OENO One
VINE AND WINE OPEN ACCESS JOURNAL
By IVES International Viticulture & Enology Society

DOI: https://doi.org/10.20870/oeno-one.2023.57.2.7431

OPEN ACCESS

ORIGINAL RESEARCH ARTICLE

Short and long-term acclimation to water status at leaf and plant level of fungus-tolerant genotypes

Luciana Wilhelm de Almeida[1,2], Anne Pellegrino[2], Bénédicte Fontez[3], Laurent Torregrosa[1,2] and Hernán Ojeda[1]

[1] Unité Expérimentale de Pech Rouge (UE 0999), INRAE, 11430 Gruissan, France
[2] UMR LEPSE, Univ Montpellier, INRAE, CIRAD, Institut Agro Montpellier, 2, place P. Viala, 34060 Montpellier Cedex, France
[3] UMR MISTEA, Univ Montpellier, INRAE, Institut Agro, Montpellier, 2, place P. Viala, 34060 Montpellier Cedex, France

This article is published in cooperation with the 22nd GiESCO International Meeting, hosted by Cornell University in Ithaca, NY, July 17-21, 2023.

Guest editors: Laurent Torregrosa and Stefanos Koundouras.

ABSTRACT

Today's viticulture faces the double challenge of reducing chemical input while adapting plant material to future climate conditions. Adopting fungus disease-tolerant varieties appears to be a long-term solution as long as they are performant under abiotic constraints, such as drought. This study aimed to study the effects of water deficit (WD) and characterise the different strategies adopted to cope with drought in six new fungus disease-tolerant varieties selected by INRAE compared to Syrah. During five consecutive seasons (2018 to 2022), a gradient of WD was applied in field conditions and monitored through vine predawn water potential measurements. Grape development was non-destructively tracked by imaging to determine the arrest of berry phloem unloading. All variables were collected at the single plant level. The impacts of WD on leaf gas exchange and intrinsic water use efficiency (WUEi), vegetative development and yield components were assessed, as well as the genotypic sensitivity to WD at the leaf, vegetative and reproductive levels. All variables were negatively impacted by WD, except for intrinsic water use efficiency and total non-structural carbohydrate concentrations. Genotypes were differently impacted by WD at the physiological and vegetative levels, while no genotype-dependent response was observed for most reproductive variables. Generally, genotypes with the highest potential (highest intercept) in a certain variable were also the most sensitive to water deficit (higher slope). The most sensitive genotype regarding physiological and reproductive variables was G5, which showed higher reductions in berry weight, photosynthesis and WUEi, contrasting with Syrah, 3159B and Floreal. In this study, we observed a diversity of strategies to cope with WD in the long term, where some genotypes opted to reduce most variables (G5 and Artaban) or to maintain functioning at higher levels (Syrah and 3176N). Others displayed mixed responses: either reducing vegetative growth more than yield (3159B) or vice-versa (G14).

KEYWORDS: water deficit, grapevine, water use efficiency, yield, quantitative analysis

*correspondence:
luciana.wilhelm-de-almeida@inrae.fr

Associate editor:
Stefanos Koundouras

Received:
25 February 2023

Accepted:
16 April 2023

Published:
17 May 2023

This article is published under the **Creative Commons licence** (CC BY 4.0).

Use of all or part of the content of this article must mention the authors, the year of publication, the title, the name of the journal, the volume, the pages and the DOI in compliance with the information given above.

INTRODUCTION

In many regions, climate change leads to an increase in air temperature combined with a reduction of rainfall, intensifying climatic demand and water deficits (WD) (Cardell *et al*., 2019; IPCC, 2022), which in turn may negatively impact grapevine development, yield and grape composition (Santos *et al*., 2020). In addition, climate change modulates disease pressure, potentially leading to yield and quality losses, besides increasing costs due to more pesticide spraying (Salinari *et al*., 2007; Santos *et al*., 2020) and reducing viticulture acceptability by consumers (Guichard *et al*., 2017). Adopting new fungus disease-tolerant varieties appears as a promising long-term solution to implement smarter viticulture, but unfortunately, little is known regarding the adaptation of these new genotypes to climate fluctuations, such as WD. Indeed, most grapevine breeding programs do not consider drought performance as a primary attribute for selection (Wilhelm *et al*., 2021). This is related to the difficulty in identifying relevant grapevine ideotypes building a drought-performant behaviour of a perennial fruit crop in the short and long terms.

The first strategy adopted by grapevines, under WD, is the reduction of vegetative growth as a way to reduce transpiring surfaces (Simonneau *et al*., 2017), reducing total leaf area by firstly limiting secondary ramification, with later effects on the main shoot (Lebon *et al*., 2006; Pellegrino *et al*., 2005), in addition to reducing leaf expansion (Doupis *et al*., 2016) and ultimately annual vegetative biomass (Zufferey *et al*., 2020).

As the water deficit increases, the vine regulates leaf water losses by closing stomata and, thus, reducing the transpiration rate per leaf area, reducing CO2 assimilation (Flexas *et al*., 2002). Genotypic variability under drought has been widely explored in photosynthesis reduction as well as in the increase in the intrinsic water use efficiency (WUEi), where both are frequently correlated to regulations in stomatal conductance (Bota *et al*., 2016; Gutiérrez-Gamboa *et al*., 2019; Tortosa *et al*., 2019). Stomata closure sensitivity to WD among varieties led to their classification in either iso or anisohydric behaviour (Chaves *et al*., 2010). However, such classification has been debated due to controversial results for the same cultivar and the fact that such behaviour was highly dependent on environmental conditions (Hochberg *et al*., 2018; Martínez-Vilalta and Garcia-Forner, 2017).

Reproductive organs are also importantly affected by seasonal WD, mostly during the same season, mostly leading to reductions in berry weight, with greater impact if WD occurs during the pre-véraison stage (Dayer *et al*., 2013; Levin *et al*., 2020; Mirás-Avalos and Intrigliolo, 2017; Ojeda *et al*., 2001). After véraison, WD can negatively affect reserve replenishment if the photosynthetic activity is not enough to sustain berry sugar demand (Rossouw *et al*., 2017).

In addition to these seasonal processes, the interseasonal effects of WD include a reduced number of clusters per shoot, number of berries per cluster and reserve replenishment (Dayer *et al*., 2013; Guilpart *et al*., 2014; Holzapfel *et al*., 2010; Levin *et al*., 2020). Reserve replenishment in grapevine perennial organs (roots and trunks) is an important process as they play a major role in sustaining spring growth and determining yield potential (Holzapfel *et al*., 2010). Low reserve status has been linked to slower shoot growth, impaired flower formation and poor fruitset (Bennett *et al*., 2005; Vasconcelos *et al*., 2009). In fact, C reserves are determinant in ensuring vineyard longevity, a factor of great importance to be considered for a perennial fruit crop.

Despite many studies addressing WD effects on grapevine development, and grape yield and composition, there is a lack of knowledge regarding the interconnected processes involved in the interannual responses to drought, especially for new fungus disease-tolerant genotypes. In the present study, an original methodological approach was proposed to reduce the experimental noise usually linked to open-field experiments. First, the phenotyping was downscaled to the individual vine level, meaning that each vine was considered a single biological replicate. This was performed to avoid averaging plants displaying different water statuses because of other sources of variations (soil heterogeneity, vegetative volume…) than the imposed water regime. Secondly, the stage of ripe berries was determined as the arrest of phloem unloading into berries when both sugar and water content per fruit are maximum (Alem *et al*., 2021; Bigard *et al*., 2019; Shahood *et al*., 2020). Phenotyping the yield at this precise physiological development point and not at a specific Brix level allowed an accurate estimation of the maximum fruit fresh weight per plant, avoiding the uncontrolled effects of berry shrivelling during technical ripening. Thus, the objectives of the present work were to quantitatively evaluate the effects of WD over four consecutive years on leaf gas exchanges, vegetative development and yield components in a set of six new fungus disease-tolerant genotypes issued from the INRAE breeding program.

MATERIALS AND METHODS

1. Plant material and growing conditions

The experiment was conducted with field-grown vines (2018–2022) at the INRAE unit of Pech Rouge, Gruissan, France (43.14° North | 3.14° East). The panel of genotypes consisted of 6 INRAE fungus disease-tolerant genotypes (*V. vinifera* L. × *M. rotundifolia* hybrids) and the *V. vinifera* var. Syrah, as the control. Artaban and Floreal (red and white fruited genotypes, respectively) (Schneider *et al*., 2019) were grafted onto 110R and planted in 2015. 3176N and 3159B (red and white fruited genotypes, respectively) were grafted onto 140Ru and planted in 2012. G14 and G5 (red and white fruited genotypes, respectively) were grafted onto 140Ru and planted in 2015. The last two scion genotypes carry the sugarless trait, previously described by Bigard *et al*. (2022) and Escudier *et al*. (2017), and it refers to those varieties in which phloem unloading arrest (and berry growth ceases) at a significantly lower sugar concentration than that observed in common wine grape varieties. Both rootstock adopted in the experiment (140Ru and 110R) are widely known to

perform well under dryer conditions to confer similar traits to scion (high vigour and delay growth cycle), besides coming from the same crossing (*V. berlandieri* × *V. rupestris*) (Pl@ntGrape, 2009). All genotypes were present in contiguous plots within the same meso-pedoclimatic unit. No fungicide was applied, except for the Syrah plot that was treated with Champ® Flo (1.2 L/ha, 360 g/L of Cu) and Fluidosoufre® S (5 L/ha, 700 g/L of S). All plots had the same plant density (4400 vines per hectare, 2.5 × 0.9 m) and row orientation (SW–NE) and were managed in VSP (vertical shoot positioning) through the same pruning method.

A total of 30 plants per genotype were individually phenotyped. Of those, from 2019 to 2021, half were manually irrigated (I) from flowering until harvest, while the other half was not irrigated (NI) and exposed to WD in the function of season weather conditions. The water supply of the I vines consisted of 20 L per plant once a week in 2019 and 2020 and twice a week in 2021, corresponding to a total of 96 mm year $^{-1}$ in both former seasons and 184 mm year $^{-1}$ in the latter season (~17 mm week $^{-1}$). During the 2022 season, all plants were under NI treatment.

2. Plant water status

The predawn water potential (Ψp) was measured weekly for all 30 plants per genotype, from flowering until harvest, between 3:00h and 5:00h using a Scholander chamber. The accumulated Ψp (Acc-Ψp) was then estimated for each plant and each year as the area under the curve of Ψp over time, divided by the number of days of the period comprised from flowering to harvest. The Ψp and Acc-Ψp were then used as the reference variable to standardise plot, vine age and rootstock variability.

3. Physiological parameters

Leaf net photosynthesis (A), stomatal conductance (gs) and transpiration (E) were assessed on the day of Ψp measurement from 2018 to 2021. Measurements were performed between 13:00h and 16:00h, targeting plants in the function of their Ψp and taking one exposed leave per plant. An infrared gas analyser system (ADC BioScientific LC Pro System), equipped with a 6.25 cm^2 chamber, at saturated light (≥ 1000 µmol m^{-2} s^{-1}) and a CO_2 concentration of 390 ppm, was used. The WUEinst and WUEi were calculated as the ratio of An and E and An and gs, respectively.

4. Determination of the physiological ripe stage

This stage was defined as the moment at which phloem unloading arrest, the stage at which berries reach maximum water and solute contents (Vmax) (Bigard *et al*., 2019; Shahood *et al*., 2020). During the 2019 and 2020 seasons, berry growth kinetics were assessed at the cluster level by following the increased volume of three clusters per genotype weekly, as described by Torregrosa *et al*. (2008). In 2021, berry growth kinetics was monitored through image analysis by counting the number of pixels per cluster over time using ImageJ software (Lopes and Cadima, 2021). For this purpose, 1 cluster per plant on 6 plants per genotype was photographed each week. Plants were chosen to cover a range of Ψp. Bunches were harvested when their estimated average berry volume or total number of pixels per grape stopped increasing. For the three years, sugar content at harvest reached ca 23° Brix for Floreal, Artaban, 3159B, 3176N and Syrah, and 20º Brix for the sugarless varieties G5 and G14. In the 2022 season, harvest was, thus, targeted from those two º Brix thresholds.

5. Vegetative and reproductive variables

The winter pruning fresh weight (pruning weight), the number of shoots per plant (number of shoots) and individual shoot weight (shoot weight) were assessed for each vine after the cropping season. At harvest, fresh yield and the number of clusters were directly determined. A triplicate of 100 berries per plant was sampled and weighed to assess average berry weight. The average cluster weight and number of berries per cluster were then calculated from the abovementioned variables. Vegetative and reproductive variables were collected from 2019 to 2022.

6. Total non-structural carbohydrates (TNC)

In the spring of 2022, 1 week prior to budburst (DOY 74), trunk samples were collected in a total of 6 plants per genotype, three of which had the lowest (NI) and highest (I) Acc-Ψp during the 2021 season. Samples were lyophilised, and then ground and the non-structural carbohydrates concentrations (starch and soluble sugars) were assayed in a 35 mg sample as described previously (Gomez *et al*., 2007).

7. Statistical analysis

All graphical processing and statistical tests were performed using R studio software. To have comparable ages among genotypes, the first year of measurements (2019) was not considered for those genotypes planted in 2015 (Artaban, Floreal, G14 and G5).

To analyse the irrigation effect on plant variables, a comparison of variables was analysed with analysis of variance (ANOVA, p-value < 0.05), with genotype, irrigation treatment and year as factors.

Accumulated values per genotype and treatment for the variables of number of berries per plant, yield per plant, number of shoots per plant and pruning weight per plant were calculated from 2020 to 2022 and later compared with ANOVA (p-value < 0.05), with genotype and irrigation treatment as factors.

To evaluate the response of each variable to Ψp variations, multiple linear regressions were fitted using the *lm ()* function. First, the physiological variables (gs, A and E), pruning weight and shoot weight responses to Ψp were linearised using log transformation.

The effects of Genotype, Year and their interaction on the intercepts (Ψp = 0) ('Genotype', 'Year', 'Genotype:Year') and Genotype on the slopes ('Ψp:Genotype') were tested for vegetative and reproductive variables. For gas exchange variables, the effects of Genotype on the intercept ('Genotype') and the slope ('Ψp:Genotype') were also tested.

The reproductive variables, including the number of clusters per shoot, number of berries per cluster, cluster weight and yield were fitted in the function of Acc-Ψp from the current ($Year_n$) and previous year ($Year_{n-1}$), while all other variables were fitted with Acc-Ψp from the current year ($Year_n$).

All regressions were performed considering each plant as one biological replicate. The proportion of variance explained by each factor was estimated by dividing its sum of squares by the total sum of squares (η^2).

Multiple comparisons of means and intercepts on one side and of slopes on the other side were performed using the *emmeans* package, with the functions *emmeans()* and *emtrends()*, respectively. All pairwise comparisons were performed with Bonferroni adjustment.

In addition to the regression models, a multivariate analysis (PCA) was used to decipher the genotypic and pluriannual responses to WD (slopes, intercepts) on a subset of plant variables. The PCA was conducted using the *FactoMiner* package, taking the slopes, intercepts (Acc-Ψp or Ψp = 0 MPa) and the values at Acc-Ψp or Ψp = –0.5 MPa.

RESULTS

1. Growing conditions

1.1. Climatic conditions

Over the 4 years of study, the minimum and maximum monthly averages temperatures for 2019, 2020, 2021 and 2022 growing seasons (April to October) were 9.2 °C/31.4 °C, 9.0 °C/29.6 °C, 9.6 °C/20.3 °C and 11.1 °C/32.7 °C, respectively (Figure S1). Extreme temperatures (Daily Tmax above 35 °C) were mostly recorded in the 2022 season, reaching 6 days, while 2021 and 2019 recorded 3 and 2 days, respectively, and in 2020, none. The 2021 and 2022 growing seasons were characterised as the driest, showing the lowest rainfall, 190 mm and 253 mm, respectively (Figure S1). Climatic water balance (∑Rainfall - ∑ET0) ranged from –716 mm and –755 mm from April to October for those two years.

1.2. Phenology

In general, the 2022 season showed the earliest budburst (~ 5 days) and véraison (~ 10 days) dates, while 2020 and 2021 showed the latest harvest and flowering dates, respectively. The 2021 season showed the longest growing season (131 days) (budburst to véraison) but the shorter sugar loading duration (31 days from véraison to Vmax), around 15 days difference from the 2020 and 2022 averages.

Considering all 3 years, budburst occurred the earliest for Artaban and the latest for 3176N and 3159N (p-value < 0.01, Table 1). Despite the 15 days difference in budburst timing between those genotypes, small differences in the time of flowering were observed (5 days maximum, p-value < 0.05, Table 1). However, bigger differences were seen in the dates of véraison (p-value < 0.001) and of the physiological ripe stage (p-value < 0.01) among the genotypes (Table 1). Véraison was first recorded in Artaban, 3176N, 3159B and Floreal, and 12 days later in G5 and G14. The first genotype to reach Vmax and to be harvested was Floreal, and the latest was G14 (+16 days) (Table 1). The time for grapes to reach the physiological ripe stage after veraison ranged from 30 days for G5 to 52 days for Artaban. Overall, the seasons (budburst to harvest) were the longest for G14 and Artaban (179 days) and the shortest for 3159B (153 days) (Table 1).

1.3. Plant water status: accumulated Ψp per day

The Ψp in all genotypes and years decreased during the season, to a greater extent, in non-irrigated vines when compared to irrigated vines (data not shown). In general, the higher Acc-Ψp values were seen in the 2019, 2020 and 2022 seasons (–0.36, –0.33 and –0.42 MPa, respectively), while 2021 had the lowest value (–0.67 MPa) for all genotypes, agreeing with the climatic data previous presented (Table S1, Figure 1). The differences in Acc-Ψp between NI and I plants were the highest in 2021, reaching, on average, for all genotypes –52 % MPa (Table 1). Interestingly, in the 2022 season, where no irrigation was performed, both Floreal and G14 showed an inverse behaviour between I and NI vines, where lower values of Acc-Ψp were seen in previously irrigated vines, although no differences between I and NI vines were observed for other varieties.

From 2020 to 2022 and for the two water treatments, the sum of Acc-Ψp for all genotypes reached the lowest value for Floreal (–1.85 MPa for I to –2.18 MPa for NI) while it was the highest in 3176N and Artaban (–1.03 MPa for I to –1.23 Mpa for NI) (Table 2). Correspondingly, the factor Year explained 46 % of the variation of Acc-Ψp while Genotype and irrigation explained, respectively, 24 % and 5 % of the total variation (Table S1). In general, NI vines showed a decrease of 25 % MPa when compared to I vines. The strongest decrease between NI and I vines was seen for G5 (–38 %) while the least for Floreal (–18 %) (Table 2).

2. Genotype and irrigation effect on reproductive and vegetative developments

Both genotype and irrigation strongly impacted total fruit and canopy production at the end of three years (2020 to 2022). The most and least productive genotypes were 3176N and Floreal, which produced, on average, 10 kg and 4.2 kg per plant, respectively. In fact, Floreal consistently showed the lowest values for both total fruit and pruning weight, while Syrah, 3176N and 3159B showed higher pruning weight when compared to others (Table 2).

The absence of irrigation (NI) consistently decreased the total number of berries (–11 %), yield (–19 %) and pruning weight (–17 %) for all genotypes (Table 2). The only variable poorly impacted by irrigation was the total number of shoots. Indeed, this variable mainly relies on pruning management which regulates the number of regular buds per vine.

In general, the genotype that was mostly affected by irrigation was Floreal, which showed the greatest reductions in the total number of berries per plant (–28 %), total yield (–33 %) and pruning weight (–30 %). In contrast, 3176N and G14 showed lower variations between NI and I vines for those variables

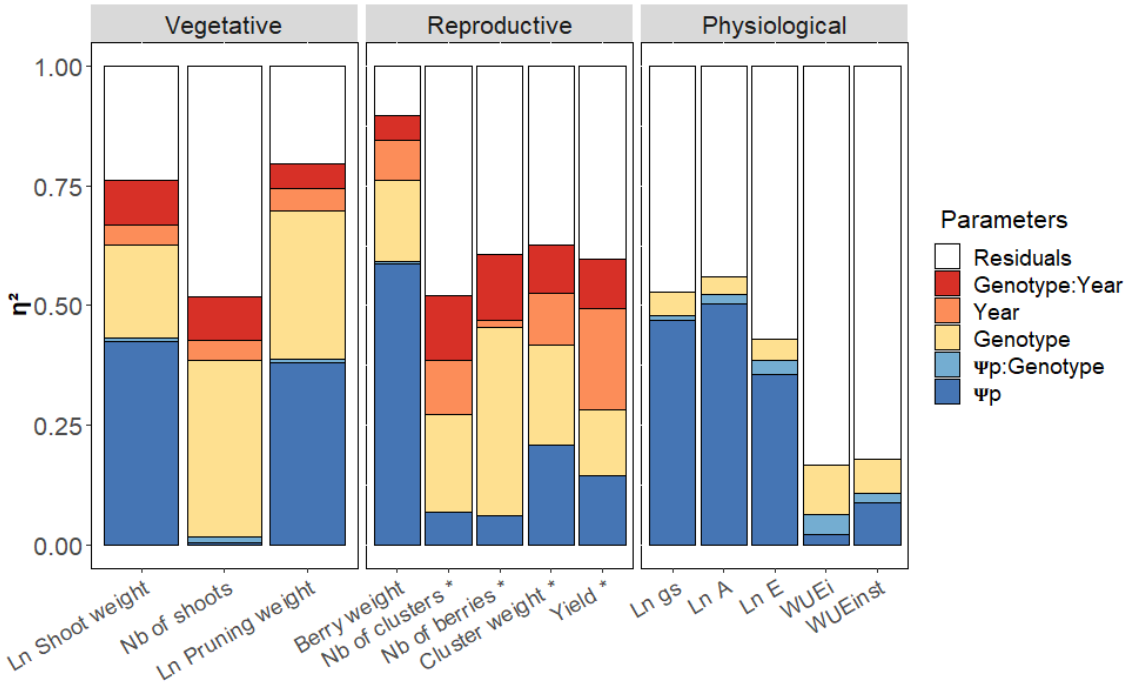


FIGURE 1. Proportion of variance explained (η^2) by each factor and its interactions (p-value < 0.05), on slopes (blue colours) and intercepts (orange colours), in each vegetative, reproductive and physiological variable.

[a]`Ln` indicates linearised variables with natural logarithm. `*` indicates variables fitted in function of the accumulated Ψp over 2 seasons period ($Year_n$+ $Year_{n-1}$).

TABLE 1. Budburst (Bud), flowering (Flo), véraison (Ver) and harvest (Vmax) mean dates (day of the year), growing cycle (budburst to veraison), sugar loading (véraison to Vmax) and whole cycle (budburst to Vmax) duration in days, per genotype and year for 6 fungus disease-tolerant genotypes and Syrah, from 2020 to 2022, Gruissan - France, 2020-2022.

		Phenology				Growing cycle	Sugar loading	Whole cycle
		Bud	Flo	Ver	Vmax			
Genotype	Syrah	85 *ab*	151 *ab*	207 *ab*	246 *ab*	122	39	161
	3176N	88 *b*	151 *ab*	202 *a*	252 *ab*	114	50	164
	3159B	88 *b*	152 *b*	205 *a*	241 *ab*	117	36	153
	Artaban	73 *a*	147 *a*	200 *a*	252 *ab*	127	52	179
	Floreal	82 *ab*	150 *ab*	203 *a*	239 *a*	121	36	157
	G14	76 *ab*	151 *ab*	216 *c*	255 *b*	140	39	179
	G5	85 *ab*	152 *b*	214 *bc*	244 *ab*	129	30	159
Year	2020	86 *b*	148 *a*	206 *b*	252 *b*	120	46	166
	2021	82 *ab*	157 *b*	213 *c*	244 *a*	131	31	162
	2022	79 *a*	146 *a*	200 *a*	245 *a*	121	45	166

*Different letters indicate statistical differences, at 0.05 significance level, among genotypes or year (Bonferroni adjustment) and 'ns' indicates no significance.

(less than 16 % between I and NI), although the differences in Acc-Yp between NI and I were higher for those varieties (–24 % to –31 %) than for Floreal (–18 %) (Table 2).

Despite the global positive effect of irrigation on the vegetative and reproductive variables, not all variables and genotypes were affected in all years. In 2019, except for Syrah, little differences were observed between I and NI vines, which was consistent with the lack of differences in Acc-Ψp during that season for 3176N and 3159B. In 2020, irrigation mainly affected yield components in Artaban, Floreal, G14 and G5 and vegetative variables in Syrah, 3176N, 3159B and Floreal. The driest season, 2021, was the one where most variables showed differences between I and NI vines agreeing with the strong effect of irrigation in Acc-Ψp (Table S1, Table S2 and Table S3).

TABLE 2. Mean and standard deviations (SD) for the accumulated values of pre-dawn water potential (Acc- p), number of berries, yield (kg), number of shoots and pruning weight (kg) per plant, and relative difference (%) between NI and I treatments, for 6 fungus disease-tolerant genotypes and Syrah under irrigated (I) and non-irrigated treatments (NI), from 2020 to 2022, Gruissan - France.

	Acc-Ψp (MPa)				Relative Diff. (%)	Nb of berries per plant (x 1000)							Relative Diff. (%)	Yield (kg)							Relative Diff. (%)			
	I		NI			G	I	G:I	I		NI			G	I	G:I	I		NI			G	I	G:I
	Mean	SD	Mean	SD		***	***	ns	Mean	SD	Mean	SD		***	***	ns	Mean	SD	Mean	SD		***	***	ns
Syrah	-1.55	*0.08*	-1.83	*0.12*	-18	*b*			6.6	*1.5*	5.2	*1.5*	-21	e			10.1	*2.5*	7.2	*2.5*	-29	*cd*		
3176N	-0.96	*0.06*	-1.25	*0.06*	-31	*d*			4.6	*0.9*	4.8	*0.9*	6	cd			10.4	*2.2*	10.0	*2.4*	-4	*d*		
3159B	-1.06	*0.08*	-1.43	*0.08*	-34	*c*			4.6	*1.2*	3.9	*0.8*	-15	bc			*7.8*	*2.3*	6.3	*1.3*	-19	*bc*		
Artaban	-1.03	*0.09*	-1.23	*0.15*	-20	*d*			4.5	*0.9*	4.2	*0.8*	*-7*	bcd			*7.0*	*1.7*	5.8	*1.2*	-18	*b*		
Floreal	-1.85	*0.12*	-2.18	*0.21*	-18	*a*			3.7	*0.7*	2.7	*0.9*	-28	a			*5.1*	*1.0*	3.4	*1.4*	-33	*a*		
G14	-1.42	*0.23*	-1.76	*0.23*	-24	*b*			*5.4*	*1.6*	5.1	*1.4*	-4	de			*8.5*	*3.0*	*7.3*	*2.8*	-14	*bc*		
G5	-0.98	*0.09*	-1.35	*0.19*	-38	*cd*			3.8	*2.1*	3.2	*0.8*	-17	ab			*7.1*	*1.6*	*5.9*	*1.6*	-18	*b*		
Mean	-1.26	*0.34*	-1.58	*0.37*	-25				*4.7*	*1.6*	4.2	*1.4*	-11				8.0	*2.7*	6.5	*2.7*	-19			

	Nb of shoots per plant				Relative Diff. (%)	Pruning weight (kg)							Relative Diff. (%)			
	I		NI			G	I	G:I	I		NI			G	I	G:I
	Mean	SD	Mean	SD		***	ns	*	Mean	SD	Mean	SD		***	***	*
Syrah	52	10	44	*10*	-14	*d*		*	2.5	1.0	2.0	*0.5*	-20	*bc*		*
3176N	46	*8*	48	*8*	5	*d*		*ns*	2.2	*0.6*	1.9	*0.7*	-16	*b*		*
3159B	39	*7*	38	*5*	-3	*c*		*ns*	2.9	*0.6*	2.3	*0.4*	-21	*c*		*
Artaban	28	*3*	29	*4*	3	*a*		*ns*	1.4	*0.3*	1.3	*0.3*	-5	*a*		ns
Floreal	39	*3*	35	*8*	-11	*bc*		*ns*	1.5	*0.3*	1.0	*0.4*	-30	*a*		*
G14	37	*3*	32	*5*	-14	*bc*		*	1.2	*0.5*	1.2	*0.4*	0	*a*		ns
G5	32	*4*	34	*5*	9	*ab*		*ns*	1.0	*0.5*	1.0	*0.3*	-2	*a*		ns
Mean	39	*9*	37	*9*	-5				1.8	*0.8*	1.50	*0.6*	-17			

*Values correspond to the accumulated data per plant and treatment 2020, 2021 and 2022 for all genotypes. No irrigation treatment was performed in 2022. 'Relative Diff (%)' indicates the difference between NI and I treatments (NI-I) / I * 100. 'G', 'I' and 'G:I' indicate the effect of genotype, irrigation and their interaction, respectively. '***', '**', '*' indicates 0.001, 0.01 and 0.05 significance levels and 'ns' indicates no significance. Different letters indicate statistical differences among genotypes (Bonferroni adjustment).

In 2022, although no differences in Acc-Ψp were noticed between I and NI vines in most genotypes (except for Floreal and G14), increases in pruning weight (Syrah, 3176N, 3159B and Floreal) and in yield (Syrah and 3159B) were recorded in I vines. The lower Acc-Ψp for I vines compared to NI for Floreal and G14 resulted in lower berry weight (Floreal) and reduced number of berries per cluster, number of clusters per shoot and cluster weight (G14) (Table S1, Table S2 and Table S3).

No differences among genotypes and irrigation were observed in trunk TNC and starch concentrations during winter (p-value > 0.05) (Table S4). Differently, soluble sugar concentrations varied from 15 mg g^{-1} to 35.5 mg g^{-1} in Syrah and 3176N, respectively (p-value < 0.05). All genotypes showed higher starch concentrations when compared to soluble sugars. The variation in TNC concentrations between I and NI vines was less than 10 % for half of the genotypes, except for Syrah and 3176N, which showed either an increase (20 %) or a decrease (15 %) (p-value > 0.05). Yet NI vines of Syrah, 3176N and 3159B showed higher soluble sugars, while G5 showed lower values when compared to I vines (p-value < 0.05) (Table S4).

3. Genotype, year and Ψp contributions in plant responses to water deficit

All plants variables were then fitted as a function of Acc-Ψp, considering each plant, per genotype and year, as a biological replication to quantify the general effects of each parameter of the study (Ψp or Acc-Ψp, Genotype and Year) on plant responses (Figure 1).

3.1. Overall Genotype and Year effects on plants responses to water deficit

The variations of Ψp and their interactions with the 'Genotype' and 'Year' could explain 52 % to 90 % of variations found for vegetative and reproductive variables (Figure 1). The lowest η^2 values were reported in physiological variables, reaching 17 % and 18 % for WUEi and WUEinst, respectively. Variations of water status (Ψp or Acc-Ψp) explained up to 59 % of variations in berry weight, 40 % in shoot weight and 50 to 36 % of gas exchanges (gs, A and E).

The variations of the slopes due to the Genotype (Ψp:Genotype) explained from 0.5 % in berry weight to a maximum of 4.3 % in WUEi. Interestingly, for those variables depending on the Acc-Ψp from $Year_n$ and $Year_{n-1}$, the effect of genotype on slopes was not significant (variables marked with an '*', Figure 1). The genotype effect on slopes (Ψp:Genotype) is illustrated by the fitted curves of leaf photosynthesis (Figure 2), individual berry weight (Figure 3) and plant pruning weight (Figure 4).

In contrast, the Genotype highly impacted the maximum values of the variables (intercept, Ψp or Acc-Ψp = 0, 'Genotype'), accounting for 3.7 % to 39 % of the variations in photosynthesis (dashed lines, Figure 2) and Nb of berries per cluster, respectively. In general, Year and the interaction Genotype:Year (which are illustrated by the dashed lines in Figures 3 and 4) showed lower contributions in intercept

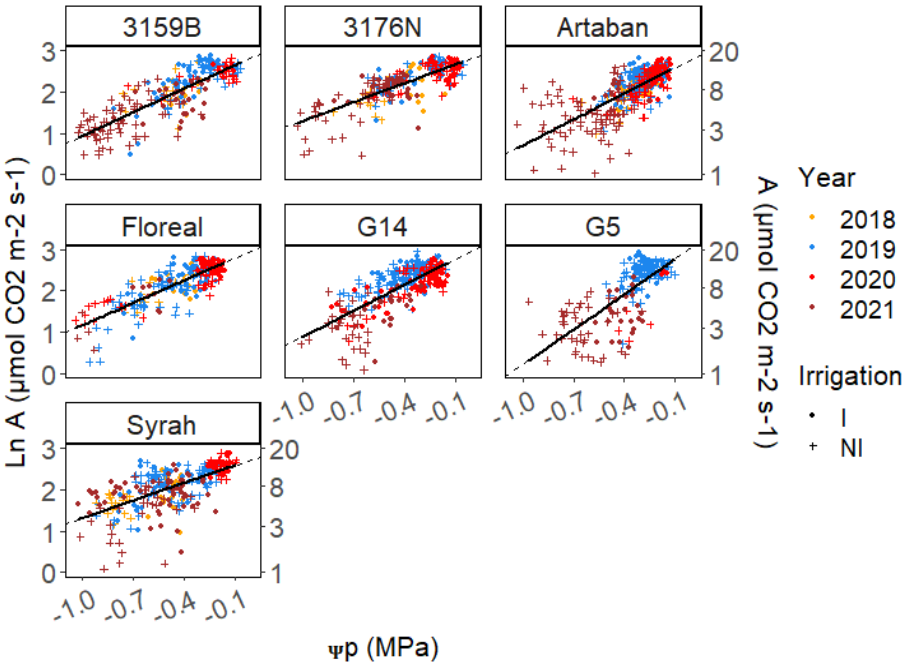


FIGURE 2. Photosynthesis response to Ψp for Syrah and 6 fungus disease-tolerant genotypes, Gruissan -France.

[a]Each point represents one plant measured in that specific year (gray, blue, red and brown colours for 2018, 2019, 2020 and 2021, respectively). Point shapes '●' and '+' indicate irrigated and non-irrigated plants, respectively. Lines are linear regressions (Ln A = **Ψ**p + Genotype + **Ψ**p:Genotype**).** Dashed lines indicate the intercepts for each genotype.

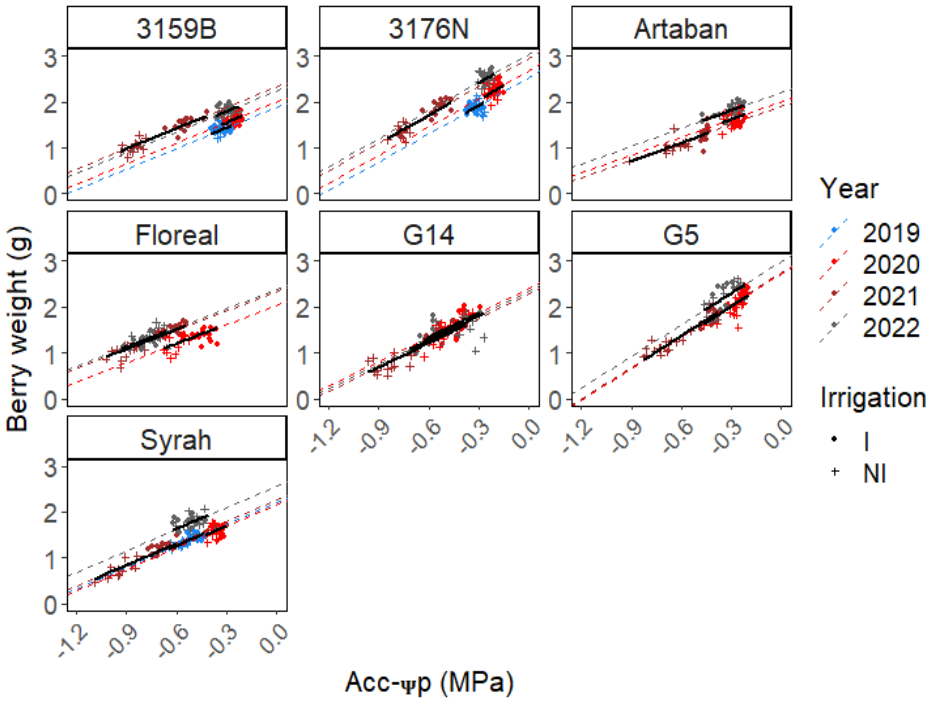


FIGURE 3. Individual berry weight (g) response to accumulated-Ψp (Acc-Ψp) for Syrah and 6 fungus disease-tolerant genotypes, Gruissan - France.

[a]Each point represents one plant measured in that specific year (blue, red, brown and dark grey colours for 2019, 2020, 2021 and 2022, respectively). Point shapes '●' and '+' indicate irrigated and non-irrigated plants, respectively. Lines are linear regressions (Berry weight = Ψp + Genotype*Year + Ψp:Genotype). Dashed lines indicate the intercepts for each year within genotype.

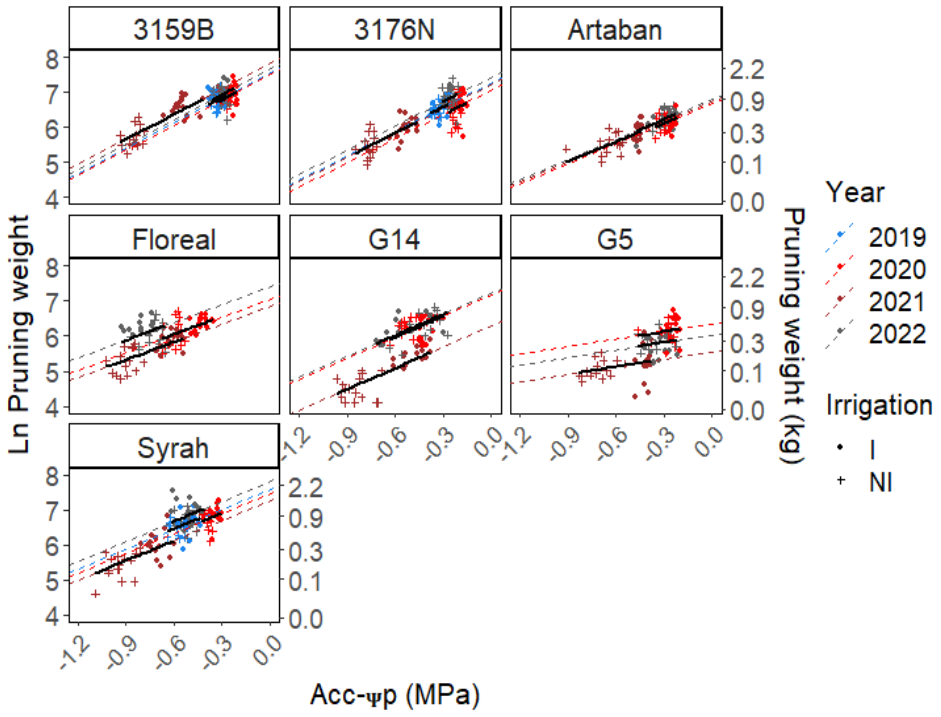


FIGURE 4. Pruning weight (g) response to accumulated-Ψp (Acc-Ψp) for Syrah and 6 fungus disease-tolerant genotypes, Gruissan - France.

[a]Each point represents one plant measured in that specific year (blue, red, brown and dark grey colours for 2019, 2020, 2021 and 2022, respectively). Point shapes '●' and '+' indicate irrigated and non-irrigated plants, respectively. Lines are linear regressions (Ln pruning weight = Ψp + Genotype*Year + Ψp:Genotype). Dashed lines indicate the intercepts for each year within genotype.

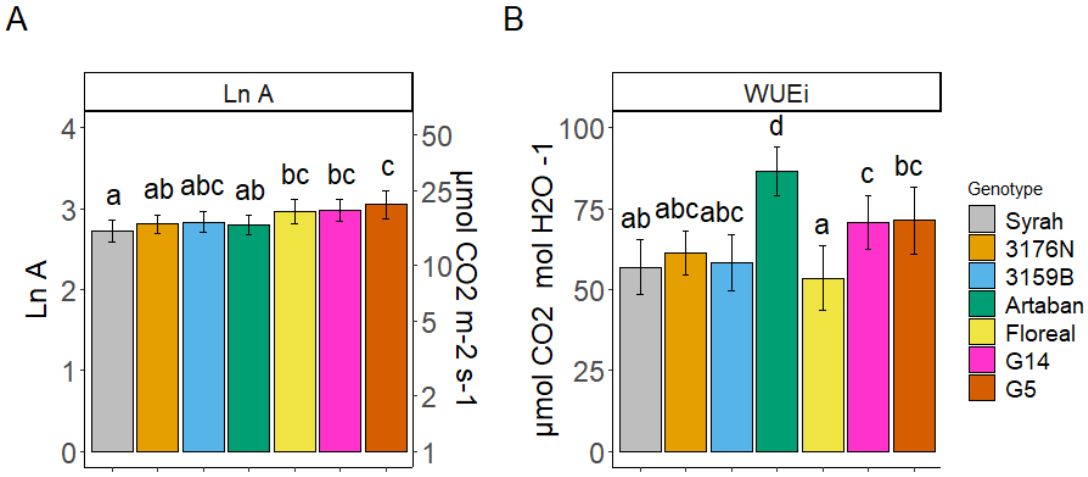


FIGURE 5. Intercept (Ψp = 0) per genotype for photosynthesis (A) and intrinsic water use efficiency, WUEi (B).

[a]Different lowercase letters indicate statistical differences among genotypes (Bonferroni adjustment, p-value < 0.05). The variable of photosynthesis was linearised with natural logarithm, the left and right y-axis display the linearised and real values, respectively.

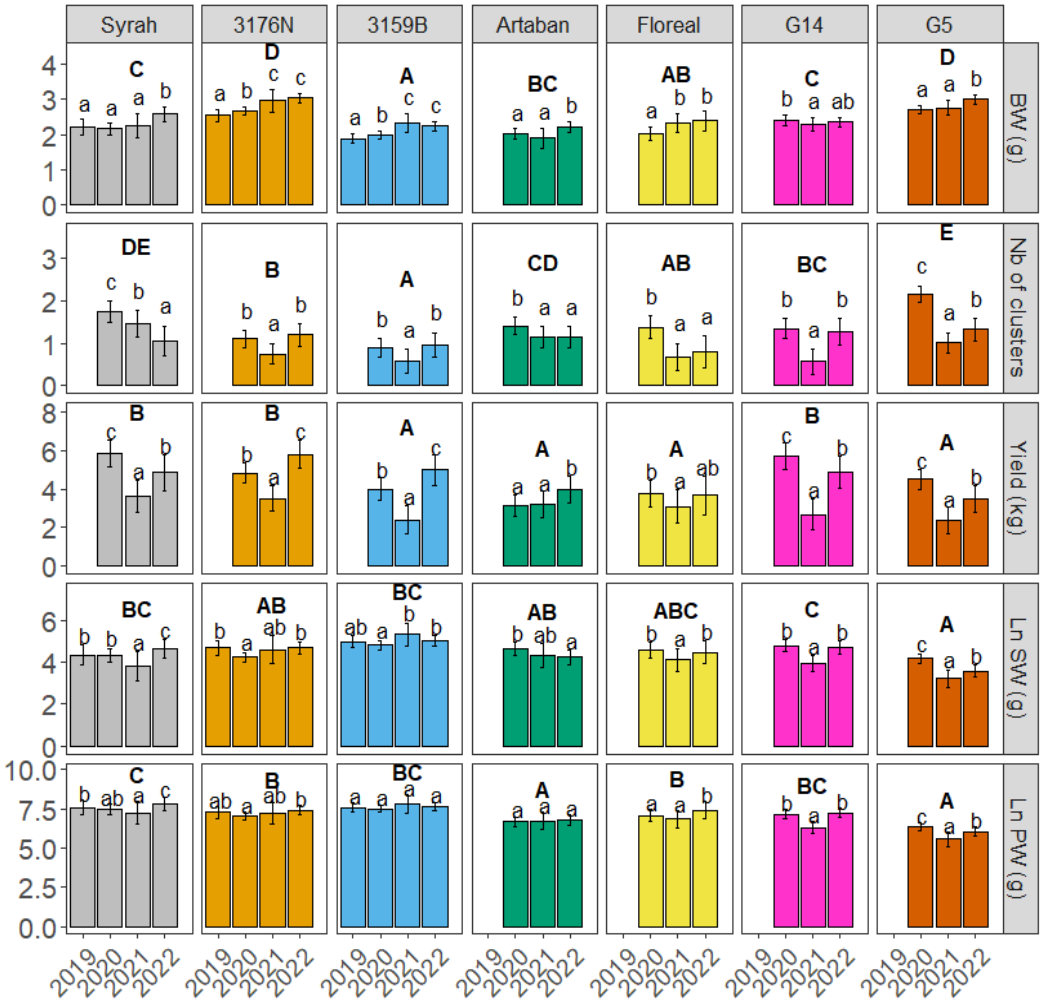


FIGURE 6. Intercept (Ψp = 0) per genotype and year for reproductive variables, berry weight ('BW (g)'), number of clusters per shoot ('Nb of clusters'), yield ('Yield (kg)'), and vegetative variables, Ln shoot weight ('Ln SW (g)'), Ln pruning weight ('Ln PW (g)').

[a]Different lowercase and uppercase letters indicate statistical differences among years within genotypes and among genotypes, respectively (Bonferroni adjustment, p-value < 0.05). The variables of shoot weight and pruning weight were linearised with natural logarithm.

variations than Genotype, except for Yield, which showed the highest η^2 for Year (21 %) (Figure 1).

3.2. Intercepts: the genotype potential in the absence of water deficit (Ψp = 0)

Based on the intercept values (Ψp or Acc-Ψp = 0), genotypes could be divided into different groups regarding their maximum photosynthetic activity (Amax) and WUEi (WUEi max). The first group, including 3159B and 3176N, displayed the lowest Amax (Ln Amax 2.8 µmol CO_2 m^{-2} s^{-1}, Amax 16 µmol CO_2 m^{-2} s^{-1}) and WUEi max (59 µmol CO_2 mol H_2O^{-1}), similarly to Syrah. The second group, formed by G14 and G5, showed higher values of Amax (Ln Amax 3.0 µmol CO_2 m^{-2} s^{-1}, Amax 20 µmol CO_2 m^{-2} s^{-1}) and WUEi max (71 µmol CO_2 mol H_2O^{-1}) (Figure 2, Figure 5A, B). Lastly, compared to Syrah, Artaban presented a similar Amax but higher WUEi max, while Floreal showed a higher Amax but similar WUEi max (Figure 5A, B).

The reproductive and vegetative variables' maximum values per genotype and year are shown in Figure 6. In general, compared to Syrah, maximum values of berry weight (BWmax) were higher for G5 and 3176N (~ 2.9 g), lower for Floreal and 3159B (~ 2.0 g) or similar for Artaban and G14 (~ 2.3 g) (Figure 3 and 6). The number of clusters per shoot and yield were either similar to or lower than those values seen in Syrah. Artaban and G5 showed a similar number of clusters (1.4), and 3176N and G14 had a similar yield (4.7 kg) as Syrah. Ultimately, 3176N, 3159B, Floreal and G14 displayed a lower number of clusters, and 3159B, Artaban, Floreal and G5 showed lower yields than Syrah (Figure 6).

With regards to vegetative variables, the only genotype to show lower vigour (shoot weight) than Syrah (Ln SW 4.4 g, SW 82g) was G5 (Ln SW 3.95g, SW 52g) (Figure 6). The maximum pruning weight was either similar (G14 and 3159B, Ln PW 7.25 kg, PW 1.4 kg) or lower (G5, Artaban, Floreal and 3176N, Ln PW 6.56 kg, PW 0.7 kg) than Syrah (Figure 4 and Figure 6).

Besides the genotype, the intercepts of reproductive and vegetative variables also varied among the years. Variation among years within each genotype was rather low for individual berry weight (Figure 3 and Figure 6) and plant pruning weight (except for G14) (Figure 4 and Figure 6), but variations were greater for individual shoot weight (Ln SW), number of clusters per shoot and yield (Figure 6). In general, the seasons of 2020 and 2022 showed higher intercept values, while 2021 was the lowest (Figure 3, Figure 4 and Figure 6).

3.3. Slopes: the genotype sensibility to variations in Ψp

To compare genotype sensibility to water deficit, we extracted the slopes from a set of plant functioning variables (Table 3). All variables decreased as Ψp dropped, with the exception of WUEi. The most and least sensitive variables were yield and number of clusters per shoot, showing a general reduction of –0.34 kg and –0.1 x 10^{-4} per 0.1 MPa decrease, respectively, for all genotypes (Table 3).

The most sensitive genotype regarding reproductive and physiological variables was G5, which showed the highest reductions in berry weight, photosynthesis (Ln A) and WUEi (–0.23 g, –0.28 µmol CO_2 m^{-2} s^{-1} and –0.81 µmol CO_2 mol H_2O^{-1}, respectively). In contrast, Syrah, 3159B and Floreal showed lower reductions of both berry weight and photosynthesis and higher increase in WUEi (Figure 2, Figure 3 and Table 3).

The highest reductions in vegetative development (Ln shoot weight and Ln pruning weight) were seen in G14, 3159B and 3176N, while the lowest was in G5 (Figure 4 and Table 3).

TABLE 3. Slopes (reductions in absolute values per 0.1 MPa decrease) per genotype (Ψp: Genotype) and general slope (Ψp) for the reproductive (berry weight, number of clusters per shoot and yield), vegetative (shoot weight and pruning weight) and physiological (linearised photosynthesis (A) and, intrinsic water use efficiency (WUEi) variables for 6 fungus disease-tolerant genotypes and Syrah.

Genotype	Berry weight (g)	Number of cluster per shoot	Yield (kg)	Ln Shoot weight (g)	Ln Pruning weight (g)	Ln A (µmol CO2 m -2 s -1)	WUEi (µmol CO2 mol H2O -1)
Syrah	-0.16 *a*	0.07	-0.60	-0.10 *ab*	-0.19 *ab*	-0.14 *a*	3.29 *b*
3176N	-0.20 *ab*	0.06	-0.28	-0.23 *ab*	-0.23 *b*	-0.15 *ab*	2.51 *bc*
3159B	-0.15 *a*	-0.05	-0.41	-0.22 *b*	-0.24 *b*	-0.19 *bc*	0.81 *bcd*
Artaban	-0.13 *a*	0.12	-0.04	-0.15 *ab*	-0.19 *ab*	-0.21 *c*	-2.42 *d*
Floreal	-0.14 *a*	-0.06	-0.27	-0.13 *ab*	-0.17 *ab*	-0.18 *abc*	7.63 *a*
G14	-0.18 *ab*	-0.02	-0.51	-0.20 *b*	-0.20 *b*	-0.21 *c*	1.77 *ns*
G5	-0.23 *b*	-0.01	-0.12	-0.08 *a*	-0.07 *a*	-0.28 *d*	-0.81 *cd*
General slope (Ψp)	-0.17	1,00E-04	-0.34	-0.16	-0.18	-0.14	6.00
Ψp: Genotype	***	ns	ns	**	**	***	***
Ψp	***	***	***	***	***	***	***

* Values indicate reductions (berry weight, Number of clusters per shoot, yield, shoot weight, pruning weight and photosynthesis) and gain (WUEi) per 0.1 MPa decrease. The variables of shoot weight, pruning weight and photosynthesis were linearised with natural logarithm. '***', '**', '*' indicates 0.001, 0.01 and 0.05 significance levels and 'ns' indicates no significance. Different letters indicate statistical differences among genotypes (Bonferroni adjustment).

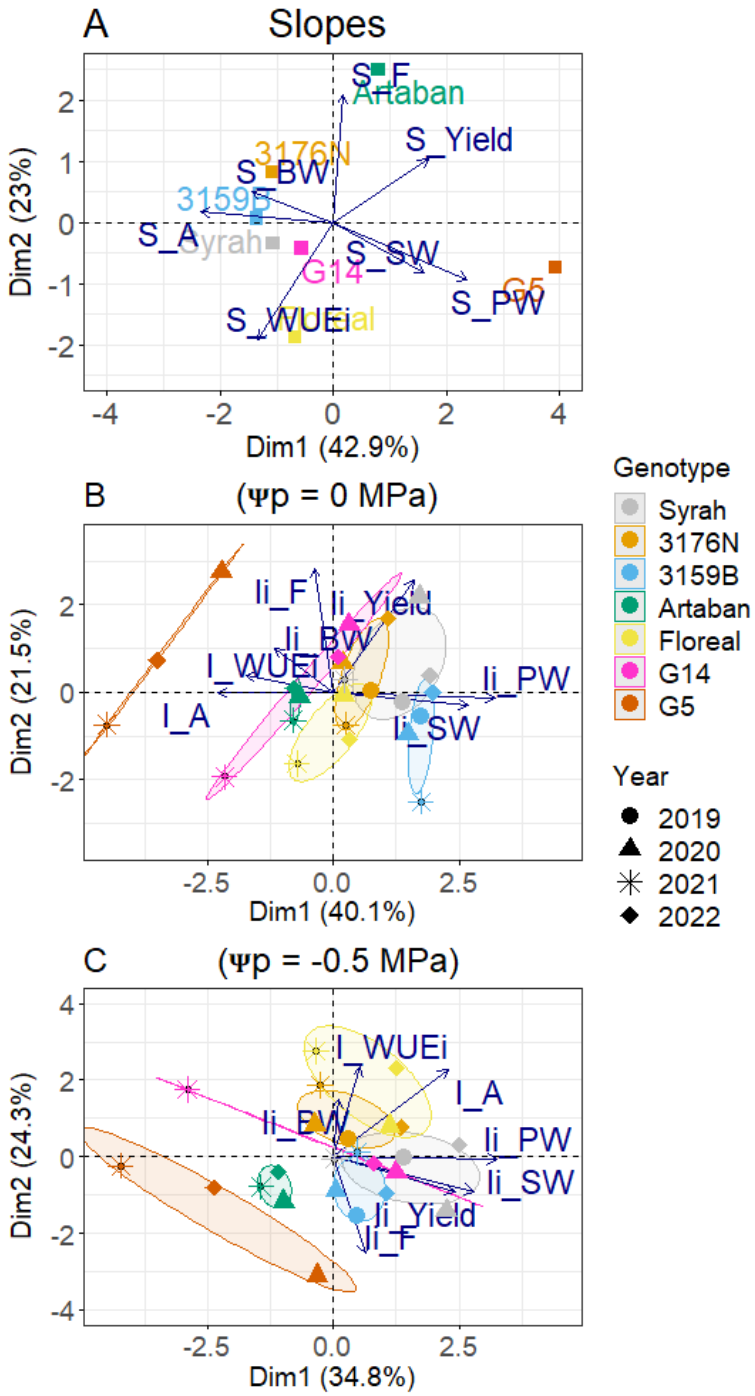


FIGURE 7. PCA of plant responses, slopes (A), intercepts at Ψp = 0 MPa (B) and at Ψp = -0.5 MPa (C), for berry weight (BW), yield, number of clusters per shoot (F), pruning weight (PW), shoot weight (SW), photosynthesis (A) and intrinsic water use efficiency (WUEi) per genotype and year, Gruissan - France.

[a] 'S', 'li' and 'I' stand for slope, intercept per year and intercept for each variable.

Artaban consistently showed intermediate values of slopes, except for WUEi, where it showed the highest reductions (–2.42 µmol CO_2 mol H_2O^{-1} per 0.1 MPa decrease) (Table 3).

Despite differences observed among genotypes in all variables, genotypes showed similar responses to Syrah (except for G5 with higher berry weight reduction). However, regarding photosynthesis response, genotypes were either similar (3176N and Floreal) or more sensitive (3159B, Artaban, G14 and G5) than Syrah (Figure 2 and Table 3). Yet, as regards to WUEi, genotypes showed higher (Floreal), lower (Artaban and G5) or similar (3176N and 3159B) increases under WD as Syrah (Table 3).

3.3. PCA of plant responses to water deficit

Principal components analysis with the slopes and intercepts extracted from the linear fitted models (Table 3, Figure 5

and Figure 6) are shown in Figure 7. The PCA with genotype sensitivity to water deficit (slopes) explained 66 % of the variation, where the first and second principal components (Dim1 and Dim2) accounted for 42.9 % and 23 % (Figure 7A). Dim1 was mainly related to the slopes of photosynthesis (S_A) and pruning weight (S_PW), and Dim2 was related to the slopes of the number of clusters per shoot (S_F) and WUEi (S_WUEi). Genotypes displayed different acclimations to water deficit. The genotype G5 showed a higher sensitivity of berry weight and photosynthesis to water deficit while fewer regulations of canopy development. Floreal and G14 (oppositely from Artaban) showed greater regulations in yield and number of clusters per shoot. Floreal also correlated with WUEi, thus, indicating an increase in this variable as the water deficit increased. Finally, 3159B and 3176N were characterised by a greater sensitivity to water deficit of vegetative development (pruning weight and shoot weight) and lower sensitivity to water deficit of WUEi for 3176N. The genotype G14 was poorly represented on this PCA (Figure 7A).

The PCA with genotype and year effects on the maximum values of plant variables, i.e., under unlimited water supply (intercepts) and under high water deficit (Ψp = –0.5 MPa), explained 60 % and 58 % of the variation, respectively. Where Dim1 and Dim2 accounted for 38.7 % and 21.5 % in the former (Figure 7B) and 34.2 % and 24.3 % in the latter (Figure 7C).

Under unlimited water supply, photosynthesis (I_A) was opposite to the pruning weight (Ii_PW) and shoot weight (Ii_SW), and all three correlated to Dim1, while Yield (Ii_Yield) and the number of clusters per shoot (Ii_F) mostly contributed to Dim2. Genotypes were mainly spread on Dim1, where Syrah and 3159B were opposite to G5, characterised, respectively, by higher vegetative biomass and photosynthetic capacity. G14, Artaban and Floreal were poorly represented by this PCA (central position). Years were mainly related to Dim2, clearly separating 2021 (correlated to lower Yield potential) from the 2019, 2020 and 2022 seasons. Indeed, the 2021 corresponded to the driest season (Figure 7B).

Under high water deficit (Acc Ψp of –0.5 MPa), pruning weight was correlated to shoot weight and yield on Dim1, while WUEi was correlated to A, and both were inversely correlated to the number of clusters on Dim2. Genotypes were mainly distributed on Dim2, while Years were spread on Dim1. Floreal and 3176N were mostly correlated to higher WUEi and A and opposite to G5. Yet, Syrah and 3159B continued showing the highest vegetative development and yield. Artaban was poorly represented by this PCA. As previously remarked, 2021, the driest season, was opposite to most of the vegetative and reproductive variables (Figure 7C).

DISCUSSION

The classical approach of studying a population of plants submitted to the same treatment (I and NI) is a challenging task when working in field conditions where many times, exterior factors are uncontrolled. Such factors as weather annual fluctuations and soil heterogeneity, for example, can contribute to a large part of the effects and cause great variation, making it hard to evaluate the real effect of the treatment applied. To reduce the usual experimental noises linked to open field experiments (weather, soil heterogeneity, plant age, rootstock, root development, etc.), we downscaled the phenotyping unit to the individual vine level (i.e. each vine was considered as a single biological replicate), taking into account the real perception of WD by the plant (Ψp). Plus, to quantify the yield per plant in terms of fresh weight, it is necessary to determine the correct sampling time at the physiological ripe stage, especially when quantifying the effect of WD on fruits. Thus, by harvesting at phloem unloading arrest, we got a better estimate of the plant's maximum water accumulation, avoiding the bias linked to water losses due to berry shrivelling (Alem *et al*., 2021; Bigard *et al*., 2019; Shahood *et al*., 2020).

1. Genotype and variables sensitivity to water deficit

In the present study, most variables (vegetative, reproductive and physiological) were reduced as Ψp decreased, except for some variables that increased or varied poorly (ex. WUE, trunk sugar content). The intensity of the responses to WD was genotype-dependent. Interestingly, the genotypes that showed the highest potential in a certain variable (highest intercept) were also the most sensitive to water deficit (higher slope).

Genotypic variability in photosynthesis and WUEi values under no WD and sensitivity to WD have been widely reported (Bota *et al*., 2016; Chaves *et al*., 2010; Gutiérrez-Gamboa *et al*., 2019; Prieto *et al*., 2010). The values of A (15.3 µmol CO_2 m^{-2} s^{-1} to 21.1 µmol CO_2 m^{-2} s^{-1}) and WUEi (54 µmol CO_2 mol H_2O^{-1} to 87 µmol CO_2 mol H2O^{-1}) under no water constraints are in accordance with those observed by Bota *et al*. (2016), who measured on a set of 23 grapevine cultivars, a range of A and WUEi from 14 µmol CO_2 m^{-2} s^{-1} to 23 µmol CO_2 m^{-2} s^{-1}, and from 60 µmol CO2 mol H_2O^{-1} to 95 µmol CO_2 mol H_2O^{-1}, respectively. G5 and Syrah were the most and least sensitive genotypes regarding CO_2 assimilation, respectively. However, the ones with the highest and lowest Amax. Yet, in all genotypes (except for Artaban and G5), the reduction of water loss was higher than the reduction of CO_2 assimilation, implying better water use. The increase in WUEi under WD was the most important in Floreal, even though it showed the lowest WUEi at Ψp = 0.

Yet studies on the genotype variability of reproductive and vegetative variables sensitivity to WD are less available. Individual shoot weight and plant pruning weight are used as indicators of shoot vigour, canopy size and vine capacity. The decrease for all genotypes in both individual shoot and pruning weight were correlated, indicating that all genotypes reduced vigour and canopy size, to a higher (3159B and G14) or lesser (G5) extent, as a response to WD. Dayer *et al*. (2013) also observed a negative relationship between pruning weight and accumulated midday stem Ψ (Ψs) in Malbec field-grown vines. Reductions in both vigour and pruning weight due to WD (lower Ψp) have been previously reported (Chaves *et*

al., 2007; Zufferey *et al*., 2017, 2020), and it is a strategy adopted by plants to avoid water losses (Simonneau *et al*., 2017). Indeed, it is the first effect observed once vines are imposed to WD, mainly during budburst and véraison. Yet, vegetative growth sensitivity to WD also depends on the developmental process and WD intensity, with secondary branching being firstly impacted (mild to moderate WD) than main shoot (moderate to high WD) (Lebon *et al*., 2006; Pellegrino *et al*., 2005).

In our study, berry weight was the only reproductive variable with genotype-dependent sensitivities, where G5 showed the highest berry weight potential and losses under WD. Similar results were reported by Mirás-Avalos and Intrigliolo (2017), who showed on a panel of 12 grapevine varieties that the varieties (as Bobal and Tempranillo) which had the highest berry weight were also the most sensitive to WD. Berry growth is highly sensitive to WD, mostly when it occurs before véraison, due to an impaired cell expansion (Ojeda *et al*., 2001). The shift of water unloading into berries from the xylem to the phloem at véraison may explain, at least partly, the lower sensitivity of berry weight to WD after this stage (Keller, 2005).

Although all genotypes reduced yield and number of clusters per shoot, such response was not genotype-dependent, as also observed by Levin *et al*. (2020), which might be due to the high variability of yield variable. Petrie *et al*. (2004) also reported reduced yield and number of clusters in plants that were subjected to WD in the previous season. However, the general decrease in the number of clusters per shoot was found to be very low despite water being the most limiting factor in bud fertility and inflorescence formation, mostly around flowering (Guilpart *et al*., 2014; Vasconcelos *et al*., 2009). Thus, the lower effect of the number of clusters per shoot observed in our study can be related to a lower WD at that stage, mainly for years such as 2019 and 2020. Other factors, such as microclimate, including light and temperature (Vasconcelos *et al*., 2009) and plant nitrogen status (Guilpart *et al*., 2014), are also known to impact yield formation. Further studies are needed to assess the ability of this indicator to replace A and gs measurements.

2. Variability in genotypes performance under high water deficit

The pros and cons of the strategy adopted by a genotype to cope with water shortage highly rely on the WD scenario (timing, intensity). To better understand the strategies adopted under water deficit, we compared the genotypes at Acc-Ψp = –0.5 MPa, corresponding to a high to severe WD (Deloire *et al*., 2004), a value which is regularly reported in vineyards in the region, and during the 4 years of the experiment.

At high WD, genotypes were mainly separated by A and the number of clusters per shoot (Figure 7C). Floreal and 3176N maintained the highest carbon assimilation at the same canopy size and number of clusters per shoot. Despite these similarities, at high WD, both genotypes showed contrasting strategies regarding yield, where Floreal showed the lowest values, and 3176N maintained yield at its maximum, mainly by keeping bigger berries. Both Syrah and 3159B maintained higher canopies under high WD, although Syrah maintained A, WUEi and berries at higher values than 3159B. Oppositely, G14 reduced canopy size and berry growth but maintained A and yield at high values.

Finally, G5 and Artaban showed the most distinctive behaviour when compared to other genotypes, displaying the highest slopes and intercepts in berry weight and A and the lowest in shoot weight and pruning weight. Under high WD, such behaviour led to the lowest A, WUEi and canopy development and the lowest yield. Despite both opting to save water on the leaf, canopy and reproductive level, G5 did not compromise berry growth, whereas Artaban did. In the short term, it is possible that the accentuated A regulation could lead to maturity blockage (Schultz, 2000). However, G5 low sugar demand at the berry level (conferred by the sugarless trait (Bigard *et al*., 2022)) could offer advantages under WD, allowing for the reduction of the berry C demand while maintaining berry growth.

From the several variables adopted, we observed that the A measurement was an effective indicator in separating genotypes. However, under field conditions, gas exchange analyses are costly and difficult to manipulate. 'Easy to measure' proxies to vine water status and photosynthetic activity, as following the apex growth and measuring the δC13 in berries, are also widely used in the industry and cover two key phenological stages, before and after véraison, respectively (Bchir *et al*., 2016; Pellegrino *et al*., 2005; Pichon *et al*., 2020). Despite individual shoot weight showing high variability among years, it has the advantages of relating to vine vigour, integrating apex growth dynamics and correlating to Ψp in all genotypes. The δC13, in addition to correlating with Ψp (Chaves *et al*., 2007), WUEi and crop WUE (yield/water consumed) (Bchir *et al*., 2016), it was proposed as a physiological trait for variety selection due to its variation among genotypes (Bota *et al*., 2016).

3. Long-term consequences of genotypes strategies under water deficit

The strategies adopted by each genotype can lead to different outcomes in the long term. The overall low dispersion among years within genotypes (Figure 7C) suggests that the genotypic strategies were rather constant, i.e., the genotype and WD had higher effects on plant responses than other seasonal variations. However, the yield and number of clusters varied between years in all genotypes. In addition, high pruning and shoot weight dispersion were observed for both sugarless genotypes (G14 and G5), indicating a higher interaction with the environment.

In the short term, G5 and Artaban down-regulated both vegetative and reproductive development under high WD. However, in the long term, NI vines showed no reductions in the accumulated pruning weight while showing the same accumulated yield losses as other genotypes (–18 %) (Table 2).

Even though Syrah and Floreal showed contrasting short-term strategies, in the long-term, both were highly impacted by WD, as seen by the highest losses in yield and pruning weight for one of the smallest differences in Acc-Ψp (Table 2). Differently, the maintenance of carbon assimilation under high WD observed in 3176N probably supported its strategy of preserving yield under high WD, as evidenced by the lowest yield losses after 3 years of experimentation (Table 2).

The short-term strategies adopted by 3159B and G14 under high WD led to different outcomes in the long term. After 3 years of experimentation, 3159B equally reduced yield and vegetative growth, as seen by the equivalent relative losses in both variables between NI and I vines (Table 2). While G14 mainly reduced yield (–14 %) with no effect on accumulated pruning weight (Table 2).

TNC concentrations are regulated by genotype, climate and vine management (Holzapfel *et al*., 2010; Pellegrino *et al*., 2014; Rossouw *et al*., 2017; Zufferey *et al*., 2012), with higher concentrations being mainly seen in roots (Zapata *et al*., 2004). Despite genotypes reaching different Ψp, no differences were seen neither among genotypes nor between I and NI vines (Table S4). The lack of differences in WD on TNC concentrations agrees with previous studies (Dayer *et al*., 2013), despite other authors observing reductions in starch concentrations in either potted (Savi *et al*., 2019) or field-grown vines (Dayer *et al*., 2013; Pellegrino *et al*., 2014). However, one could expect that the total TNC storage in trunks could be impacted by WD. As plant vigour decreases under WD, the total trunk volume could be negatively impacted and ultimately reduce total reserve storage.

Low C reserve status at the beginning of the current season has been linked to reduced shoot growth and total pruning weight, besides negatively impacting flowering and productivity (Bennett *et al*., 2005). In the present study, the reductions observed at the canopy and reproductive level were mainly linked to seasonal WD acclimation rather than different reserve C status at the beginning of the season (Table S4).

CONCLUSION

In the present study, we focused on general physiological, vegetative and yield responses to WD in a rather integrative approach, considering the general effects of seasonal, interannual and multi-annual WD. All variables were negatively affected by decreases in Acc-Ψp or Ψp, with the exception of WUEi and total non-structural carbohydrates. Among these variables, we identified those that were mostly explained by Acc-Ψp or Ψp, such as berry weight, gs, A, E, shoot weight and pruning weight, from those related to season variations, such as yield, cluster weight and the number of clusters per shoot. Physiological and vegetative variables showed great variability regarding genotype sensitivities to WD, while from reproductive variables, only berry weight responses were found to be genotype-dependent.

This report provides data to rank genotypes regarding their sensitivities to water deficit and better understand their strategies to cope with it. It was possible to distinguish those genotypes that show a similar physiological behaviour under WD to Syrah (3176N and Floreal) from those showing a contrasting response (G5, G14, 3159B and Artaban). However, even if two contrasting groups were formed regarding their physiological response to WD when considering integrative variables, such division became blurred where some genotypes, such as Artaban and G5, reduced all functioning variables while others maintained functioning at higher values, such as 3176N and Syrah. However, others displayed combined responses, such as reducing reproductive development more than vegetative (G14) and vice-versa (3159B) or reducing both vegetative and reproductive functioning but keeping gas exchanges at higher values, such as in Floreal. This highlights the complexity when integrating whole plant seasonal and interannual regulations and multi-annual acclimation to WD.

In the same study, we also studied responses to WD of the same genotypic panel at the fruit level. For this, during the 4 years of experiments, we have analysed, at a single plant level, the grape composition in main primary (sugars, organic acids, nitrogen compounds), secondary metabolites (anthocyanins, aromatic glycosylated precursors), major cations and the variations of carbon isotopic incorporation in sugars (δC13). This will provide additional information about the effect of WD on fruit physiology and the accumulation of compounds of interest by this panel of new fungus disease-tolerant genotypes.

ACKNOWLEDGEMENTS

The authors thank Emmanuelle García-Adrados for her essential technical support and management of the experimental fields and the students Emilie Barnett, Martina Baliani and Bruno Izquierdo for their support in data acquisition. The study was funded by the Occitanie Region, the Conseil Interprofessionnel des Vins du Languedoc—CIVL, the International Organisation of Vine and Wine—OIV, INRAE and Institut Agro Montpellier (France).

REFERENCES

Alem, H., Ojeda, H., Rigou, P., Schneider, R., & Torregrosa, L. (2021). The reduction of plant sink/source does not systematically improve the metabolic composition of Vitis vinifera white fruit. *Food Chemistry*, *345*, 128825. https://doi.org/10.1016/j.foodchem.2020.128825

Bchir, A., Escalona, J. M., Gallé, A., Hernández-Montes, E., Tortosa, I., Braham, M., & Medrano, H. (2016). Carbon isotope discrimination (δ13C) as an indicator of vine water status and water use efficiency (WUE): Looking for the most representative sample and sampling time. *Agricultural Water Management*, *167*, 11–20. https://doi.org/10.1016/j.agwat.2015.12.018

Bennett, J., Jarvis, P., Creasy, G. L., & Trought, M. C. T. (2005). Influence of Defoliation on Overwintering Carbohydrate Reserves, Return Bloom, and Yield of Mature Chardonnay Grapevines.

American Journal of Enology and Viticulture, *56*(4), 386–393. https://doi.org/10.5344/ajev.2005.56.4.386

Bigard, A., Romieu, C., Ojeda, H., & Torregrosa, L. J.-M. (2022). The sugarless grape trait characterised by single berry phenotyping. *OENO One*, *56*(3), Article 3. https://doi.org/10.20870/oeno-one.2022.56.3.5495

Bigard, A., Romieu, C., Sire, Y., Veyret, M., Ojéda, H., & Torregrosa, L. (2019). The kinetics of grape ripening revisited through berry density sorting. *OENO One*, *53*(4), Article 4. https://doi.org/10.20870/oeno-one.2019.53.4.2224

Bota, J., Tomás, M., Flexas, J., Medrano, H., & Escalona, J. M. (2016). Differences among grapevine cultivars in their stomatal behavior and water use efficiency under progressive water stress. *Agricultural Water Management*, *164*, 91–99. https://doi.org/doi.org/10.1016/j.agwat.2015.07.016

Cardell, M. F., Amengual, A., & Romero, R. (2019). Future effects of climate change on the suitability of wine grape production across Europe. *Regional Environmental Change*, *19*(8), 2299–2310. https://doi.org/10.1007/s10113-019-01502-x

Chaves, M. M., Santos, T. P., Souza, C. R., Ortuño, M., Rodrigues, M. I., Lopes, C. M., Maroco, J. P., & Pereira, J. S. (2007). Deficit irrigation in grapevine improves water-use efficiency while controlling vigour and production quality. *Annals of Applied Biology*, *150*(2), 237–252. https://doi.org/10.1111/j.1744-7348.2006.00123.x

Chaves, M. M., Zarrouk, O., Francisco, R., Costa, J. M., Santos, T., Regalado, A. P., Rodrigues, M. L., & Lopes, C. M. (2010). Grapevine under deficit irrigation: Hints from physiological and molecular data. *Annals of Botany*, *105*(5), 661–676. https://doi.org/10.1093/aob/mcq030

Dayer, S., Prieto, J. A., Galat, E., & Peña, J. P. (2013). Carbohydrate reserve status of Malbec grapevines after several years of regulated deficit irrigation and crop load regulation. *Australian Journal of Grape and Wine Research*, *19*(3), 422–430. https://doi.org/10.1111/ajgw.12044

Deloire, A., Carbonneau, A., Wang, Z., & Ojeda, H. (2004). Vine and water: A short review. *OENO One*, *38*(1), 1. https://doi.org/10.20870/oeno-one.2004.38.1.932

Doupis, G., Bosabalidis, A. M., & Patakas, A. (2016). Comparative effects of water deficit and enhanced UV-B radiation on photosynthetic capacity and leaf anatomy traits of two grapevine (Vitis vinifera L.) cultivars. *Theoretical and Experimental Plant Physiology*, *28*(1), 131–141. https://doi.org/10.1007/s40626-016-0055-9

Escudier, J.-L., Bigard, A., Ojeda, H., Samson, A., Caillé, S., & Romieu, C. (2017). De la vigne au vin: Des créations variétales adaptées au changement climatique et résistant aux maladies cryptogamiques. *Revue des Oenologues*, *165*, 6.

Flexas, J., Bota, J., Escalona, J. M., Sampol, B., & Medrano, H. (2002). Effects of drought on photosynthesis in grapevines under field conditions: An evaluation of stomatal and mesophyll limitations. *Functional Plant Biology*, *29*(4), 461. https://doi.org/10.1071/PP01119

Gomez, L., Bancel, D., Rubio, E., & Vercambre, G. (2007). The microplate reader: An efficient tool for the separate enzymatic analysis of sugars in plant tissues - Validation of a micro-method. *Journal of the Science of Food and Agriculture*, *87*, 1893–1905. https://doi.org/10.1002/jsfa.2924

Guichard, L., Dedieu, F., Jeuffroy, M.-H., Meynard, J.-M., Reau, R., & Savini, I. (2017). Le plan Ecophyto de réduction d'usage des pesticides en France: Décryptage d'un échec et raisons d'espérer. *Cahiers Agricultures*, *26*(1), Article 1. https://doi.org/10.1051/cagri/2017004

Guilpart, N., Metay, A., & Gary, C. (2014). Grapevine bud fertility and number of berries per bunch are determined by water and nitrogen stress around flowering in the previous year. *European Journal of Agronomy*, *54*, 9–20. https://doi.org/10.1016/j.eja.2013.11.002

Gutiérrez-Gamboa, G., Pérez-Donoso, A. G., Pou-Mir, A., Acevedo-Opazo, C., & Valdés-Gómez, H. (2019). Hydric behaviour and gas exchange in different grapevine varieties (Vitis vinifera L.) from the Maule Valley (Chile). *South African Journal of Enology and Viticulture*, *40*(2). https://doi.org/10.21548/40-2-3224

Hochberg, U., Rockwell, F. E., Holbrook, N. M., & Cochard, H. (2018). Iso/Anisohydry: A Plant–Environment Interaction Rather Than a Simple Hydraulic Trait. *Trends in Plant Science*, *23*(2), 112–120. https://doi.org/10.1016/j.tplants.2017.11.002

Holzapfel, B. P., Smith, J. P., Field, S. K., & Hardie, W. J. (2010). Dynamics of Carbohydrate Reserves in Cultivated Grapevines. In *Horticultural Reviews* (Jules Janick ed., Vol. 37, pp. 143–211). Wiley-Blackwell.

IPCC (2022). *Climate Change 2022: Impacts, Adaptation and Vulnerability* (p. 3056). Contribution of working group II to the Sixth Assessment Report of the Intergovernmental Panel on Climae Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambrigne University Press. https://doi.org/10.1017/9781009325844

Keller, M. (2005). Deficit Irrigation and Vine Mineral Nutrition. *American Journal of Enology and Viticulture*, *56*(3), 267–282. https://doi.org/10.5344/ajev.2005.56.3.267

Lebon, E., Pellegrino, A., Louarn, G., & Lecoeur, J. (2006). Branch Development Controls Leaf Area Dynamics in Grapevine (Vitis vinifera) Growing in Drying Soil. *Annals of Botany*, *98*(1), 175–185. https://doi.org/10.1093/aob/mcl085

Levin, A. D., Matthews, M. A., & Williams, L. E. (2020). Effect of Preveraison Water Deficits on the Yield Components of 15 Winegrape Cultivars. *American Journal of Enology and Viticulture*, *71*(3), 208–221. https://doi.org/10.5344/ajev.2020.19073

Lopes, C., & Cadima, J. (2021). Grapevine bunch weight estimation using image-based features: Comparing the predictive performance of number of visible berries and bunch area. *OENO One*, *55*(4), 209–226. https://doi.org/10.20870/oeno-one.2021.55.4.4741

Martínez-Vilalta, J., & Garcia-Forner, N. (2017). Water potential regulation, stomatal behaviour and hydraulic transport under drought: Deconstructing the iso/anisohydric concept: Deconstructing the iso/anisohydric concept. *Plant, Cell & Environment*, *40*(6), 962–976. https://doi.org/10.1111/pce.12846

Mirás-Avalos, J. M., & Intrigliolo, D. S. (2017). Grape Composition under Abiotic Constrains: Water Stress and Salinity. *Frontiers in Plant Science*, *8*, 8. https://doi.org/10.3389/fpls.2017.00851

Ojeda, H., Deloire, A., & CARBONNEAU, A. (2001). Influence of water deficits on grape berry growth. *Vitis*, *40*, 141–145.

Pellegrino, A., Clingeleffer, P., Cooley, N., & Walker, R. (2014). Management practices impact vine carbohydrate status to a greater extent than vine productivity. *Frontiers in Plant Science*, *5*. https://doi.org/10.3389/fpls.2014.00283

Pellegrino, A., Lebon, E., Simonneau, T., & Wery, J. (2005). Towards a simple indicator of water stress in grapevine (Vitis vinifera L.) based on the differential sensitivities of vegetative growth components. *Australian Journal of Grape and Wine Research*, *11*(3), 306–315. https://doi.org/10.1111/j.1755-0238.2005.tb00030.x

Petrie, P. R., Cooley, N. M., & Clingeleffer, P. R. (2004). The effect of post-veraison water deficit on yield components and maturation of irrigated Shiraz (*Vitis vinifera* L.) in the current and following

season. *Australian Journal of Grape and Wine Research*, *10*(3), 203–215. https://doi.org/10.1111/j.1755-0238.2004.tb00024.x

Pichon, L., Brunel, G., Payan, J.-C., & Tisseyre, B. (2020). Apex-Vigne: A mobile application to facilitate the monitoring of growth and estimate the water status of the viticulture plots. *IVES Technical Reviews, vine and wine*. https://doi.org/10.20870/IVES-TR.2020.3558

Pl@ntGrape (2009). *Catalogue des vignes cultivées en France, IFV - INRAE - l'Institut Agro | Montpellier SupAgro*. https://plantgrape.plantnet-project.org/es/credits

Prieto, J. A., Lebon, É., & Ojeda, H. (2010). Stomatal behavior of different grapevine cultivars in response to soil water status and air water vapor pressure deficit. *J. Int. Sci. Vigne Vin*, 12. https://doi.org/10.20870/oeno-one.2010.44.1.1459

Rossouw, G. C., Smith, J. P., Barril, C., Deloire, A., & Holzapfel, B. P. (2017). Carbohydrate distribution during berry ripening of potted grapevines: Impact of water availability and leaf-to-fruit ratio. *Scientia Horticulturae*, *216*, 215–225. https://doi.org/10.1016/j.scienta.2017.01.008

Salinari, F., Giosuè, S., Rossi, V., Tubiello, F. N., Rosenzweig, C., & Gullino, M. L. (2007). Downy mildew outbreaks on grapevine under climate change: Elaboration and application of an empirical-statistical model. *EPPO Bulletin*, *37*(2), 317–326. https://doi.org/10.1111/j.1365-2338.2007.01126.x

Santos, J. A., Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., Dinis, L.-T., Correia, C., Moriondo, M., Leolini, L., Dibari, C., Costafreda-Aumedes, S., Kartschall, T., Menz, C., Molitor, D., Junk, J., Beyer, M., & Schultz, H. R. (2020). A Review of the Potential Climate Change Impacts and Adaptation Options for European Viticulture. *Applied Sciences*, *10*(9), 3092. https://doi.org/10.3390/app10093092

Savi, T., García González, A., Herrera, J. C., & Forneck, A. (2019). Gas exchange, biomass and non-structural carbohydrates dynamics in vines under combined drought and biotic stress. *BMC Plant Biology*, *19*(1), 408. https://doi.org/10.1186/s12870-019-2017-2

Schneider, C., Onimus, C., Prado, E., Dumas, V., Wiedemann-Merdinoglu, S., Dorne, M. A., Lacombe, M. C., Piron, M. C., Umar-Faruk, A., Duchêne, E., Mestre, P., & Merdinoglu, D. (2019). INRA-ResDur: The French grapevine breeding programme for durable resistance to downy and powdery mildew. *Acta Horticulturae*, 207–214. https://doi.org/10.17660/ActaHortic.2019.1248.30

Schultz, H. (2000). Physiological mechanisms of water use efficiency in grapevines under drought conditions. *Acta Horticulturae*, *526*, 115–136. https://doi.org/10.17660/ActaHortic.2000.526.9

Shahood, R., Torregrosa, L., Savoi, S., & Romieu, C. (2020). First quantitative assessment of growth, sugar accumulation and malate breakdown in a single ripening berry. *OENO One*, *54*(4), 1077–1092. https://doi.org/10.20870/oeno-one.2020.54.4.3787

Simonneau, T., Lebon, E., Coupel-Ledru, A., Marguerit, E., Rossdeutsch, L., & Ollat, N. (2017). Adapting plant material to face water stress in vineyards: Which physiological targets for an optimal control of plant water status? *OENO One*, *51*(2), 167. https://doi.org/10.20870/oeno-one.2016.0.0.1870

Tortosa, I., Douthe, C., Pou, A., Balda, P., Hernandez-Montes, E., Toro, G., Escalona, J. M., & Medrano, H. (2019). Variability in Water Use Efficiency of Grapevine Tempranillo Clones and Stability over Years at Field Conditions. *Agronomy*, *9*(11), 701. https://doi.org/10.3390/agronomy9110701

Vasconcelos, M. C., Greven, M., Winefield, C. S., Trought, M. C. T., & Raw, V. (2009). The Flowering Process of Vitis vinifera: A Review. *American Journal of Enology and Viticulture*, *60*(4), 411–434. https://doi.org/10.5344/ajev.2009.60.4.411

Wilhelm, L., Pellegrino, A., Ojeda, H., & Torregrosa, L. (2021). La Revue des Œnologues | Caractériser la tolérance à la sécheresse des nouvelles variétés résistantes aux maladies fongiques. *La Revue des Œnologues*, *181*, 4.

Zapata, C., Deleens, E., Chaillou, S., & Magne, C. (2004). Partitioning and mobilization of starch and N reserves in grapevine (*Vitis vinifera* L.). *Journal of Plant Physiology*, *161*, 1031–1040. https://doi.org/10.1016/j.jplph.2003.11.009

Zufferey, V., Murisier, F., Vivin, P., Belcher, S., Lorenzini, F., Spring, J. L., & Viret, O. (2012). Carbohydrate reserves in grapevine (Vitis vinifera L. 'Chasselas'): The influence of the leaf to fruit ratio. *VITIS - Journal of Grapevine Research*, *51*(3), 103–110. https://doi.org/10.5073/VITIS.2012.51.103-110

Zufferey, V., Spring, J.-L., Verdenal, T., Dienes, A., Belcher, S., Lorenzini, F., Koestel, C., Rösti, J., Gindro, K., Spangenberg, J., & Viret, O. (2017). Influence of water stress on plant hydraulics, gas exchange, berry composition and quality of Pinot noir wines in Switzerland. *OENO One*, *51*(1), 17–27. https://doi.org/10.20870/oeno-one.2017.51.1.1314

Zufferey, V., Verdenal, T., Dienes, A., Belcher, S., Lorenzini, F., Koestel, C., Blackford, M., Bourdin, G., Gindro, K., Spangenberg, J. E., Rösti, J., Viret, O., Carlen, C., & Spring, J.-L. (2020). The influence of vine water regime on the leaf gas exchange, berry composition and wine quality of Arvine grapes in Switzerland. *OENO One*, *54*(3), 553–568. https://doi.org/10.20870/oeno-one.2020.54.3.3106.