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SPECIAL ISSUE

Water use efficiency of Chardonnay under different grafting combinations in the viticultural area of Franciacorta

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ABSTRACT

Drought poses a challenge to future viticulture, exacerbated by climate change, which increases the frequency and severity of water shortages. The use of tolerant rootstocks is considered a sustainable strategy for facing drought, increasing water use efficiency and preserving the production and the quality of grapes and wines. Rootstocks M1 and M4 have recently been selected for facing abiotic stresses, and their stress tolerance is currently under investigation, considering interactions with scion, climate, and soil properties. This study aims to investigate the effect of these new rootstocks on Chardonnay's water-stress response in the Franciacorta viticultural area and to explore their potential under changing climate scenarios.

The study was conducted in three consecutive years in a pilot vineyard of Chardonnay located in Franciacorta, under four grafting combinations, *i.e.*, M1, M4, SO4, and 1103P. During the three experimental seasons, water status and physiological response were monitored in terms of water potential, gas exchange, and intrinsic Water Use Efficiency (iWUE). At harvest, main productive and qualitative parameters of grapes were analysed.

The three experimental years varied in water availability levels during the vegetative seasons, allowing testing of grafting combinations under different environmental conditions. Overall, across the three years, M4 limited water consumption of Chardonnay compared with 1103P, maximising iWUE, especially under non-stress conditions. Under water deficit, 1103P generally maintained the highest water potential and stomatal conductance, achieving the highest yield. A strong positive relationship was found between iWUE and grape quality potential, in terms of technological ripening. Responses induced by M1 and SO4 were intermediate between those observed in M4 and 1103P, although M1 was more similar to M4, while SO4 was closer to 1103P.

These results suggest that the tested new rootstocks could be suitable for the Franciacorta region for sparkling wine production under a changing climate, as valuable alternatives to traditional rootstocks. Further studies on adaptive strategies of drought-tolerant rootstocks will provide better insights into their performance under different environmental conditions and grafting combinations.

KEYWORDS: grapevine, rootstocks, climate change adaptation, sustainable viticulture, Franciacorta, GreenWINE 2025

INTRODUCTION

Franciacorta is a viticultural area located in northern Italy, covering the moraine ridges in the southern area of Lake Iseo. According to the Köppen–Geiger classification, the climate of Franciacorta is temperate and humid (Cfa) (Beck *et al*., 2018), and water stress was not typically considered a limiting factor for grapevine growing. However, during recent decades, an increase in mean temperatures and a modified precipitation pattern were observed, in line with other viticultural regions worldwide (Droulia & Charalampopoulos, 2022). Moreover, increasingly drier and warmer conditions over wine regions are expected in the near future, leading to higher susceptibility of viticulture to drought (Essa *et al*., 2023). Although the grapevine is generally considered tolerant to water stress, extreme conditions might negatively affect yield and quality of production. Grape quality is a multiparametric concept encompassing technological ripening (sugar accumulation and organic acid balance), as well as phenolic composition and aromatic profile coming from secondary metabolism, which are strongly influenced by environmental factors (Poni *et al*., 2018). In the Franciacorta region, the primary oenological goal is the production of sparkling wines, which generally require low pH and high titratable acidity to enhance wine freshness, an attribute expected to decline under future climate change scenarios (Jones *et al*., 2014). Grape ripening is strongly related to the balance between vegetative growth and grape production, which is affected by vine water status (Chacón-Vozmediano *et al*., 2020). Thus, agronomic adaptation strategies are required to preserve the suitability of threatened wine regions and the quality standards of production (van Leeuwen *et al*., 2024).

The physiological response of grapevine to drought involves several mechanisms, such as canopy regulation and transpiration control to reduce water loss, or the expansion of the root system to increase water availability. Although grapevine is generally considered an isohydric species, partially preserving leaf water potential through stomatal regulation, cultivar-specific differences have been reported, with behaviours ranging from near-isohydric to near-anisohydric (Chaves *et al*., 2010). Since modern viticulture is based on grafting to improve tolerance to biotic and abiotic stresses, these response mechanisms are further affected by the rootstock genotype (Tsegay *et al*., 2014). Thus, the selection of tolerant rootstocks is considered a promising adaptation strategy to face drought conditions (Bianchi & Brancadoro, 2021; Costa *et al*., 2016).

In the Franciacorta region, Chardonnay is the most widely grown cultivar and is commonly grafted onto rootstock Selection Oppenheim 4 (SO4), which is suitable for the local climate and soils. Due to the increasing risk of drought, a current trend is to replace SO4 – susceptible to water stress – with 1103 Paulsen (1103P), which is traditionally considered drought-tolerant (Zhang *et al*., 2016). Recently, new rootstocks have been selected to face abiotic stress conditions, including rootstocks M1 and M4. Specifically, M1 was obtained by crossing Kober 5BB × Teleki 5C (Migliaro *et al*., 2019) and was selected to improve adaptation to calcareous soils and reduce the risk of iron chlorosis, whereas M4 was selected from a progeny of rootstock 1103P to cope with drought. In the first studies, conducted under controlled conditions, these new rootstocks showed high efficiency in water use (Bianchi *et al*., 2020; Galbignani *et al*., 2016; Zamboni *et al*., 2016). Nevertheless, their performance under field conditions and in different grafting combinations is currently under investigation.

This study aims to: i) to evaluate the intrinsic water use efficiency of M1 and M4 rootstocks in comparison to SO4 and 1103P in an experimental field located in Franciacorta and in grafting combination with Chardonnay, which is the predominant variety in the region; ii) to investigate the relation between rootstock-induced water use efficiency and the productive and qualitative potential of Chardonnay grapes; and iii) to explore the potential of new rootstocks for sparkling wine production under changing climate scenarios.

MATERIALS AND METHODS

1. Experimental design

The study was conducted in three consecutive years, from 2022 to 2024, in a pilot vineyard of Chardonnay cv. located in Adro (Brescia, Italy), included in the viticultural area of Franciacorta. The vineyard lies at 226 m above sea level (5°36’42.1” N 9°57’25.6” E) on a loamy clay soil. More details on soil characteristics are reported in Table 1. In the experimental field, the scion Chardonnay was grafted onto different rootstocks, with about 50 vines per combination. Vines were planted in 2014 and were trained using the Guyot system with a planting layout of 2.0 m between rows and 0.9 m within rows. The rows are oriented NE-NW, and the inter-row area is covered by spontaneous grass.

During the three experimental seasons, water availability, vine water status, and physiological activity were monitored. In particular, measurements of vine water status began at the end of bunch closure (BBCH 79) in all three years and

TABLE 1. Soil properties of the experimental field. OM = Organic Matter; CEC = Cation Exchange Capacity; ESP = Exchangeable Sodium Percentage.

Soil layer	Sand [%]	Silt [%]	Clay [%]	OM [%]	CEC [cmol (+) kg^{-1}]	ESP [%]	pH	Total limestone [%]
Topsoil (0.0–0.4 m)	43	37	20	2.10	11.9	0.3	6.6	0.8
Subsoil (0.4–1.0 m)	41	25	34	0.53	12.7	0.2	6.7	0.4

were repeated when water deficit conditions were predicted, based on soil water balance calculations and weather forecasts (BBCH 81 in 2022; BBCH 82 and BBCH 89 in 2024). Overall, water status measurements were performed on DOYs 188 and 195 in 2022, on DOY 175 in 2023, and on DOYs 193, 204, and 235 in 2024.

At harvest, which took place on DOY 224 in 2022, DOY 233 in 2023, and DOY 236 in 2024, productive and qualitative parameters of grapes were measured. Pruning weight was measured during dormancy for vigour assessment.

2. Soil water balance

Meteorological data of daily maximum and minimum temperature and precipitation were made available by the Franciacorta Consortium. The weather station is located 300 m from the experimental vineyard. Soil Water Content (SWC) was calculated for the 3 seasons with a single-layer reservoir water balance model (Cola *et al*., 2014), using a time-discrete continuity equation (Jones & Tardieu, 1998). The model took into account the measured soil hydrological properties to determine the Potential Available Water (PAW = 178 mm). Due to the availability of daily data on temperature and precipitation, reference evapotranspiration ET0 was obtained by the Hargreaves and Samani method (Allen, 2000). Grapevine phenology, modelled by means of the Iphen model (Mariani *et al*., 2013), drove the dynamic crop coefficient Kc for the calculation of maximum grapevine evapotranspiration. Inter-row cropping water competition was considered following the FAO paper 56 approach (Allen, 2000).

3. Grapevine water status and gas exchange

At each measurement time, stem water potential (Ψs) was assessed on 4 vines per grafting combination using a Scholander pressure chamber (Scholander *et al*., 1965), produced by PMS Instrument Company, Corvallis, Oregon (USA). For Ψs analysis, four leaves of about 30 days old per grafting combination – belonging to different vines – were chosen from primary shoots and wrapped in a plastic bag supplied with aluminium bags for 1 hour. From the same vines selected for Ψs determination, gas exchange was measured on 4 sunny leaves using a leaf portable photosynthesis system (CIRAS-3, PP Systems, Amesbury, MA, USA) equipped with PLC6 (U) cuvette 18 mm circular (2.5 cm^2 head plate), under constant saturating PPFD of 1500 µmol photons m^{-2} s^{-1}, CO_2 concentration of 400 µmol mol^{-1}, block temperature of 25 °C and relative humidity between 60 % and 70 % allowing ~1.5 kPa of VPD inside the leaf chamber. Quantified gas exchange traits were carbon assimilation (A), stomatal conductance (gs), transpiration rate (E), and the vapour pressure deficit in the substomatal chamber (VPD). In correspondence with gas exchange, chlorophyll fluorescence was measured using the CFM-3 Chlorophyll Fluorescence Module connected to CIRAS-3, quantifying the operative photochemical efficiency of Photosystem II (ΦPSII) and the thylakoid electron transport rate (J). Stem Ψ and gas exchange were analysed between 11:00 am and 1:00 pm solar time. Maximum transpiration (Emax) was considered as the highest E value recorded in each experimental year. Intrinsic Water Use Efficiency (iWUE) was assessed as the ratio between A and gs (Seibt *et al*., 2008).

4. Vine vigour and grape production

At harvest of each experimental year, the yield of 7 representative vines per grafting combination was assessed as the weight of total grape production per vine. Border vines and visually atypical vines were excluded to avoid edge effects and reduce variability within grafting combinations. Two bunches from central shoots of each vine were collected and pressed together to obtain one must per plant. To prevent fermentation, 0.2 ‰ sodium azide (NaN_3, VWR, Radnor, USA) was added to each must. The pruning weight of the same vines was measured during dormancy as an index of vine vigour. Ravaz index (RI) was calculated as the ratio between grape production per plant and the relative pruning weight.

The quality of the must was analysed considering the total soluble solids (TSS) as an index of sugar content, pH, and titratable acidity (TA). TSS were determined via refractometry using a digital refractometer (DBR 35 SALT, Udine, Italy) and expressed as Brix (°Bx). Must pH and TA were measured using an automatic titrator (FLASH Automatic Titrator, Steroglass, Perugia, Italy). Briefly, 7.5 mL of juice for each sample was diluted to 50 mL with ultrapure water (resistivity > 18.2 MΩ.cm at 25 °C) for TA analysis. According to AOAC (Association of Official Analytical Chemists) guidelines, samples were titrated with 0.1 M NaOH to a pH of 8.3. TA was expressed as g L^{-1} of tartaric acid equivalent.

5. Statistical analysis

Obtained data were analysed using Microsoft Office Excel and R software (R Core Team 2023, s.d.). General linear models were used to compare years and grafting combinations on analysed parameters, checking homogeneity of variance using Levene's test. When heteroscedasticity was not accounted for, a Generalised Least Squares (GLS) model was fitted. Significant effects were considered for $0.01 < p \leq 0.05$ (*), $0.001 < p \leq 0.01$ (**), and $p \leq 0.001$ (***). When significant effects were detected, differences between years and grafting combinations were investigated using the Tukey *post-hoc* test at $p \leq 0.05$ using the glht() function in the multicomp package (Hothorn *et al*., 2008). Principal Component Analysis (PCA) was carried out on analysed parameters using the prcomp() function in the R environment, with centred and scaled data. Traits included in PCA were yield, vigour, TSS, pH, and TA. Biplots of loadings and scores for the first two components were drawn using ggplot2 (Wickham, 2016), ggfortify, and ggrepel R packages. Quadratic models were fitted between SWC–Ψs and gs–Ψs, whereas linear models were fitted between PC1–E_{max} and PC1–iWUE, to evaluate the coefficient of determination (R^2) and *p*-value.

RESULTS

1. Water availability and water status

The three experimental years differed in terms of temperature and precipitation, inducing different water dynamics in the soil. As shown in Figure 1A, the modelled SWC was higher than 80 % of PAW during spring for all the considered years. In 2022, SWC decreased below 80 % of PAW in the middle of June (DOY 167), reaching 40 % at DOY 197 and 20 % at DOY 223. The minimum SWC, amounting to 17 % of PAW, was reached at DOY 228. The following rainfall events, which occurred in August, were not sufficient to recover the SWC above 40 % before the end of the month. In 2023, the SWC level was maintained above 80 % until the end of June. Due to the frequent and intense precipitation that occurred in Franciacorta during summer 2023, the minimum level of SWC was about 60 % of PAW. Finally, in 2024, SWC was maintained above 80 % for a longer period compared to the previous seasons, until DOY 197. During the following weeks, SWC progressively decreased, reaching 60 % at DOY 209 and 40 % at DOY 234. The minimum SWC of 2024, amounting to 35 %, was reached at DOY 238. The rainfall at the end of August refilled the SWC above 50 % of PAW.

Considering the measurement times across the three years, SWC in the soil was strictly related to grapevine water status. A significant quadratic regression (p = 0.001) was fitted between SWC and Ψs, reporting a coefficient of determination of R^2 = 0.988 (Figure 1B). As a result of this relation, under the experimental conditions, a Ψs level of –0.9 MPa corresponds to a SWC level of 61 %, and a Ψs level of –1.1 MPa corresponds to a SWC level of 42 %. Thus, according to the model, in 2022 the number of days below the Ψs threshold of –0.9 MPa amounted to 63, of which 49 days were below –1.1 MPa. In 2023, only 4 days had Ψs below –0.9 MPa, and no days were below –1.1 MPa. In 2024, a number of 33 days were estimated with Ψs below –0.9 MPa, of which 7 days were below –1.1 MPa.

2. Rootstock-induced stomatal control and gas exchange

The water stress level reached by Chardonnay among the three experimental years was significantly affected by the grafting combination. An interaction was found between the rootstock and the water status. In particular, a significant effect of rootstock on Ψs was found whenever the mean Ψs was below the threshold of –0.9 MPa, therefore on DOYs 188 and 195 in 2022, and on DOY 235 in 2024. Any significant effect of rootstock was found in 2023 and on DOYs 193 and 204 in 2024. When a significant effect was detected, 1103P induced the highest Ψs in the scion, with significant differences with respect to M1 (on DOY 188 of 2022 and DOY 235 of 2024) and SO4 (on DOY 188 of 2022), which induced the lowest water potential (Table 2).

A strict relation was observed between Ψs and stomatal conductance across the three years (Figure 2). As shown in the figure, a significant quadratic regression was fitted. According to the model, gs decreased below 250 mmol H_2O m^{-2} s^{-1} when Ψs reduced below –0.9 MPa, and below 100 mmol H_2O m^{-2} s^{-1} when Ψs dropped to –1.16 MPa. A significant effect on stomatal conductance was found in all three experimental years, on DOYs 188, 175, and 235, within the respective years 2022 to 2024. On DOY 188 in 2022, the gs level induced by 1103P was 343 ± 41 mmol H_2O m^{-2} s^{-1}, significantly higher than other grafting combinations, whose gs levels were lower than 250 mmol H_2O m^{-2} s^{-1}. On the same day, a similar trend was observed in terms of transpiration, higher for 1103P than other rootstocks, but no significant differences were

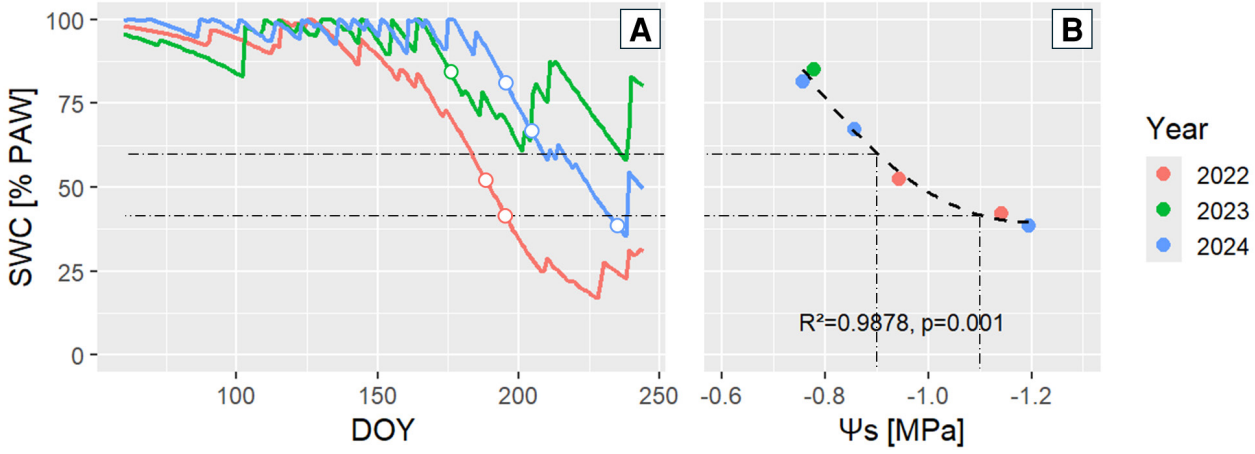


FIGURE 1. A) Dynamics of Soil Water Content (SWC) as a percentage of Potential Available Water (PAW) in the three experimental seasons (A). Empty dots represent the SWC at each measurement time; solid dots represent the relationship between stem water potential (Ψs) of Chardonnay and SWC during measurement times (B). Solid dots are the mean values of all grafting combinations. The quadratic regression model is shown as a dashed line. Thresholds of Ψs = –0.9 MPa and Ψs = –1.1 MPA for weak to moderate- and high-water stress, respectively (van Leeuwen *et al.*, 2009) are shown as dash-dotted lines, as well as the corresponding values of SWC according to the obtained regression model.

observed in photosynthetic activity. On DOY 175 in 2023, no rootstock effect was found on Ψs, but the highest stomatal conductance was induced by SO4, exceeding 500 mmol H_2O m^{-2} s^{-1}, which showed a significant difference from the gs levels induced by M1 and M4, amounting to 300 ± 39 and 254 ± 43 mmol H_2O m^{-2} s^{-1}, respectively. On the same day, SO4 also reported higher transpiration than M4, but no effect of rootstock was observed on photosynthetic activity. On DOY 235 in 2024, a similar trend to 2022 was observed, with 1103P inducing higher gs and E than other rootstocks. In this case, a significant effect of rootstock was also observed on photosynthetic activity, with higher A maintained by the 1103P combination, and lower J performed by the M1 combination with respect to SO4 (Table 2). Considering the measurement times, maximum transpiration (E_{max}) was detected on DOY 187 in 2022, on DOY 175 in 2023, and on DOY 193 in 2024.

3. Intrinsic water use efficiency

Intrinsic WUE, assessed as the ratio between carbon assimilation and stomatal conductance, was significantly affected by the year and by the grafting combination. As shown in Figure 3A, iWUE ranged between 25–60 µmol CO_2 mol^{-1} H_2O in 2022, between 28–73 µmol CO_2 mol^{-1} H_2O in 2023, and between 26–78 µmol CO_2 mol^{-1} H_2O in 2024. Average levels of iWUE amounted to 43 µmol CO_2 mol^{-1} H_2O in 2022, 46 µmol CO_2 mol^{-1} H_2O in 2023, and 54 µmol CO_2 mol^{-1} H_2O in 2024. A significant difference was reported between mean iWUE in 2022 and mean iWUE in 2024.

TABLE 2. Mean levels of gas exchange induced by rootstocks on Chardonnay in different years and measurement times. The respective phenological stage is reported in brackets using the BBCH scale. Ψs = stem water potential [MPA]; gs = stomatal conductance [mmol H_2O m^{-2} s^{-1}]; E = transpiration [mmol H_2O m^{-2} s^{-1}]; A = carbon assimilation [µmol CO_2 m^{-2} s^{-1}]; iWUE = intrinsic water use efficiency [µmol CO_2 mol^{-1} H_2O]; J = thylakoid electron transport rate [µmol e^- m^{-2} s^{-1}]. Rootstock had significant effects at $0.01 < p \leq 0.05$ (*), $0.001 < p \leq 0.01$ (**), and $p \leq 0.001$ (***). Ns = non-significant effect. Results of Tukey's multiple comparisons are reported as letters.

Year	DOY (BBCH)	Rootstock	Ψs	gs	E	A	J	iWUE
2022	188 (79)	1103P	–0.84 a	343 a	6.78 a	13.65	188	41.9
		M1	–1.00 b	233 b	4.81 b	10.10	158	43.5
		M4	–0.91 ab	247 b	5.01 b	11.25	180	45.8
		SO4	–1.02 b	237 b	4.82 b	12.10	177	50.9
		Sig.	**	**	**	ns	ns	ns
2022	195 (81)	1103P	–1.02 a	120	2.79	3.93	129	34.9
		M1	–1.20 b	94	2.24	3.83	89	40.8
		M4	–1.11 ab	95	2.15	3.78	110	43.6
		SO4	–1.15 b	93	2.23	4.25	124	45.6
		Sig.	**	ns	ns	ns	ns	ns
2023	175 (79)	1103P	–0.72	383 ab	10.10 a	13.87	189	35.8 b
		M1	–0.87	300 b	8.36 a	15.83	176	53.4 a
		M4	–0.79	254 b	5.40 b	15.70	160	63.3 a
		SO4	–0.76	501 a	10.42 a	16.07	186	32.3 b
		Sig.	ns	*	***	ns	ns	***
2024	193 (79)	1103P	–0.76	421	10.34 a	14.10	180	35.8 b
		M1	–0.78	361	7.71 b	15.98	208	44.2 ab
		M4	–0.77	300	9.33 a	15.48	196	51.7 a
		SO4	–0.73	398	9.25 ab	14.30	207	36.9 b
		Sig.	ns	ns	**	ns	ns	**
2024	204 (82)	1103P	–0.91	225	5.24	12.42	170	53.2
		M1	–0.90	198	5.70	13.60	197	69.0
		M4	–0.81	244	6.97	13.48	151	56.1
		SO4	–0.81	234	6.01	14.23	183	61.7
		Sig.	ns	ns	ns	ns	ns	ns
2024	235 (89)	1103P	–1.03 a	170 a	6.16 a	8.60 a	90 ab	55.6
		M1	–1.37 b	49 b	2.13 b	2.74 b	70 a	58.3
		M4	–1.24 ab	68 b	2.52 b	4.11 b	82 ab	64.7
		SO4	–1.24 ab	77 b	2.75 b	5.25 b	92 b	68.7
		Sig.	*	***	***	***	*	ns

A significant effect on iWUE was also reported for rootstock. The grafting combination with 1103P showed a range of iWUE between 25–66 µmol CO_2 mol^{-1} H_2O. Furthermore, iWUE ranged between 29–78 µmol CO_2 mol^{-1} H_2O in combination with M1, between 29–96 µmol CO_2 mol^{-1} H_2O in combination with M4, and between 28–78 µmol CO_2 mol^{-1} H_2O in combination with SO4 (Figure 3B). The average iWUE amounted to 42 µmol CO_2 mol^{-1} H_2O for 1103P, 52 µmol CO_2 mol^{-1} H_2O for M1, 54 µmol CO_2 mol^{-1} H_2O for M4, and 49 µmol CO_2 mol^{-1} H_2O for SO4. A significant difference was reported between the mean iWUE induced by M4 and the mean iWUE induced by 1103P.

An interaction was found between the grafting combination and the measurement times. In particular, a significant effect of the rootstock on iWUE was found at DOY 175 in 2023, higher for M1 and M4 than other grafting combinations, and at DOY 193 in 2024, higher for M4 than 1103P and SO4 (Table 2).

4. Water use and grape production

Grape production of Chardonnay was evaluated in terms of yield and quality across the three experimental years. In 2022 and 2023, rootstock reported a significant effect on yield, in both cases higher for 1103P (Table 3). In 2022, a significant effect was also found on the Ravaz index, ranging between 4.16 for M4 and 6.09 for 1103P. Grape quality was affected by rootstock in 2022, with higher TSS induced by M4 with respect to 1103P and M1. A miscellaneous effect of rootstock was found on grape acidity between years: in 2022, 1103P induced higher TA than M4 and SO4, as well as lower pH than other rootstocks; in 2023, a non-significant effect was detected; in 2024, the highest TA was induced by M4, as well as a lower pH than SO4 (Table 3).

The PC analysis – conducted including yield, vigour, Ravaz index, TSS, pH, and TA – identified two significant principal components, overall explaining 84.2 % of total variance.

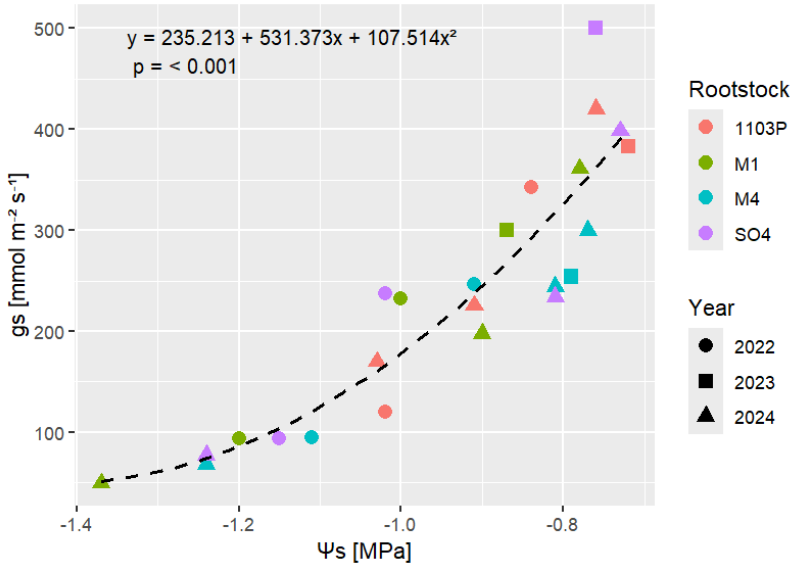


FIGURE 2. Relation between stem water potential (Ψs) and stomatal conductance (gs) measured on Chardonnay in the three experimental years (dot shapes) and in different grafting combinations (dot colours). Each dot represents the average of four leaves from different vines. The quadratic regression model is shown as a dashed line.

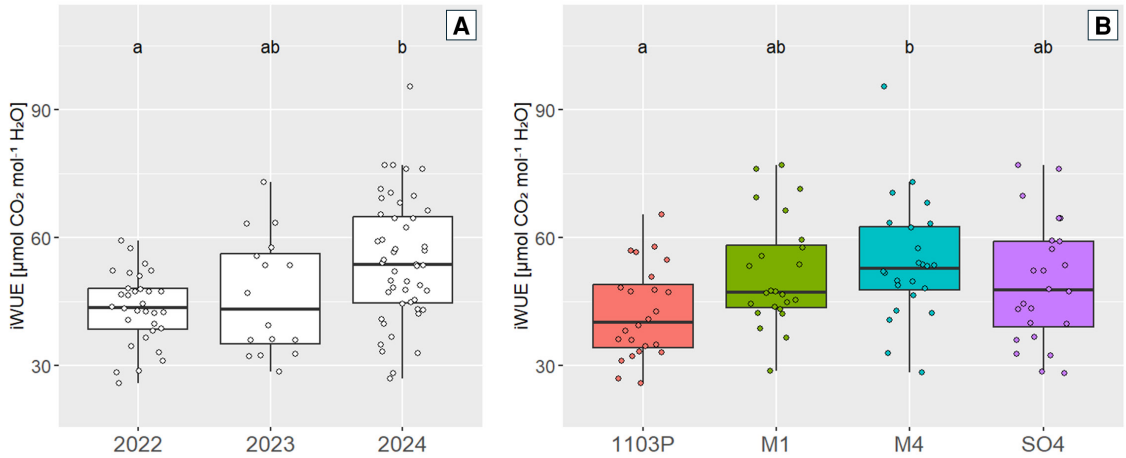


FIGURE 3. Boxplots of intrinsic Water Use Efficiency (iWUE) of Chardonnay in the three experimental years (A) and in different grafting combinations (B). Results of Tukey's multiple comparisons are reported as letters. Dots represent single analysed leaves.

The first component (PC1), explaining 64.6 % of the total variance, was positively affected by pH, TSS, and Ravaz index, whereas negatively affected by vigour, TA, and yield. The second component (PC2) explained 19.6 % of the variance and was mainly affected by yield and Ravaz index. Thus, qualitative traits are more related to PC1 and more separated from quantitative traits by PC2. A biplot of the first two components is shown in Figure 4. Alongside PC1, 2022 was clearly differentiated from the other experimental years, reporting lower vigour and higher sugar and pH in the must. On the other hand, the year 2023 was clearly differentiated from 2024 along PC2, reporting higher yield and lower TSS in the must.

Both PC1 and PC2 were significantly affected by rootstocks ($p \leq 0.05$). A significant linear regression ($R^2 = 0.967$; $p = 0.017$) was obtained between the mean value of PC1 for each grafting combination and the mean value of maximum E observed each year from the different rootstocks (Figure 5A). In particular, at increasing levels of E_{max}, the ripening potential represented by PC1 gradually decreased. A significant linear regression ($R^2 = 0.983$; $p = 0.008$) was also obtained between the mean value of PC1 for each rootstock and the mean value of iWUE (Figure 5B). In this case, PC1 gradually increased at increasing levels of iWUE.

DISCUSSION

1. Environmental effect on Chardonnay performance

Water dynamics in the soil of the pilot vineyard strongly differed between the three experimental years: the first year (2022) was characterised by a long drought period, starting in mid-June and continuing until harvest; the second year (2023) was characterised by high SWC during the whole season, due to frequent and intense rainfall; the last year (2024) was also characterised by high SWC in the first part of the season, but a long drought period occurred starting from July, about one month later than 2022. Different water availability over the years led to variable water status of vines. Based on the strict relation obtained between SWC and Ψs and thresholds reported in literature (van Leeuwen *et al*., 2009), in 2022 vines experienced 14 days of weak to moderate-WS and 49 days under high-WS, amounting to a total of 63 days under stress; in 2023 only 4 days under moderate-WS occurred; in 2024 were estimated 26 days under moderate-WS and 7 days under high-WS, counting a total of 33 days under stress. Length and severity of water stress affected the physiological response of the vine across the three experimental years. In the literature, Chardonnay is generally considered an isohydric variety, due

TABLE 3. Mean vegetative and productive parameters of Chardonnay for each grafting combination in the three experimental years. RI = Ravaz Index; TSS = Total soluble solids; TA = titratable acidity. Significant effect of rootstock was reported for $0.01 < p \leq 0.05$ (*), $0.001 < p \leq 0.01$ (**), and $p \leq 0.001$ (***). ns = non-significant effect. Results of Tukey's multiple comparisons are reported as letters.

Year	Rootstock	Yield [kg]	Vigour [kg]	RI	TSS [°Bx]	pH	TA [g L^{-1}]
2022	1103P	2.98 a	0.49	6.09 a	20.2 b	3.4 c	5.30 a
	M1	2.03 b	0.43	4.71 ab	20.1 b	3.66 ab	4.89 ab
	M4	1.55 c	0.38	4.16 b	21.8 a	3.71 a	4.34 b
	SO4	2.2 b	0.46	4.97 ab	20.9 ab	3.64 b	4.47 b
	Sig	***	ns	*	**	***	***
2023	1103P	4.86 a	1.12	4.08	19.4	3.29	6.55
	M1	3.31 b	1.00	2.89	20.8	3.31	6.05
	M4	3.35 b	0.92	3.81	20.6	3.32	6.64
	SO4	3.48 b	1.04	3.40	19.7	3.31	6.81
	Sig	**	ns	ns	ns	ns	ns
2024	1103P	2.45	1.02	2.81	20.4	3.41 ab	7.02 b
	M1	2.43	0.85	3.02	20.0	3.33 b	6.70 b
	M4	1.82	0.89	2.56	20.0	3.32 b	7.74 a
	SO4	2.69	0.87	3.21	18.6	3.43 a	7.09 b
	Sig	ns	ns	ns	ns	**	*

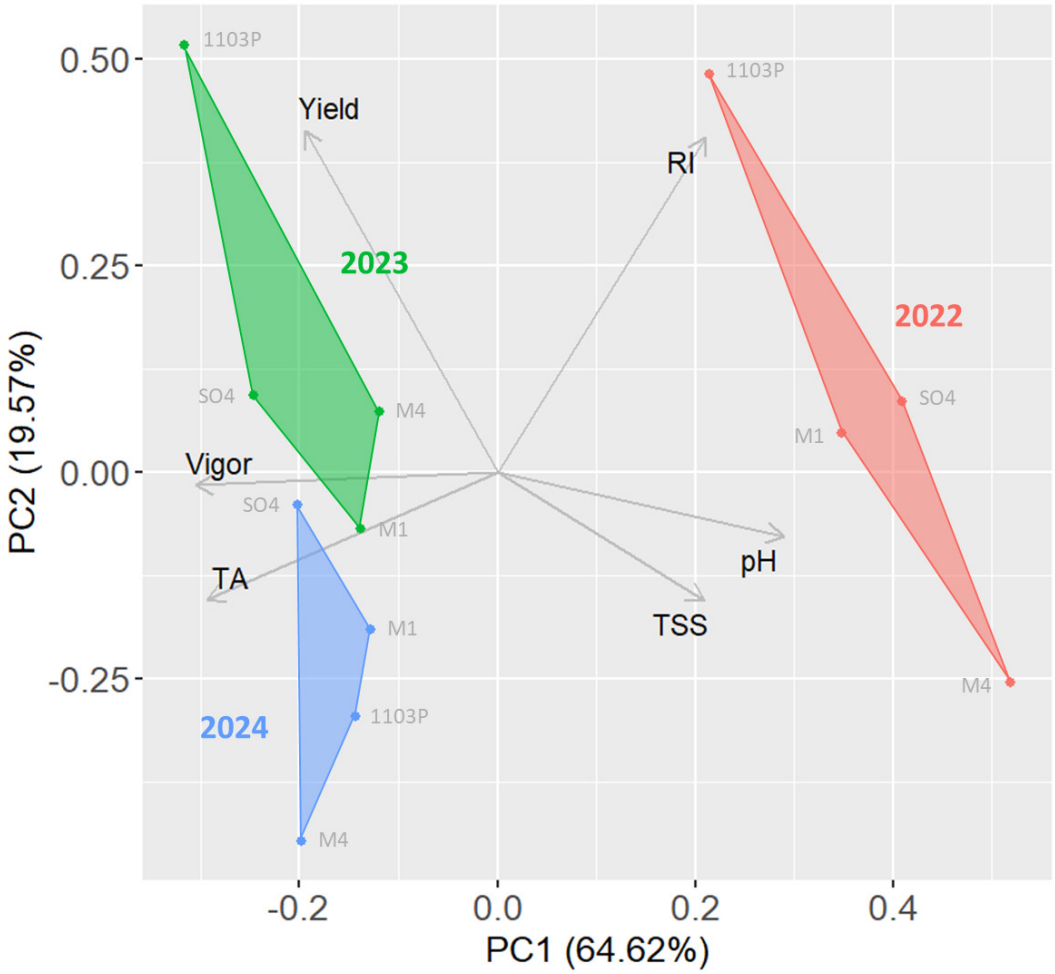


FIGURE 4. Biplot of the two main components obtained in the Principal Component Analysis (PCA) based on vegetative and productive parameters of Chardonnay. The percentage of variance explained by the two PCs is reported in brackets. Polygons represent the three experimental years in the PC-dimensional space.

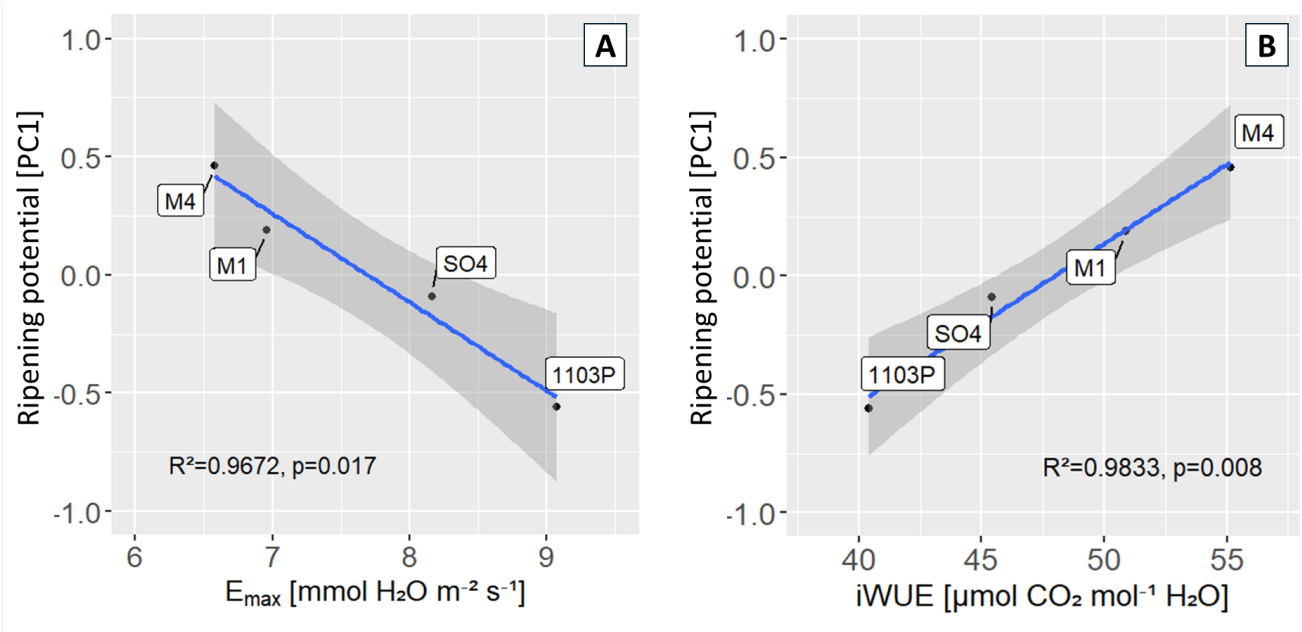


FIGURE 5. Relation between Principal Component 1 (PC1) and A) the maximum transpiration (Emax) and B) the intrinsic Water Use Efficiency (iWUE). Dots represent the general mean value for each rootstock. Blue lines represent the linear regression models. The shaded band around the curves indicates the 95 % confidence intervals.

to the delay in stomatal closure when water potential decreases (Chaves *et al*., 2010). Thus, under brief and mild WS, gas exchange in Chardonnay was not expected to decline, as observed in 2023 on DOY 175 (Table 2). A long period under moderate WS induced stomatal closure, as occurred in 2024, but Chardonnay preserved carbon assimilation, resulting in a rise in iWUE. An increment of iWUE under moderate water deficit is reported in the literature (Bota *et al*., 2016). Under a long period of drought, as in 2022, both carbon assimilation and stomatal conductance were reduced (on DOY 195: gs = 101 mmol H_2O m^{-2} s^{-1}; A = 2.35 µmol CO_2 m^{-2} s^{-1}), resulting in iWUE at a standard level (Figure 3a). Vine water status affects the balance between vegetative growth and grape production (Chacón-Vozmediano *et al*., 2020), as highlighted in this study by the PC analysis. Considering PC1, long and high-WS induced low vigour in Chardonnay in 2022, high accumulation of sugar and pH, as well as low titratable acidity. Considering PC2, the yield was maximised in 2023, when only brief and mild-WS occurred. In 2024, the vigour was enhanced by the wide water availability in the first part of the season (Figure 1), but yield and grape quality probably suffered from the drought period that occurred during ripening (Figure 4).

2. Rootstock effect on Chardonnay performance

The large differences described for the three experimental years allowed testing the effect of the grafting combination with Chardonnay under various environmental conditions (Figure 1).

In particular, a significant effect of rootstock on water potential was found whenever a moderate- or high-WS was reported; therefore, when the mean Ψs was below the threshold of –0.9 MPa (*i.e.*, DOYs 188 and 195 in 2022, and on DOY 235 in 2024). Nevertheless, at the other measurement times, water stress was not detected, but significant differences were observed in terms of stomatal conductance and transpiration (Table 2). These differences, which occurred under well-watered conditions, could further affect the response induced in the scion when water stress is manifested, modulating the total water consumption during the whole season (Gambetta *et al*., 2020). Under no-stress conditions (2023, DOY 175 and 2024, DOY 193), a similar pattern was observed in terms of transpiration, increased by 1103P, and iWUE, maximised by M4. When water stress occurred (2022, DOYs 188 and 195; 2024, DOY 235), 1103P generally maintained the highest water potential and stomatal conductance. The model reported in Figure 2 highlighted a conserved response of Chardonnay gas exchange to stem water potential under water deficit. Indeed, for a given level of Ψs, Chardonnay shows a similar regulation of stomatal conductance, irrespective of the rootstock. Thus, rootstock seemed not to modify this functional relationship itself, but rather to delay or prevent the decline in Ψs as soil water decreases (Figure 2).

Considering E_{max} as a proxy, water consumption under weak water deficit conditions was strictly related to the final vigour and ripening potential (PC1) induced by each rootstock, as shown in Figure 5a. According to this relation, greater water use (E_{max}) led to increased vigour and reduced TSS accumulation. Limiting transpiration when water is available, and consequently improving the water use efficiency, can allow water to be preserved in the soil and postpone the drought period (Shahbaz *et al*., 2025). Considering the observed relations of PC1 with E_{max} and iWUE, an opposite behaviour was induced in Chardonnay by M4 and 1103P. In particular, according to the regression models reported in Figure 5, M4 limited E_{max} and vigour, improving iWUE and promoting grape ripening, whereas 1103P maximised water consumption, vigour, and yield. Rootstocks M4 and 1103P are generally considered drought-tolerant (Fattorini *et al*., 2025); nevertheless, they seem to adopt different strategies to cope with water stress. Both rootstocks are able to promote root development as an adaptation strategy to water deficit (Pérez-Álvarez *et al*., 2023; Smart *et al*., 2006), but the main differences between them appear at the aboveground level. In a recent study, the two rootstocks affected the theoretical hydraulic conductance in stems and petioles of Pinot blanc by differently modifying the xylem anatomy (Bianchi *et al*., 2025). In the cited study, 1103P induced a reduction in xylem vessel area and an increase in vessel number in response to water deficit, whereas any modification in xylem anatomy was induced by M4, which preserved hydraulic conductance by promptly modulating stomatal conductance after water deprivation and rehydration. It is interesting to notice that under well-watered conditions, xylem vessel diameter in the petiole of Pinot blanc was larger when grafted onto 1103P, which could be related to higher water flux (E_{max}), as obtained in the present study (Bianchi *et al*., 2025). Finally, M1 and SO4 exhibited intermediate behaviour compared to the other two rootstocks, leaning respectively toward M4 (low vigour and high iWUE) and 1103P (high vigour and E_{max}). In line with previous studies conducted under controlled conditions (Frioni *et al*., 2020; Galbignani *et al*., 2016), under these operative field conditions M4 induced higher water use efficiency in the scion with respect to 1103P (Figure 3).

3. Oenological implication for Franciacorta wines

The viticultural area of Franciacorta is worldwide known for the production of sparkling wines. For this specific oenological goal, low pH, and high titratable acidity are desirable, especially considering the negative trend of these parameters predicted in future climatic scenarios (Jones *et al*., 2014). The choice of drought-tolerant rootstocks, which are also able to preserve acidity in the grape, is a sustainable strategy to adapt viticultural regions to a warmer climate (Costa *et al*., 2016). In this study, an interaction between rootstocks and years was observed: in 2022, when long period under high water stress occurred, rootstock 1103P reported a delay in grape ripening, leading to lower TSS than M4 and lower pH than other rootstock at harvest (Table 3); in 2023, when water availability seemed not to be limiting, grape acidity was not affected by rootstock; in 2024, when weak to moderate water deficit prevailed during ripening, TA was maximised by

rootstock M4 (Table 3). Besides the ripening potential, summarised by PC1, rootstock also affected yield and the vegetative-productive balance, mainly described by PC2 (Figure 4). In this study, a significant effect on yield was observed in 2022 and 2023, mainly driven by 1103P (Table 3), which, in standard vineyard conditions, may require specific agronomic practices to control production and avoid exceeding the protocol limits of PDO Franciacorta. These results suggest that the use of drought-tolerant rootstocks, such as 1103P and M4, can enhance acidity in grapes with respect to the widely used SO4 rootstock, although their effect is not consistent over the years (Table 3). Overall, the new rootstocks M1 and M4 could be a valuable alternative to traditional rootstocks in the context of Franciacorta, generally reducing water consumption by improving water use efficiency (Figure 3), containing vigour and production, and providing grapes with technological characteristics in line with high-quality wine production. However, the choice of rootstock is strongly dependent on the oenological goal, the interaction with the scion variety, and the site-specific environmental factors (Migicovsky *et al*., 2025). Thus, further studies are required to characterise the effect of these new rootstocks in other pedo-climatic conditions of the Franciacorta region, as well as to investigate their potential in other viticultural regions and different grafting combinations.

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