown under various agrosystems combining different soil (cover-crop, irrigation & fertilization) and plant (winter & spring prunings) management practices. The study mainly relied on high-throughput phenotyping based on chlorophyll fluorescence, porometry and Near InfraRed Spectrometry (NIRS).These measurements allowed the estimation of photosynthetic efficiency under contrasted combinations of water (predawn water potential), nitrogen (leaf nitrogen content) and carbohydrate (leaf starch and soluble sugar contents) status. From these relationships, the optimal trade-off to maintain sufficient carbon production with minimal water and nitrogen input for each genotype could be identified.

## MATERIAL AND METHODS

### Description of experimental sites

The study was carried out in 2023 and 2024 on three varieties tolerant to downy and powdery mildews: Artaban, Vitis hybrid obtained in the Resdur-1 program, 3159-2-12-B and 3197-81-B, Vitis hybrids obtained by Alain Bouquet. The latter is known to accumulate less hexoses in berries at harvest, producing low ethanol levels wines. In this study the two last varieties are named 3159B and VQDA-G5 respectively.

The vineyards were located at INRAE Pech Rouge experimental unit in Gruissan, France (43.14'/3.14''W), subjected to a hot and semi-arid Mediterranean climate. Year 2023 was very hot and dry (average temperature of 21.0°C and total rainfall of 116.5 mm between April and August) while 2024 was a bit less warm and dry (average temperature of 20.4°C and total rainfall of 152 mm between April and August).

Eight treatments involving various cultural practices at both the plant and plot scales were imposed to alter water, nitrogen, and carbon availability, on ten plants each. For the first treatment, weekly irrigation (representing 87, 55, 98 mm in 2023 and 72, 44, 77 mm in 2024 for VDQA-G5, 3159-B and ARTABAN respectively) and organic fertilization (40kgN/ha were applied for the three genotypes in 2024 only) were implemented, and all inter-row vegetation was removed. For the second treatment, a cover crop was sown in the inter-row in September and removed in mid-April. Irrigation (representing 68, 17, 36 mm in 2023 and 34, 16, 50 in 2024 for VDQA-G5, 3159-B and ARTABAN respectively) was applied every two weeks, while no fertilization was added, in order to create a moderate water and nitrogen constraint. For the last treatment, applied only on VDQA-G5 and ARTABAN, a spontaneous cover crop was left to grow in the inter-row until June. No irrigation or fertilization was applied, creating high water and nitrogen constraints.

These cultural practices were coupled to contrasted plant management practices : vertical shoot positioning (VSP) system with regular canopy height (from 75 to 105 cm) and VSP with reduced canopy height. This reduction in canopy height, representing a decrease of around 30% to 60%, was carried out in late June for the three treatments. Grapevines subjected to minimal pruning were also considered for 3159B, only. These three treatments of high and low VSP and minimal pruning were applied to modify the carbon source:sink balance at the plant scale.

### Plant measurements

Measurements were carried out on monitoring plots of 10 plants per modality, selected through homogeneity criterion based on different variables (overall plant health, trunk circumference, vegetative expression and mean shoot weight, number of buds per plant).

### Photosynthetic activity

From late May to mid-August, every three weeks, on sunny days between 9 and 12:30 AM, ecophysiological measurements were carried out on the 10 plants par modality. Chlorophyll fluorescence and stomatal conductance, known as proxies of leaf photosynthetic activity, were measured with a Licor-600 on 10 to 20 leaves per modality. Concomitantly, CO2 maximal assimilation level (Amax), was measured with ambient CO2 concentration conditions (400 $\mu mol.mol^{-1}$) with a Licor-6800 on 3 leaves per modality, setting climatic conditions of the measurement chamber on optimal ranges for leaf photosynthetic activity (temperature : 27,5°C, VPD : 1.5 to 2 kPa, saturated light conditions : 1800 $\mu mol.m^2.s^{-1}$). A calibration model between Amax and variables measured with L600 (Electron Transport Rate (ETR), Photosystem II activity, stomatal conductance, leaf temperature, VPD) was built to estimate the potential Amax at 27.5°C on all leaves measured only with L600.

### Carbon and nitrogen related traits

Near Infrared Spectroscopy (NIRS) was used to estimate nitrogen (N) and non-structural carbohydrates (NSC) contents. All the leaves measured for ecophysiological measurements were sampled and dried in an oven. For three leaves per modality, a part of the leaf sample was frozen for subsequent classical assays of starch, soluble sugars and nitrogen contents. NIRS measurements were performed with a laboratory near infrared spectrometer equipped with optic fiber on the dried samples from these three leaves. PLS regression models were then built to estimate starch, soluble sugars contents (LS and LSS respectively) and nitrogen per area unit concentrations (LN) from NIRS spectra.

### Water-related traits

Pre-dawn leaf potential (Ψpd) (indicator of plant and soil water status) was measured at each ecophysiological measurement session with a Scholander chamber on one leaf belonging to six different plants of each modality.

### Statistical analysis

All statistical analyses and graphics and calibration models were done with R software. For each variable the genotype and year effect was assessed with two ways ANOVA with interaction. To analyze the effect of carbon, nitrogen and water related traits on photosynthesis activity, regression analyses were performed.

## RESULTS

### Robustness of calibration model to estimate nitrogen, carbon related variables and photosynthesis

PLS predictive models were built between leaf C and N related variables and NIRS spectra or pre-processed NIRS spectra (i.e. smoothed spectra, standard normal variate spectra, and first derivative and second derivative of the spectra). The selected models had $R^2$ of 0.81 and RMSE of 0.01 mg $cm^{-2}$ for nitrogen surface concentration, $R^2$ of 0.92

and RMSE of 3.94 mg $gMS^{-1}$ for starch concentration, $R^2$ of 0.71 and RMSE of 6.83 mg $gMS^{-1}$ for soluble sugars concentration. Predictive calibration model for Amax built from high throughput measurements with L600 was selected and had $R^2 = 0.71$ and RMSE = 2.8 µmol $m^{-2}$ $s^{-1}$.

### Variability of water, nitrogen and carbon status between cultivar and years

The experimental design allowed to obtain a wide range of predawn leaf water (Ψpd), of leaf starch and soluble solids concentrations (LS and LSS, respectively), and to a lower extent of leaf nitrogen surface concentration (LN) (Fig. 1). The values of these variables were notably influenced by the genotype. VDQA-G5 and 3159-B showed a large range of Ψpd, VDQA-G5 reaching the lowest values (-1.43MPa), whereas ARTABAN generally presented higher Ψpd (-1.02Mpa). VDQA-G5 showed lower LN compared to ARTABAN and 3159-B, and ARTABAN had lower variability in the observed valuesty. Regarding carbon-related variables, VDQA-G5 was characterized by a wider range of values both for LS or LSS. ARTABAN reached the lowest values for LSS and G5 the lowest values for LS. Some values estimated by the selected models could be negative due to bias in the estimations of variables coming from NIRS or fluorescence data. Nevertheless, these variables remained adequate to compare the differences between the treatments as the bias in the estimated values did not depend on the treatment that is considered (data not shown).

### Impacts of water, nitrogen and carbon related variables on leaf photosynthetic maximal net activity after veraison

In general, ARTABAN presented higher levels of Amax, with a mean of 14.4 µmol $m^{-2}$ $s^{-1}$ against 11 and 12.3 µmol $m^{-2}$ $s^{-1}$ for VDQA-G5 and 3159-B, regardless of water, nitrogen and carbon status (Fig. 2).

The impact of carbon, nitrogen and water related variables on Amax was studied independently. Ψpd had a positive and highly significant effect on Amax across all genotypes, showing an overall $R^2$ value of 0.21. Similarly, LN showed a strong positive impact on Amax, particularly for VDQA-G5 and 3159-B and to a lesser extent for ARTABAN, with R-squared values of 0.2, 0.41 and 0.06 respectively. LS had a significant positive effect on Amax for ARTABAN and 3159-B only (with low R-squared values of 0.11 and 0.15 respectively) whereas it did not significantly affect Amax for VDQA-G5. LSS also had a significant positive effect on Amax for Artaban and 3159B (with low R-squared values around 0.1) but this effect was not observed for VDQA-G5.

### Interactions between leaf status variables

Ψpd and LN show a strong positive relationship, particularly for VDQA-G5 and 3159-B, with $R^2$ values of 0.37 and 0.39 respectively. Carbon related variables were also positively correlated with Ψpd for every genotype, with $R^2$ values of 0.26 and 0.34 for LS and LSS respectively. LN and LS are significantly correlated only for 3159-B, showing a low $R^2$ value of 0.07. Conversely LN and LSS are positively correlated across all genotypes with an overall $R^2$ value of 0.27.

## CONCLUSION

In this study, the contrasted cultural practices allowed to reach a wide range of combined water, nitrogen and carbon leaf status. Generally, Ψpd and LN showed the strongest impacts on Amax, while LS and LSS had lower impacts. ARTABAN photosynthesis activity was generally higher regardless of W, N or C status, showing a potential higher adaptability to environmental and internal constraints. Statistical methods, such as partial correlations or structural equation modeling, could provide valuable insights to better understand leaf physiology under various combined W, N and C conditions and to identify the optimal trade-off between the reduction in W and N use and leaf photosynthesis.

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## FIGURES

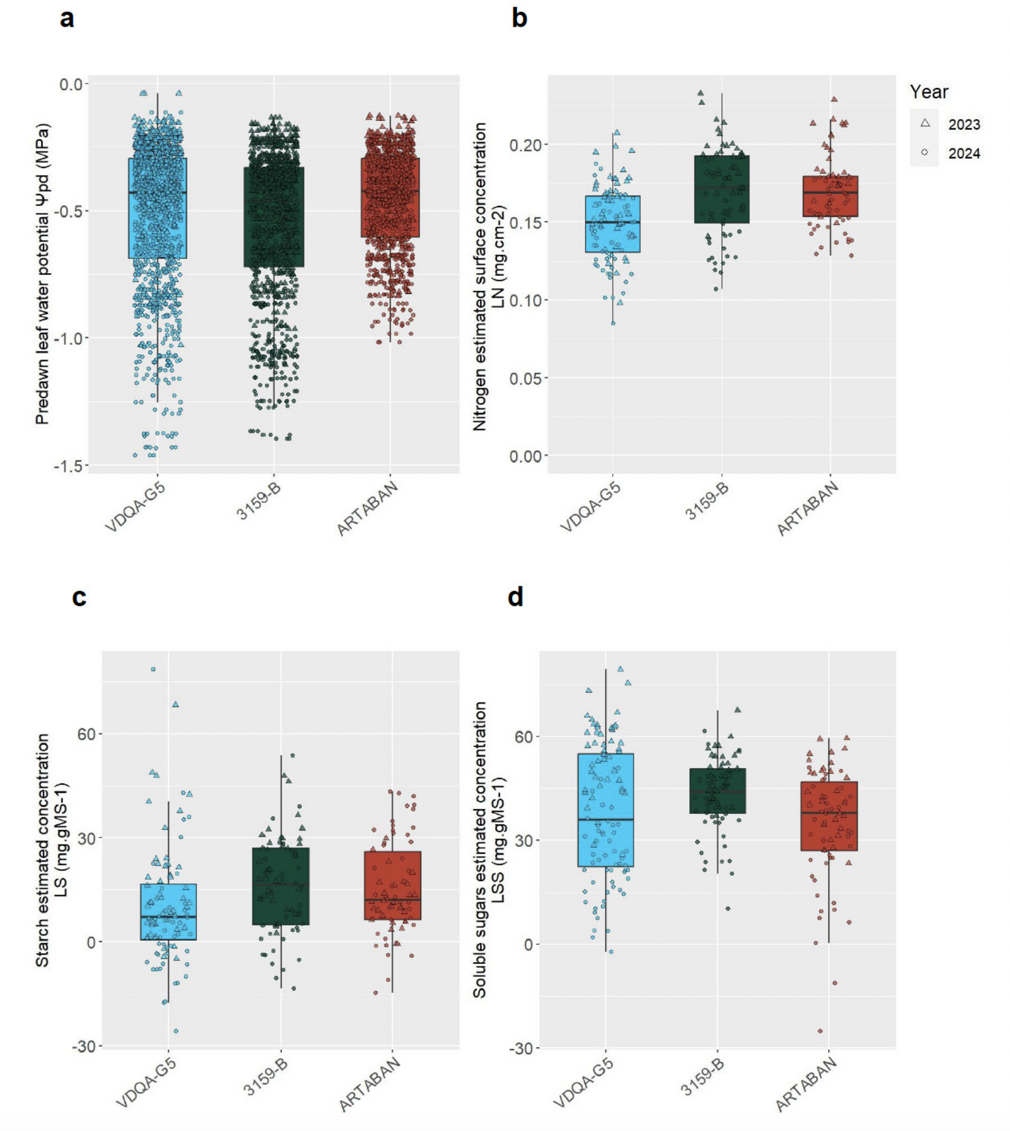


**Figure 1.** Distribution of the variables assessing for water, nitrogen and carbon leaf status in 2023 and 2024 for VDQA-G5, 3159-B and ARTABAN : predawn leaf potential Ψpd for water status (a), nitrogen estimated surface leaf concentration (b) for nitrogen status, starch estimated leaf concentration (c) and soluble sugars estimated leaf concentration (d) for carbon status.

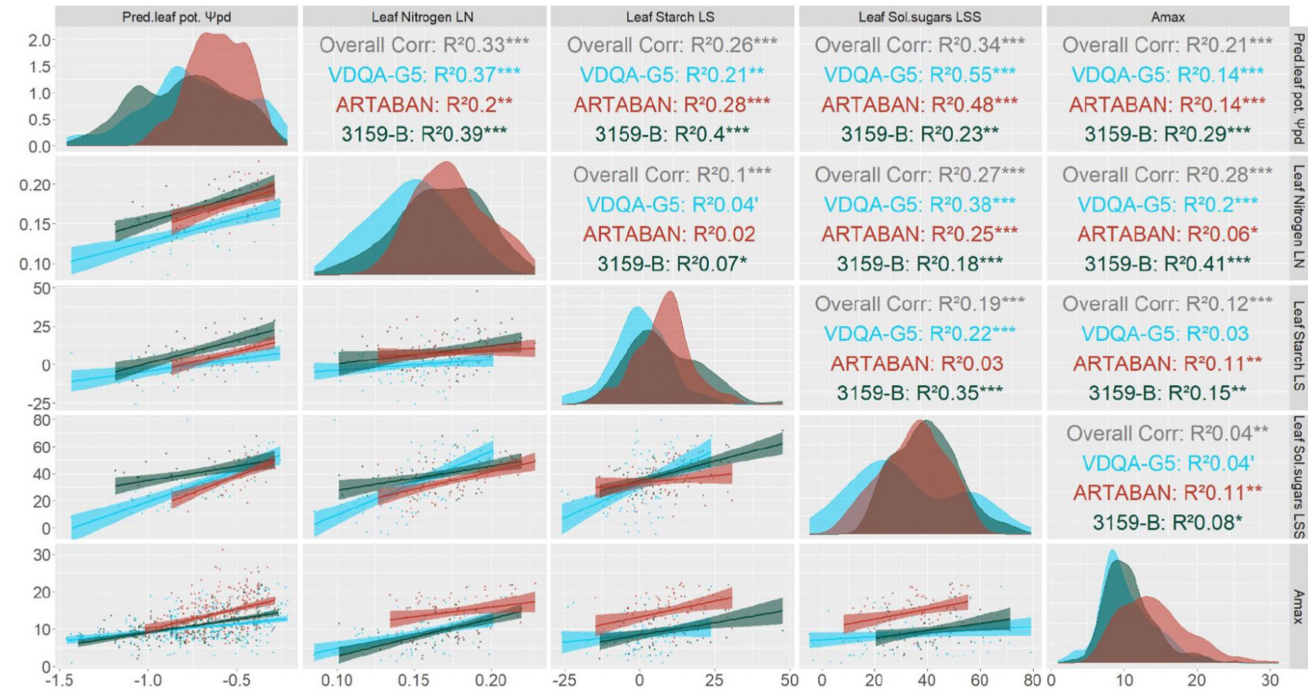


**Figure 2.** Correlations and statistical significativity of relations between photosynthetic maximal net activity (Amax) and leaf water, nitrogen and carbon status, assessed by predawn leaf potential Ψpd, nitrogen estimated surface leaf concentration, starch and soluble sugars estimated leaf concentration respectively, for VDQA-G5, 3159B and ARTABAN after veraison.