

Research Article

How Rootstocks Impact the Scion Vigour and Vine Performance of *Vitis vinifera* L. cv. Tempranillo

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Background and Aim. The genetically determined characteristics of grapevine rootstocks are important factors that affect scion performance. This 3 years' field study aimed to characterize the influence of four well-established 30-year-old rootstocks (110 Richter, 1103 Paulsen, 41B, and 161-49Couderc) on the Tempranillo cultivar in the semiarid wine growing region of D.O.Ca. Rioja, North-Eastern Spain. **Methods and Results.** Nutrient concentrations of mineral elements at flowering and veraison were determined in the vines, jointly with grapevine-water status, gas exchange, vine vigour, and chlorophyll content. Moreover, grapevine yield and grape quality were determined at harvest. The least vigorous rootstocks (41B, 161-49C) conferred drought adaptability traits by increasing water use efficiency (WUE) and decreasing whole-plant water conductance per unit leaf area (K_{plant}) compared to the more vigorous rootstocks (1103-P, 110-R). In contrast, the more vigorous rootstocks increased water transport capacity, which led to higher plant performance and nutrient uptake efficiency. At flowering, 1103-P and to a lesser extent, 110-R were most efficient at taking up nutrients, while 161-49C had the lowest concentrations for most nutrients. At veraison, 41B exhibited closer behaviour to 110-R than 161-49C, while 1103-P and 161-49C remained the most differentiated rootstocks, with higher and lower nutrient uptake, respectively. In addition, compared to the more vigorous rootstocks, the yield was up to 1.6 kg lower for the less vigorous rootstocks but the grape composition was improved. Notably, 161-49C led to higher total soluble solids, total acidity, and polyphenol content. **Conclusions.** Overall, grafting onto specific rootstocks represents a strategy to confer differential regulation of grapevine water-saving strategies, yield, berry quality, and nutrient uptake potential. **Significance of the Study.** This information may be useful for growers seeking to develop a site-specific selection of rootstocks for the grafted Tempranillo cv.

1. Introduction

Vitis vinifera scions are commonly grafted onto various rootstocks of other *Vitis* species (i.e., *Vitis berlandieri*, *Vitis rupestris*, and *Vitis riparia*) to influence scion vigour and provide resistance to biotic and abiotic stresses (Marín et al. 2021).

As evapotranspiration and the water needs of vines are predicted to increase as a result of climate change [1], much research has focused on the identification of new adaptation mechanisms to save water and improve the water use efficiency of crops without negatively affecting the quality of

crops [2, 3]. The responses of different grapevine rootstocks to water stress conditions have been widely investigated to obtain a better understanding of the mechanisms involved in drought responses [4] and the differential responses that grafted plants attain due to rootstock behaviour [5–9]. This knowledge could enable specific rootstocks to be used to increase crop quality in areas that are susceptible to drought as an efficient strategy to avoid permanent drought damage to vineyards. However, in addition to drought stress, other factors should be also considered when choosing the best rootstock for a specific soil. Soil characteristics and cultural practices also represent important controlling factors in the

development of viticulture. Thus, depending on the wine growers' interests and the soil characteristics, a variety of requirements need to be solved in order to obtain the desired vine and grape qualities for specific sites. It is widely known that certain rootstocks produce the best results in soils with specific characteristics. Thus, several studies have studied the effects of various rootstocks under different conditions on fruit quality, nutrient uptake, plant growth, root development, cold tolerance, water stress adaptation, and resistance to different diseases (reviewed in [10]).

The ideal rootstock will increase reproductive growth or yield, without leading to an excessive increase in vegetative growth. However, the effects of rootstocks on berry composition are generally considered to be an indirect result of the impact of the rootstock on vegetative and reproductive growth, for example, by altering the uptake of water or nutrients [11, 12]. Indeed, water and nutrient uptake were identified as two key processes that differed between own-rooted and grafted plants [12–14]. Thus, rootstocks differently modulate water supply and nutrient uptake and have an impact on the performance of grafted vines.

On the one hand, water supply to the grapevine may be modulated by root growth dynamics. Differences in root quantity, distribution, and/or the apparent efficiency of water uptake and transport would promote better grapevine-water relations [15, 16]. Indeed, compared to drought-sensitive rootstocks, drought-tolerant rootstocks formed more new roots in the soil profile during a dry and hot season which increased the uptake of water by the grapevines [13]. Root hydraulic conductance has been employed to describe changes in water uptake from the root-soil interface to the apoplast in the leaves [17]. Higher hydraulic conductance is observed in more drought-tolerant rootstocks, which exhibit improved development of xylem and lower vessel embolization; these properties can confer higher conductance [13]. According to Tramontini et al. [18], the hydraulic system is not only influenced by genetics but can also be affected by the soil type, which can significantly impact the development of xylem tissue, and thereby affect hydraulic conductance in the whole plant.

On the other hand, an efficient root system is advantageous for nutrient uptake by grapevines and allows the vine to better exploit the nutrient resources available in the soil. Indeed, it is well known that the rootstock confers vigour to the scion, and higher vigour is related to a higher nutrient uptake capacity [19]. However, some rootstocks allow excessive uptake of a number of nutrients that can be damaging to grapevines, including sodium, chloride, and boron [20]. More recent reports showed that rootstocks modified the mineral composition of the scion petioles and blades [7–9, 14, 19]. Gautier et al. [14] reported that the genetic background of a rootstock can modify the concentrations of phosphorus, magnesium, and sulfur in scion petioles. Moreover, several authors [12, 19] presented updated information on how different rootstocks influence the absorption of nutrients and the composition and sensory properties of the wine. Indeed, other researchers used different rootstocks to achieve lower pH and higher titratable acidity (TA) in grape juice by reducing berry potassium

concentrations [21]. Overall, this strategy may serve as a tool or criterion to establish guidelines for fertilization by considering the rootstock employed, and thus, may help to increase the efficiency of fertilizers use, reduce costs, and avoid environmental contamination [19]. However, given the complex interactions between the rootstock, cultivar, and environment, local long-term studies are necessary before a specific type of rootstock can be recommended for a specific edaphoclimatic condition (Marín et al. 2021). This implies that the results obtained for a particular cultivar-rootstock combination in a specific environment cannot be widely extrapolated to other situations [22, 23]. In this context, these specificities may explain the contradictory results obtained in previous studies. Moreover, although data from pot experiments and controlled conditions are highly valuable for the comparison of genotypes, such data must be considered with caution before extrapolation to the field [24].

To address these issues, the present field study conducted in a 30-year-old vineyard aimed to assess the effects of four well-established rootstocks, 41B Millardet et de Grasset (41B), 161-49 Couderc (161-49C), 110 Richter (110-R), and 1103 Paulsen (1103-P), on the vine performance and fruit composition of the scion cultivar Tempranillo, one of the most widely cultivated black grapes in Spain. 1103-P and 110-R have previously been classified as more vigorous rootstocks and 41B and 161-49C as low-vigour rootstocks [5, 25]. Moreover, by evaluating the vines across three years, we aimed to account for the effects of seasonal climatic variability on the performance of the four scion-rootstock combinations. Finally, we demonstrate the practical application of the nitrogen balance index (NBI), which has been proven to be a useful tool for monitoring nutrient absorption capacity, to rapidly obtain, accurate, objective non-destructive information, and in almost real-time.

2. Materials and Methods

2.1. Site Characteristics and Plant Material. The trial was carried out over three growing seasons (2018–2020) in a 30-year-old “Tempranillo” (*Vitis vinifera* L.) vineyard located in Aldeanueva de Ebro, La Rioja (Spain). Randomized soil sampling was performed in the first year at 0–30 and 30–60 cm depth for soil characterization purposes. In each of the four experimental units, three single samples were taken using a stainless steel drill to make a pooled sample representative of each depth. In the laboratory, the soil samples were desegregated, air-dried to constant mass, sieved (2 mm), and stored until chemical analysis. The soil was classified as a *Haplocalcids* typical [26] which corresponds to loam soil with the following average characteristics: clay 23.1%, silt 45.2%, sand 31.7% (USDA classification), organic matter 0.94%, and pH of 8.3 (additional information on the soil profile is given in Supplementary Table 1). The climate is between warm and temperate, with hot and dry summers and mean annual rainfall of about 500 mm·year⁻¹. The drought period usually lasts from May to September, but its length is highly variable from year to year. Meteorological data were provided by an automatic meteorological station

belonging to the AgroClimatic Information Service of La Rioja (SIAR) located 1.4 km from the experimental site.

The grapevines (*Vitis vinifera* cv. Tempranillo) were grafted onto seven different rootstocks, and the vine spacing was 2.7 m × 1.2 m. Two vigorous (1103-P and 110-R) and two less vigorous (41B and 161-49C) rootstocks were selected for the current study. Rootstocks 1103-P and 110-R (both *Vitis berlandieri* × *Vitis rupestris* parentage) are commonly characterized as high vigour and drought-resistant rootstocks [5, 25], rootstock 41B (*Vitis berlandieri* × *Vitis vinifera* parentage) is characterized as having moderate vigour and medium tolerance to drought, and rootstock 161-49C (*Vitis berlandieri* × *Vitis riparia* parentage) is characterized as having low vigour and drought intolerant [5]. The experimental design was a randomized complete block divided into four experimental units ($n = 4$). Each experimental unit consisted of 48 vines per rootstock-scion combination, distributed within one row. Buffer vines with the same rootstock (R99) were distributed between rows.

The training system was a vertical shoot positioning trellis with movable wires, and the vines were spur-pruned on a bilateral royat cordon system, leaving an average of 10 to 12 buds per plant. Shoots were trimmed twice a year, between bloom and veraison, at a height of about 1.0 m. All grapevines were rain-fed until veraison; thereafter, the irrigation dosage was adjusted using a drip system up to 30% of the reference evapotranspiration (ET_0) as calculated from the Penman-Monteith relationship and adjusted using a grape crop coefficient (Kc) and evaporation from a Class A pan [27]. Thus, irrigation began on different dates in each year depending on the weather conditions: irrigation began in July 2018, June 2019, and August 2020.

2.2. Grapevine Water Status and Gas Exchange. One of the four experimental units was selected for water status and gas exchange measurements. In the selected experimental unit, with 48 vines per rootstock, six ($n = 6$) well-established plants along the row (one per post) were selected to monitor vine water status. Predawn and midday leaf water potential (Ψ_{PD} and Ψ_{MD} , respectively) were measured ones using a Scholander pressure chamber (Soilmoisture Equipment Corp., Santa Barbara, CA, USA) at two phenological stages: (i) flowering and (ii) veraison. On the same day, stomatal conductance (g_s), transpiration rate (E), and net photosynthesis (A_N) were measured on six mature, healthy, sun-exposed leaves from six different plants per rootstock ($n = 6$) using a portable open gas exchange system (Li-6400; Li-Cor Inc., Lincoln, NE, USA) with a CO_2 concentration of 400 mmol CO_2 mol⁻¹ air in the cuvette. Measurements were taken at midmorning, from 10:00 am to 12:00 pm, on sunny days.

Intrinsic water use efficiency (WUE), obtained from instantaneous measurements, was calculated as the ratio between A_N and g_s .

2.3. Whole-Plant Hydraulic Conductance Per Unit Leaf Area. Whole-plant hydraulic conductance per unit leaf area (K_{plant}) was estimated on the basis of Ohm's law analogy for

the soil-plant-atmosphere continuum as described by Nardini and Salleo [28] as follows:

$$E_{max} = K_{plant} \times (\Psi_{soil} - \Psi_{leaf}), \quad (1)$$

where E_{max} , Ψ_{leaf} , and Ψ_{soil} are the maximum diurnal transpiration rate, leaf water potential, and soil water potential, respectively. Ψ_{PD} was taken as a proxy for Ψ_{soil} and Ψ_{MD} was taken as Ψ_{leaf} [29].

2.4. Measurements of Vine Vigour and Chlorophyll Content Using Optical Sensors. DUALEX (Force-A, Paris, France) is a hand-held device for measuring the chlorophyll and polyphenols contents of leaves. The chlorophyll content is estimated through the leaf transmittance ratio of two wavelengths in the red and infrared bands of the spectrum, and the flavonol content is measured by a chlorophyll fluorescence screening method at 375 nm [30].

The DUALEX calculates the nitrogen balance index (NBI) as the ratio between the content of chlorophyll and flavonoids [31, 32]. This index introduces the flavonoid content as a stress factor and indicates possible nutritional deficiencies in the plant. In each of the four experimental units per rootstock, 15 measurements were taken using this hand-held sensor, at two phenological stages: flowering and veraison.

Vine vigour was assessed using a Crop-Circle ACS-430 (Holland Scientific, Inc., Lincoln, NE, USA), an active light sensor, independent of natural light conditions, that emits radiation and measures reflectance in three wavelengths: 670, 730, and 780 nm (NIR). In addition to the reflectance values, the device generates NDVI values as an estimate of vegetation cover [33].

Using this equipment, continuous measurements (10 measurements per second) of each replicate were carried out at both flowering and veraison.

2.5. Leaf Chemical Analysis. Thirty complete, healthy leaves were sampled per experimental unit in each rootstock, at a rate of one leaf per plant on fruit-bearing shoots of average vigour from visually representative vines along the row. Leaves were collected opposite to the first bunch at flowering and opposite to the second bunch at veraison [34]. Both sides of the trellis were alternatively considered.

Leaf blades and petioles were separated, washed three times with tap water, rinsed with distilled water, oven-dried (Dry-big, J.P. Selecta, Barcelona, Spain) at 70°C for 48 hours, ground in an ultracentrifugal mill (ZM1, Retsch, Haan, Germany), and passed through a 0.5 mm mesh.

To determine total N (N-organic + N-NH₄⁺) in leaf blades and petioles, 0.20 g of ground samples were subjected to dry combustion analysis (Leco CNS, St. Joseph, MI, USA) using the Dumas method [35]. For the remaining nutrients, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and boron (B)- 0.20 g samples were subjected to wet digestion with H₂SO₄ (95%) and H₂O₂ (30%) [36] and analysed by inductively coupled plasma-optical emission spectrometry (Optima 3000DV, PerkinElmer, Norwalk, CT,

USA). Double deionised water (Milli-Q, Millipore, Bedford, MA, USA) was used for all dilutions. Concentrations were expressed on a dry mass basis.

2.6. Grapevine Yield and Grape Quality. At harvest, the number of clusters, the total yield per vine, cluster mass, berry number per cluster, and berry mass were recorded on six plants per replicate ($n = 4$), i.e., in 24 plants per rootstock per experimental year. Annually, during flowering, well-formed homogeneous plants were selected for sampling. The number of shoots per vine and the annual shoot mass per vine was determined at pruning after the growth cycle. Furthermore, the Ravaz index was calculated by dividing the total yield by the pruning mass recorded per vine. Vine vigour parameters were only recorded in 2019 and 2020.

Each year, the evolution of grape maturity was evaluated by random berry sampling. In each of the four experimental units, 500 berries per rootstock were sampled within the entire row to analyse the evolution of technological and phenolic maturity. Technological maturity was analysed by determining the total soluble solids (TSS) by refractometry, pH, total acidity, malic acid, potassium content, and colour intensity (CI) according to EEC methods [37], and tartaric acid, according to the Rebelein method [38] in the juice of berries crushed using a blender. The evolution of phenolic maturity was assessed by extracting phenolic compounds from the grapes; briefly, 200 berries were weighed, extracted with 50 mL of 1% HCl twice in a mixer without breaking the seeds, and the paste was heated with shaking up to 40°C, another fraction of 1% HCl was added, and the paste was heated and shaken again up to 60°C. The paste was cooled to 10°C in an ice bath, filtered through a cloth and the extract volume was measured. After dilution of the extracts, total phenolics were determined as total polyphenol index (TPI) by spectrophotometric absorbance at 280 nm. TPI was determined by the spectrometer Helios Omega (ThermoFisher Scientific). Total anthocyanins were determined by decoloring using sulfur dioxide [39].

2.7. Statistical Analysis. Pearson's correlations were calculated with Graphpad Prism software (GraphPad Software, Inc., La Jolla, CA, USA). The differences between means were assessed by both one and two-way analysis of variance (ANOVA) using SPSS 22.0 (IBM Corp., Armonk, NY, USA); if the differences were significant, multiple comparisons were performed using Duncan's post hoc test ($P < 0.05$) in SPSS 22.0.

Principal component analysis (PCA) was performed using the FactoMinR package with RStudio software, version 1.1.463, to visualize the grouping of rootstocks based on macronutrients and micronutrients at flowering and veraison for the petiole and leaf blade separately.

3. Results

3.1. Climate and Soil Water Status. The mean annual rainfall was 554, 373, and 482 mm in 2018, 2019, and 2020, respectively (Figure 1). Spring precipitation (from April to

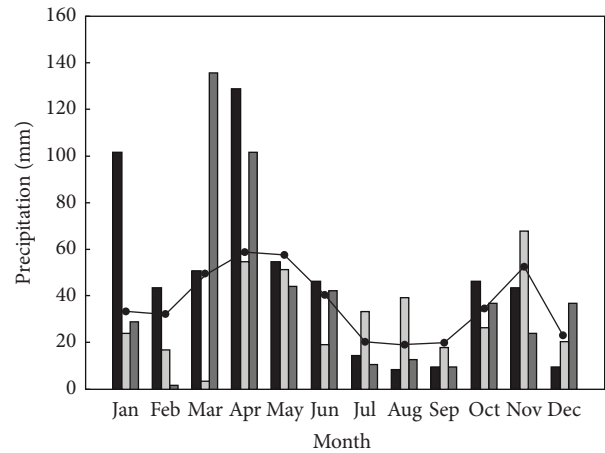


FIGURE 1: Average monthly precipitation (●) for the 2005–2020 period and monthly precipitation for the three experimental years, 2018 (■), 2019 (■), and 2020 (■). The data were obtained from the AgroClimatic Information Service station of La Rioja (SIAR, Spain) located in Aldeanueva de Ebro.

June) varied greatly from year to year. Precipitation was below average in spring 2019 (124 mm), and above average in 2018 and 2020 (240 mm in 2018 and 188 mm in 2020). Moreover, the distribution of precipitation varied between years. While rainfall mainly occurred in early spring and was scarce in summer in 2018 and 2020, accumulated precipitation was lower during spring but continued to accumulate during summer in 2019 (Figure 1). Indeed, the dynamics of plant water status reflected here by predawn water potential (Supplementary Table 2), which is assumed to represent the mean soil water potential next to the roots, varied according to the annual rainfall conditions. Thus, the 2018 vintage showed the highest Ψ_{PD} values at flowering and veraison.

3.2. Leaf Gas Exchange and Whole Plant Hydraulic Conductance. Table 1 shows the gas exchange parameters. At veraison, vines grafted on 1103-P maintained a significantly higher leaf photosynthesis rate (A_n) than those grafted on 41B in every year. Rootstocks 110-R and 161-49C generally, behaved similarly and presented intermediate A_n values, with some variation each year. In contrast to the above, stomatal conductance (g_s) differed between the high-vigour rootstocks (1103-P and 110-R) and low-to-moderate vigour rootstocks (41B and 161-49C) at both phenological stages, though no marked differences in g_s were obtained between rootstocks at flowering in 2019 and 2020. 110-Rigorous In addition, K_{plant} was also substantially lower for the 41B and 161-49C rootstocks than 1103-P and 110-R rootstocks at both stages, however, some differences were observed for this parameter in each year. Thus, in general, the fine stomatal regulation observed in Tempranillo plants grafted on the two least vigorous rootstocks resulted in higher WUE at the leaf level.

The year had a significant effect on all parameters measured. Therefore, a significant interaction between the rootstock and the year was observed at both phenological

TABLE 1: Mean values of gas exchange parameters measured early-mid morning at flowering and veraison for four rootstocks and from 2018 to 2020 and their interactions.

		Photosynthesis rate (A_n) [$\mu\text{mol}/(\text{m}^2\cdot\text{s})$]		Stomatal conductance (g_s) [$\text{mol}/(\text{m}^2\cdot\text{s})$]		Water use efficiency (WUE) ($\mu\text{mol CO}_2/\text{mol H}_2\text{O}$)		Whole plant hydraulic conductance (K_{plant}) [(mmol-m)/(MPa-s)]	
		Flowering	Veraison	Flowering	Veraison	Flowering	Veraison	Flowering	Veraison
Two-way ANOVA									
Rootstock	1103-P	19.10 a	14.56 a	0.35 a	0.18 a	57.67 b	83.56 b	4.28 a	2.77 a
	110-R	18.69 ba	14.82 a	0.32 a	0.17 a	60.73 b	85.69 b	3.54 b	2.41 b
	161-49C	17.41 ba	13.79 a	0.27 b	0.14 b	66.88 a	102.04 a	3.00 c	2.03 c
	41B	17.06 b	11.51 b	0.24 b	0.12 c	73.43 a	101.61 a	3.14 c	2.0 c
Year	2018	20.33 a	15.52 a	0.346 a	0.175 a	61.62 b	93.37 ba	3.52 b	2.72 a
	2019	18.36 b	14.13 b	0.323 a	0.148 b	57.25 b	97.08 a	4.22 a	1.76 c
	2020	15.18 c	11.25 c	0.204 b	0.131 b	76.76 a	88.67 b	2.60 c	2.45 b
	R	ns	***	***	***	***	***	***	***
	Y	***	***	***	***	***	*	***	***
	R * Y	**	*	**	*	*	ns	**	***
One-way ANOVA									
2018	1103-P	23.12 a	16.47 a	0.438 a	0.221 a	53.07 b	74.87 b	4.14 a	3.76 a
	110-R	21.57 ba	17.32 a	0.399 a	0.205 a	54.72 b	85.94 b	3.57 ba	2.55 b
	161-49C	18.95 ba	15.73 a	0.310 b	0.146 b	62.52 b	108.47 a	3.27 b	2.26 b
	41B	17.66 b	12.57 b	0.237 b	0.128 b	76.14 a	104.18 a	3.10 b	2.30 b
2019	1103-P	16.34 b	15.97 a	0.329	0.173 a	49.92 c	92.70 cb	5.33 a	2.08 a
	110-R	19.67 a	14.66 ba	0.347	0.168 a	56.86 b	87.74 c	4.40 b	1.94 a
	161-49C	19.12 a	12.73 b	0.299	0.121 b	64.16 a	106.35 a	3.32 c	1.44 b
	41B	18.30 ba	13.14 b	0.318	0.130 b	58.03 b	101.55 ba	3.82 cb	1.59 b
2020	1103-P	17.19 a	11.22 a	0.235	0.139 a	76.17 ba	83.10 b	2.93	2.46 ba
	110-R	14.83 ba	12.48 a	0.217	0.152 a	70.60 b	83.40 b	2.67	2.74 a
	161-49C	14.14 b	12.74 a	0.194	0.146 a	73.96 ba	89.16 ba	2.41	2.47 ba
	41B	15.22 ba	8.81 b	0.179	0.090 b	86.11 a	99.11 a	2.50	2.12 b

*, **, and *** indicate significant differences at the 0.05, 0.01, and 0.001 levels of probability, respectively. In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at the 95% confidence level.

stages except for WUE at veraison. In 2018, which was the wettest year in spring, gas exchange values differed between rootstocks depending on their vigour, with the high-vigour rootstocks always showing higher values. This effect was less pronounced in 2019 and even less so in 2020, which coincided with lower gas exchange values in these years and therefore less pronounced vegetative development.

3.3. Effects of the Rootstocks on Vine Growth. Early grapevine vegetative growth, i.e., the ground-based normalized difference vegetation index (NDVI), was evaluated at two distinct grapevine phenological stages: flowering (May or June) and veraison (August) for the four grapevine rootstocks. The NDVI values discriminated the vigour of the plants, with high-vigour rootstocks (1103-P and 110-R) obtaining higher NDVI values than the rootstocks that are commonly considered to have low-to-moderate vigour (41B and 161-49C) (Figure 2 and Supplementary Table 3). The same pattern occurred over the three consecutive years of flowering ($P < 0.01$). However, at veraison, different NDVI responses were obtained within the Tempranillo plants grafted on the four rootstocks, which may be a consequence of a noticeable overall decline in NDVI throughout the season or saturation of the NDVI values, which occur when crops show high physiological potential (Figure 2 and Supplementary Table 3).

The vine vigour parameters at pruning were consistent with the NDVI values. Vines grafted on the rootstock 1103-P, and to a lesser extent on 110-R, had higher pruning mass (PM) than vines grafted on the 41B and 161-49C rootstocks. However, this difference was only significant for the 1103-P rootstock (Table 2) as 110-R and 161-49C rootstocks exhibited intermediate values during both years. Indeed, the main shoot mass (MSM) was also higher for vines grafted on the 1103-P rootstock (Table 2), although the number of main shoots per vine (NMS) was lower for this rootstock and the number of basal buds was similar for all rootstocks (Table 2). Furthermore, the Ravaz index, i.e., the ratio of yield and pruning mass, was higher for vines on the 41B than 1103-P, 110-R, or 161-49C. The different vine balances observed in this study indicate that each of the rootstocks led to different crop load ratios, which was lower than the optimum range for 1103-P (low yield and larger vine), and higher than the expected range for 41B (more fruit and smaller vine).

3.4. Berry Yield and Fruit Composition. At harvest, the grape yield was higher on the rootstocks 1103-P and 110-R than 161-49C and 41B (Table 3). For vines on the 1103-P rootstock, this difference was mainly due to the higher cluster mass, and not the number of clusters per vine (Table 3). However, the high yield of vines on 110-R was due to

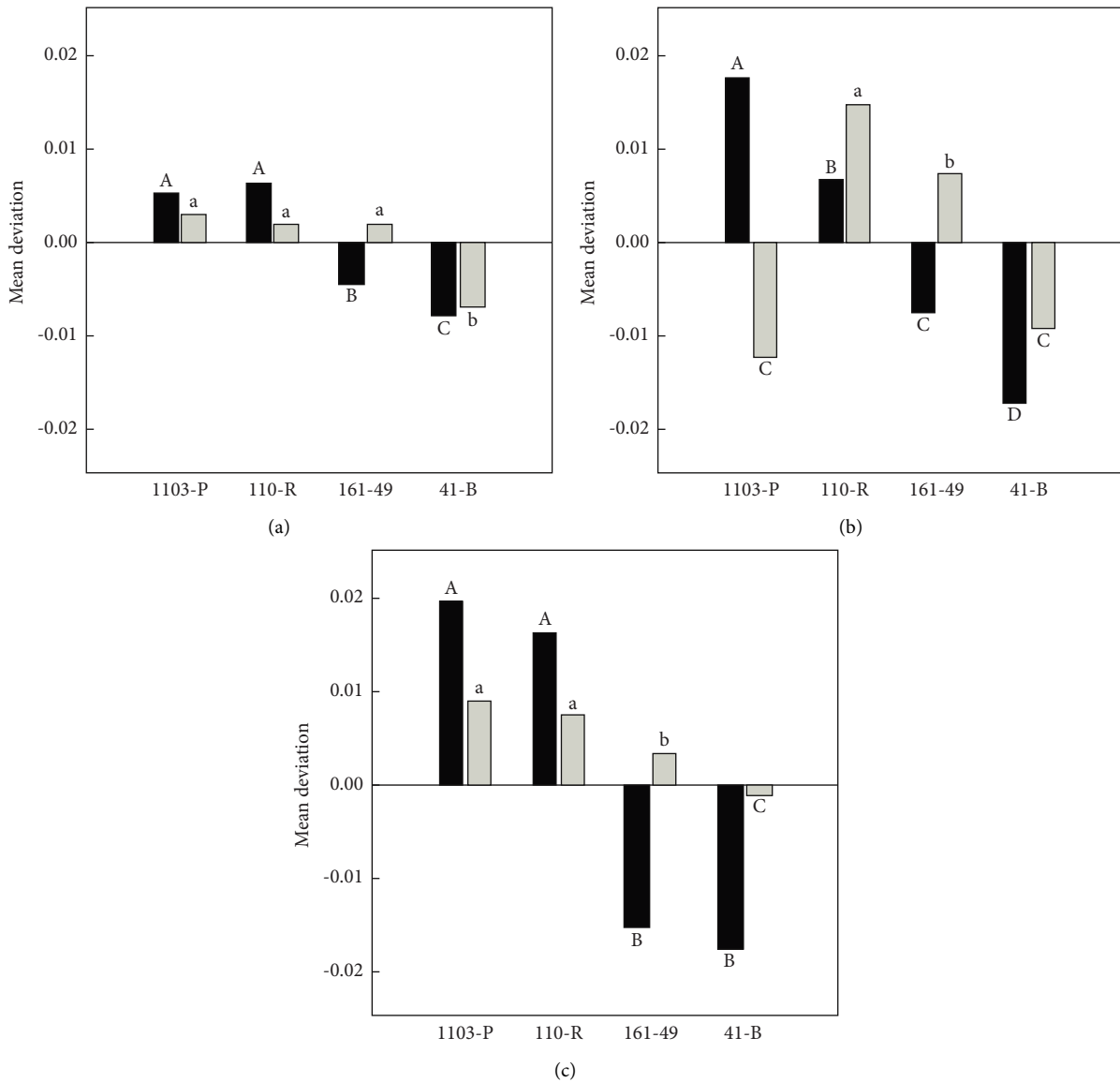


FIGURE 2: Mean deviation of normalized difference vegetation index (NDVI) from measured values for the four rootstocks during flowering (■) and veraison (■) for the three experimental years, (a) 2018, (b) 2019, and (c) 2020. Different letters indicate a significant difference at P 0.05 at flowering and veraison.

a higher number of clusters compared to vines on less-vigorous rootstocks. However, the grape yield was higher in 2018 and 2020 than in 2019. The grape yield, number of clusters per vine, mean cluster mass, and 100-berry mass were higher in 2018 and 2020 than in 2019, which may be directly related to the lower precipitation recorded during t late spring of 2019 (Figure 1). Even when taking into account the higher irrigation dosage applied at the beginning of June (before flowering) in 2019 in an attempt to compensate for increased water demand, higher predawn leaf water potential values were obtained at flowering in 2019 than in 2020 (Supplementary Table 2)

The marked differences in grape yield and in pruning mass (Tables 2 and 3), which was up to two-fold higher for the 1103-P rootstock than 41B in 2019 and 2020, with intermediate values for 110-R and 161-49C rootstocks-led to

high and low Ravaz indexes for the 41B and 1103-P rootstocks, respectively, although the Ravaz index for 1103-P was similar to 110-R and 161-49C (Table 2).

Moreover, must composition at harvest was also influenced by the rootstock, as can be seen in Table 4. Vines grafted on the 41B rootstock produced the lowest TSS content, total acidity, malic acid, and yeast-assimilable nitrogen (YAN). Contrary, must from vines grafted on 1103-P showed the highest pH, total acidity, tartaric acid, and malic acid contents. Vines grafted on 161-49C produced the berries with the highest total polyphenol index (TPI). Indeed, higher TPI values were observed for the vines on this rootstock in all three years of the study. In general, higher potassium concentrations and consequently higher pH values, which are related parameters [40–42], differentiated the more vigorous rootstocks from the less vigorous rootstocks.

TABLE 2: Mean values of vegetative development parameters for four rootstock, year, and from 2018 to 2020 separately.

	NBB	NMS	PM (kg vine ⁻¹)	MSM (g)	Ravaz index
Two-way ANOVA					
Rootstock					
1103-P	5.03	10.73 b	0.84 a	69.4 a	6.62 b
110-R	5.50	12.05 a	0.59 b	42.6 b	8.43 b
161-49C	5.15	11.07 ba	0.52 b	42.7 b	8.47 b
41B	5.45	11.80 a	0.33 c	26.0 c	11.24 a
Year					
2019	5.38	9.91	0.39 b	33.38 b	8.60
2020	5.19	12.91	0.75 a	56.94 a	8.78
R	ns	*	***	***	**
Y	ns	***	***	***	ns
R-Y	ns	ns	*	ns	ns
One-way ANOVA					
2019					
1103-P	5.1	9.0 b	0.575 a	52.7 a	6.13 b
110-R	5.5	10.3 ba	0.386 ba	30.8 ba	7.98 ba
161-49C	5.3	9.8 ba	0.358 b	30.7 b	9.21 ba
41B	5.7	10.7 a	0.242 c	19.3 c	11.08 a
2020					
1103-P	5.0	12.5	1.100 a	86.0 a	7.10 b
110-R	5.5	13.9	0.797 b	54.3 b	8.88 ba
161-49C	5.1	12.4	0.690 b	54.7 b	7.73 ba
41B	5.3	12.9	0.427 c	32.7 c	11.40 a

*, **, and *** indicate significant differences at the 0.05, 0.01, and 0.001 levels of probability, respectively. In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at the 95% confidence level. Regarding the year effect, for each factor, different letters denote statistically significant differences between years based on the Student's *t*-test ($P < 0.05$). NBB: number of shoots arising from basal buds/vine, NMS: number of main shoots/vine, MSM: main shoot mass (g), PM: pruning mass (kg/vine), and Ravaz Index: yield/pruning mass.

Berry composition also varied according to the year of the study, with the year having a higher seasonal effect on fruit composition that was independent of the rootstock (Table 4). TSS and the anthocyanin content were higher and total acidity, tartaric acid, malic acid, and YAN were lower in 2019 compared to 2018 and 2020, can be directly related to the lower precipitation recorded at flowering and late summer of 2019, which resulted in lower yields.

3.5. Leaf and Petiole Mineral Nutrition. To better understand the effect of the rootstock on scion mineral nutrition, principal component analysis (PCA) was performed on the micro and macroelements analysed during three consecutive vintages (2018, 2019, and 2020) at flowering (Figure 3) and veraison (Figure 4) on both the leaf petiole and blade.

At flowering, the first principal component (PC1), which accounted for 51.7% and 47.2% of the variability in the petiole and the leaf blade, respectively, was positive for all nutrients (Figures 3(a) and 3(b)). The rootstocks were grouped according to their vigour. 1103-P and 110-R were higher in the analysed mineral compounds (Figure 3), whereas 161-49C and 41B had the lowest concentrations of those nutrients.

At flowering, the second principal component (PC2) explained 16.8% and 21.8% of the variability in the petiole and leaf blade, respectively. Within the petiole, PC2 segregated the 110-R and 161-49C rootstocks from the two other rootstocks. PC2 was mainly explained by Mg and Na which are opposite to each other, indicating lower concentrations of these elements in the vines on the 110-R and 161-49C rootstocks. However, in the leaf blade, 110-R appeared to be displaced independently from the other three rootstocks. PC2 in the leaf blade is explained by K and Mg.

Some differences in the PCA of the petiole and leaf blade were also obtained at veraison (Figure 4). In the leaf blade, PC1 clearly segregated 1103-P and 161-49C by a gap of around five units of distance, whereas 110-R and 41B grouped together between 1103-P and 161-49C. Thereby, the PCA showed that 1103-P, on the positive side of PC1, led to higher Zn, P, Na, and Cu concentrations at veraison, whereas 161-49C, led to a lower concentration of these nutrients. Overall, these results suggest that Tempranillo vines grafted onto the 1103-P rootstock have higher nutrient concentrations at veraison while vines grafted on 161-49C have lower nutrient concentrations.

Within the leaf petiole, there was a clear segregation between 161-49C and the other three rootstocks, which was explained by PC2. 1103-P and 41B were positively correlated with the Mg, Cu, Na, and Zn concentrations, while 110-R was positively correlated with K and negatively correlated with Mg.

3.6. Dependence of Optical Indices on Individual Leaf Macro and Micronutrient Compositions. The dependence of the optical indices on leaf blade macro- and microelement concentrations was analysed for all of the Tempranillo plants grafted on the four rootstocks over three years period (2018–2020) (Table 5). Pearson's correlation tests confirmed a strong negative correlation between the abundance of chlorophyll and the leaf N concentration, with chlorophyll more strongly correlated to N than the flavonol content or NBI. Moreover, significant correlations were also observed between chlorophyll and other nutrients such as P, K, Ca, Fe, and Mn ($P < 0.01$).

Significant correlations were also observed between flavonols and the N, P, and K concentrations (Table 5). The NBI is the ratio of chlorophyll to flavonols, thus significant negative correlations ($P < 0.01$) were also observed between this index and the N and K concentrations (Table 5).

3.7. Influence of Rootstock and Vintage on Scion Leaf Composition Determined by a Noninvasive Chlorophyll Fluorescence Sensor and Nitrogen Status. As significant correlations were observed between the leaf composition determined by using either the noninvasive chlorophyll fluorescence sensor or chemical mineral determination (Table 5), we assessed the effects of the rootstock and season on optical indices and leaf N (Table 6) to determine if these optical indices could be used to discriminate the capacity of the four rootstocks to concentrate nutrients within the leaf blade. In general, both

TABLE 3: Mean values of yield components at harvest for four rootstocks and from 2018 to 2020 and their interaction.

		Yield (kg·vine ⁻¹)	Clusters (number·vine ⁻¹)	Cluster mass (g·cluster ⁻¹)	100-berry mass (g)
Two-way ANOVA					
Rootstock	1103-P	5.85 a	13.94 ba	408 a	224
	110-R	5.42 a	15.36 a	349 b	210
	161-49C	4.22 b	12.94 b	329 b	228
	41B	4.31 b	14.12 ba	303 b	211
Year	2018	5.64 a	14.67 a	387 a	238 a
	2019	3.13 b	12.33 b	255 b	170 b
	2020	6.08 a	15.28 a	399 a	247 a
R		***	ns	**	ns
Y		***	***	***	***
R·Y		ns	ns	ns	ns
One-way ANOVA					
2018	1103-P	6.27 a	13.96 b	449 a	245
	110-R	6.62 a	17.25 a	390 ba	222
	161-49C	4.31 b	13.00 b	335 b	240
	41B	5.37 ba	14.46 ba	373 ba	243
2019	1103-P	3.50	11.21	305 a	174
	110-R	2.99	12.92	235 ba	174
	161-49C	3.30	12.54	265 ba	168
	41B	2.75	12.67	215 b	163
2020	1103-P	7.79 a	16.67	468 a	252
	110-R	6.64 ba	15.92	420 ba	235
	161-49C	5.06 b	13.29	388 ba	276
	41B	4.81 b	15.24	319 b	226

*, **, and *** indicate significant differences at the 0.05, 0.01, and 0.001 levels of probability, respectively. In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at the 95% confidence level.

rootstock and year significantly influenced the leaf parameters determined using both the noninvasive sensor and by directly measuring N at the leaf level.

The 2018 vintage had the highest spring precipitation and 2019 had the highest summer precipitation (Figure 1). Consequently, a higher chlorophyll index and lower flavonol index were obtained flowering in 2018, resulting in a higher NBI, whereas a higher NBI value was obtained at veraison in 2019. In accordance with the negative correlation observed between NBI and leaf N (Table 5), leaf N at veraison was lower in 2019 than in 2018 and 2020.

As described above, NDVI values (Figure 2) distinguished the rootstocks into two groups based on vigour, with 1103-P and 110-R being the most vigorous (with higher NDVI) and 161-49C and 41B the least vigorous (with lower NDVI). At veraison, the chlorophyll index and NBI were higher for Tempranillo leaves on 1103-P and 110-R than 161-49C and 41B. However, this pattern was not observed at flowering, as the more vigorous rootstocks led to higher leaf N than the less vigorous rootstocks, while later the opposite trend was observed at veraison. Thus, at veraison, Tempranillo leaves on 1103-P and 110-R had lower N values, suggesting a dilution effect on scion leaf composition.

Looking at the NBI values over different years, it was only possible to distinguish between the rootstocks with the highest or the lowest vigour in 2019, which was the year with the highest NBI, suggesting that NBI is not strictly related to the vigour conferred by the rootstocks to the scion.

4. Discussion

The role of root systems in scion performance is a subject of intense interest to vine-growers. Variations in genetic pedigree, are assumed to alter the ability of grapevine roots to explore deeper and more humid soil layers [15] and tolerate several biotic and abiotic stresses [43].

4.1. How Rootstocks Differently Influence Scion Vigour. We studied 30-year-old Tempranillo scions grafted onto four field-grown rootstocks over three consecutive years. Previous reports indicated that different rootstocks may confer low, moderate, or high vigour to the scion Galet 1988, [44, 45]. The 1103-P and 110-R rootstocks conferred higher vigour overall than the 161-49C and 41B rootstocks (Table 2). The low vigour imparted by 161-49C and 41B has previously been reported by Romero et al. [7]. Moreover, the two more vigorous rootstocks (1103P and 110-R) led to higher NDVI values than the two lower vigour rootstocks (Figure 2 and Supplementary Table 3). NDVI is frequently used in agricultural applications to estimate various crop-related parameters such as biomass [46] and leaf area index (LAI) [47], and for crop management [48, 49] and mapping vigour zones [50, 51]. This study confirms the potential of NDVI to evaluate vine vegetative development, as previously reported by Acevedo-Opazo et al. [49]. Thus, NDVI has the potential as a reliable index to estimate vigour and also to estimate pruning wood mass (PM). It is worth noting that two distinct grapevine phenological stages were selected for

TABLE 4: Must physical-chemical parameters of Tempranillo grapes at harvest, for four rootstocks from 2018 to 2020.

		TSS (Brix)	pH	TA	Tartaric acid	Malic acid (g/L)	Potassium	YAN	Anthocyanins	TPI
Two-way ANOVA										
Rootstock	1103-P	21.2 a	3.64 a	4.08 a	6.72 a	2.23 a	1.55 a	0.231 a	1.17 b	67.20 b
	110-R	21.7 a	3.57 b	3.92 b	6.55 b	1.83 b	1.44 a	0.191 b	1.35 a	67.48 b
	161-49C	21.4 a	3.55 cb	3.86 b	6.63 ba	1.51 c	1.24 b	0.237 a	1.24 ba	73.78 a
	41B	20.0 b	3.52 c	3.69 c	6.52 b	1.28 d	1.21 b	0.168 c	1.09 b	62.63 b
Year	2018	20.3 b	3.51 b	3.99 a	6.57 b	2.11 a	1.33	0.215 b	1.1 b	62.14 c
	2019	22.3 a	3.61 a	3.70 b	6.16 c	1.61 b	1.38	0.152 c	1.49 a	68.23 b
	2020	20.5 b	3.59 a	3.96 a	7.08 a	1.42 c	1.37	0.253 a	1.04 b	72.95 a
R		***	***	***	**	***	***	***	*	***
Y		***	***	***	***	***	ns	***	***	***
R·Y		Ns	*	**	***	ns	ns	ns	*	ns
One-way ANOVA										
2018	1103-P	20.7 ba	3.55	4.33 a	6.72 a	2.64 a	1.49	0.246 a	1.18 ba	60.48 b
	110-R	20.5 ba	3.47	4.18 a	6.59 ba	2.20 b	1.35	0.210 ba	1.17 ba	59.36 b
	161-49C	21.1 a	3.56	3.74 b	6.53 ba	1.96 cb	1.28	0.230 ba	1.21 a	73.45 a
	41B	19.0 b	3.48	3.76 b	6.45 b	1.65 c	1.23	0.176 b	0.84 b	55.29 b
2019	1103-P	22.5 ba	3.70 a	3.88 a	6.31 a	2.16 a	1.58 a	0.179 a	1.38 b	68.91 ba
	110-R	23.1 a	3.63 ba	3.70 ba	6.04 b	1.73 b	1.47 a	0.132 b	1.63 a	68.34 ba
	161-49C	22.1 ba	3.53 c	3.78 a	6.39 a	1.26 c	1.20 b	0.175 a	1.56 ba	72.91 a
	41B	21.8 b	3.59 cb	3.45 b	5.91 b	1.27 c	1.27 b	0.122 b	1.41 b	62.76 b
2020	1103-P	20.5 ba	3.68 a	4.05 a	7.14	1.88 a	1.58 a	0.268 ba	0.94 b	72.22
	110-R	21.6 a	3.63 ba	3.87 b	7.04	1.57 b	1.51 a	0.232 cb	1.26 a	74.74
	161-49C	21.0 a	3.56 cb	4.05 a	6.96	1.31 b	1.25 b	0.306 a	0.95 b	74.99
	41B	19.1 b	3.48 c	3.88 ba	7.19	0.93 c	1.14 b	0.208 c	1.03 b	69.83

*, **, and *** indicate significant differences at the 0.05, 0.01, and 0.001 levels of probability, respectively. In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at the 95% confidence level. TPI: total phenolic index and YAN: yeast-assimilable nitrogen.

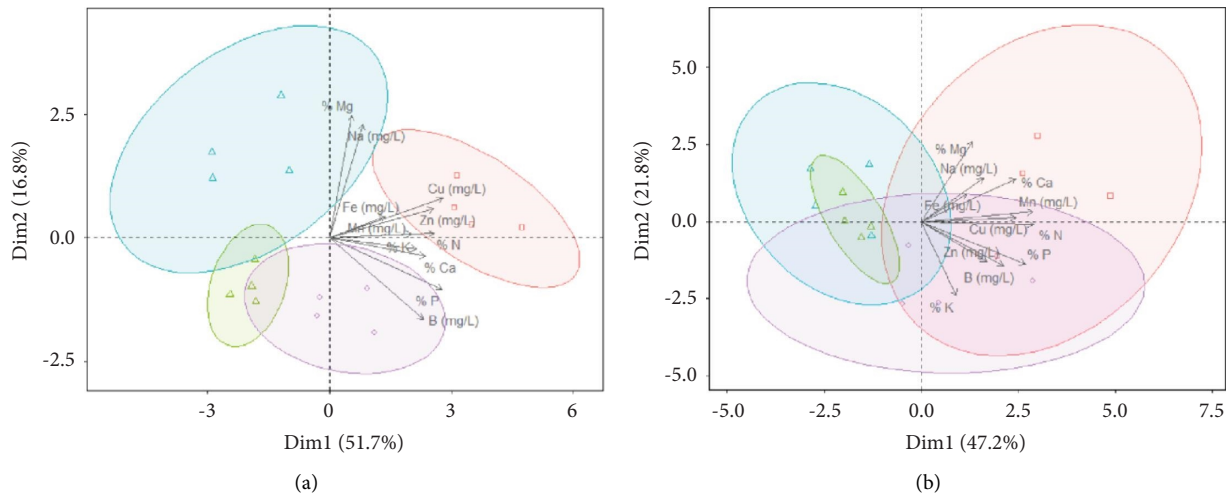


FIGURE 3: Principal component analysis biplots of macro- and micronutrients within the (a) Petiole and the (b) leaf blade of tempranillo grafted onto the rootstocks 1103P (■), 161-49C (▲), 41B (▲), and R-110 (●) at flowering for the experimental years 2018, 2019, and 2020.

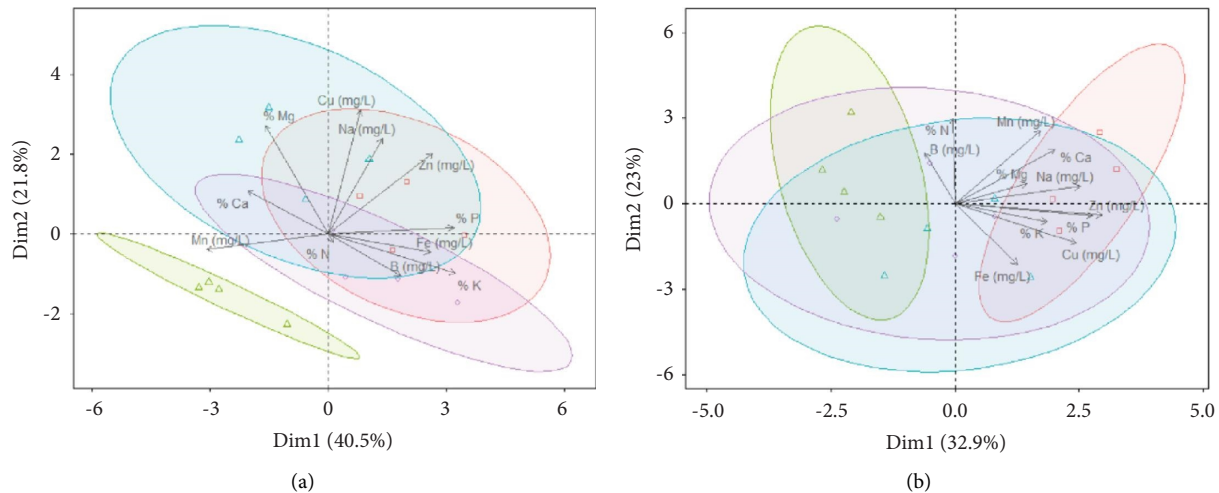


FIGURE 4: Principal component analysis biplot of macro- and micronutrients within the. (a) Petiole and the (b) leaf blade of Tempranillo scions grafted onto the 1103P (■), 161-49C (▲), 41B (●), and R-110 (◆) at veraison for the experimental years 2018, 2019, and 2020.

TABLE 5: Correlations between leaf macro and micronutrient concentrations and leaf optical indices at flowering and veraison in Tempranillo scions grafted onto four selected rootstocks during three seasons.

	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B	Na	C	Chl	Flav	NBI
Chl	-0.83**	-0.67**	-0.67**	0.68**	0.45*	0.62**	0.57**	-0.06	0.09	0.02	-0.10	-0.14	1		
Flav	-0.66**	-0.60**	-0.51*	0.50*	0.38	0.17	0.43*	-0.27	0.21	0.13	-0.27	-0.18	0.61**	1	
NBI	-0.57**	-0.39	-0.52**	0.46*	0.29	0.70**	0.44*	0.04	0.05	-0.08	0.05	-0.05	0.77**	-0.02	1

$n = 24$; Values indicate Pearson's coefficient of correlation. * $P < 0.05$ and ** $P < 0.01$; $P < 0.01$. Chl: chlorophyll, Flav: flavonol, and NBI: nitrogen balance index.

data acquisition in this study: flowering (May or June) and veraison (August). PW correlated better with NDVI values collected at flowering than with NDVI values collected at veraison, in agreement with a previous study [52]. The higher correlation between PW and NDVI at flowering in our trial (Supplementary Table 4) was probably related to the more even distribution of the vine canopy by midseason, which leads to saturation of the NDVI by the end of the season [53]. Viña et al. [54] also reported that NDVI measurements become less sensitive for estimating biomass as vegetative growth increases. Consequently, the relationship between early-season NDVI and PW may provide grape growers with a useful tool for yield estimation, as the higher the NDVI, the greater the PW, and therefore the greater the vigour. Higher vigour correlates with increases in other agronomic parameters, including grape yield (Supplementary Table 4), although this finding needs to be confirmed in future studies.

4.2. Rootstocks Influence Water Uptake and Leaf Gas Exchange. Water stress induces complex physiological regulation in grapevines at both the root and shoot levels (especially leaves). Therefore, the interrelationship between scions and rootstocks is difficult to predict. Indeed, the significant scion X rootstock interactions indicate that the scion cultivar must be taken into account during the selection and classification of rootstocks Ferlito et al. 2020,

[14]. This study demonstrates that different rootstocks confer different levels of vigour to Tempranillo cv. Compared to 1103-P and 110-R, the 161-49C and 41B rootstocks conferred lower vigour and led to smaller vines, potentially to reduce transpiration and, hence, decreased water requirements due to the development of smaller canopies [55]. In this study, the scions grafted onto the two more vigorous rootstocks (1103-P and 110-R) exhibited higher photosynthesis and stomatal conductance rates, compared to the same scion grafted onto the two least-vigorous rootstocks (161-49C and 41B) (Table 1). Similar results were reported by Alsina et al. [15], Romero et al. [7], and Lovisolo et al. [5], who attributed the improvements in root water uptake and transport capacity to the presence of *Vitis rupestris* in the genotypic background of 1103-P and 110-R compared to less vigorous rootstocks, such as 41B or 161-49C produced by crossing with *Vitis riparia*. The effects of the rootstocks on leaf photosynthesis and stomatal conductance were associated with the K_{plant} values, which were higher for 1103-P and 110-R than 161-49C and 41B, at both flowering and veraison. Similarly, Gambetta et al. [56] described higher fine root hydraulic conductance, even under well-watered conditions, in 1103-P and 110-R rootstocks, compared to other less vigorous rootstocks, such as 420A and 101-14. For the more vigorous rootstocks, increased hydraulic conductance of the fine roots correlated with a higher leaf area and higher transpiration rates in the scion. Collectively, this data suggests that different

TABLE 6: Mean values for leaf optical indices measured early-mid morning at flowering and veraison for four rootstocks and from 2018 to 2020 and their interactions.

		Chlorophyll index		Flavonol index		NBI		Leaf N (g/100g DM)	
		Flowering	Veraison	Flowering	Veraison	Flowering	Veraison	Flowering	Veraison
		Two-way ANOVA							
Rootstock	1103-P	34.73 b	42.51 ab	1.66	1.78 b	21.46 a	24.51 a	3.29 a	2.41 ba
	110-R	34.08 b	42.81 a	1.71	1.83 ab	20.30 b	23.82 ab	3.17 b	2.35 cb
	161-49C	34.55 b	41.77 b	1.66	1.86 a	21.45 a	22.88 bc	3.08 cb	2.45 a
	41B	36.44 a	39.28 c	1.68	1.82 ab	22.19 a	22.35 c	3.02 c	2.31 c
Year	2018	37.18 a	37.54 c	1.62 c	1.72 c	24.14 a	22.75 b	3.06 b	2.46 a
	2019	35.61 b	44.68 a	1.75 a	1.81 b	20.51 b	25.20 a	2.92 c	2.27 b
	2020	32.10 c	42.57 b	1.66 b	1.92 a	19.44 c	22.28 b	3.43 a	2.40 a
R		***	***	ns	***	***	***	***	***
Y		***	***	***	***	***	***	***	*
R * Y		***	ns	ns	ns	***	*	ns	*
		One-way ANOVA							
2018	1103-P	35.67 c	38.07 ab	1.61	1.70 ab	23.37 b	23.32	3.14	2.52 ba
	110-R	36.50 bc	38.89 a	1.68	1.72 ab	22.68 b	23.11	3.10	2.35 b
	161-49C	38.79 a	37.18 ab	1.57	1.80 a	26.03 a	21.35	3.02	2.63 a
	41B	37.77 ab	36.02 b	1.61	1.67 b	24.48 ab	23.21	2.98	2.35 b
2019	1103-P	35.23 b	46.60 a	1.76	1.75 b	20.13 b	27.30 a	3.05 a	2.22 b
	110-R	34.96 b	45.54 ab	1.77	1.81 ab	19.84 b	25.60 b	2.92 ba	2.30 ba
	161-49C	34.09 b	44.58 b	1.74	1.84 a	19.73 b	24.62 bc	2.90 ba	2.36 a
	41B	38.19 a	41.95 c	1.72	1.83 ab	22.36 a	23.25 c	2.81 b	2.21 b
2020	1103-P	33.28 a	42.84 a	1.61 b	1.88 b	20.87 a	23.05 a	3.67 a	2.48
	110-R	30.89 b	43.99 a	1.68 a	1.95 a	18.46 b	22.74 a	3.50 ba	2.40
	161-49C	30.89 b	43.54 a	1.66 ab	1.93 a	18.69 b	22.67 a	3.32 cb	2.36
	41B	33.39 a	39.87 b	1.70 a	1.95 a	19.80 ab	20.58 b	3.25 c	2.36

*, **, and *** indicate significant differences at the 0.05, 0.01, and 0.001 levels of probability, respectively. In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at the 95% confidence level. DM: dry mass and NBI: nitrogen balance index.

rootstock-scion combinations may explain the varied near-iso/anisohydric behaviours of certain cultivars reported in different studies [57, 58]. Indeed, several factors contribute to the drought response of the rootstock, including root anatomy, growth patterns, and chemical and physical signals related to stomatal regulation. Smart et al. [22] found that differences in the proportion of roots in the different soil layers, rather than differences in the ability of rootstocks to develop roots at depth, conferred high-vigour rootstocks with improved access to water and minerals from the deeper soil profile. Thus, it is likely that a combination of all of these factors contribute to the drought response when a rootstock is subjected to drought conditions.

Water use efficiency (WUE) based on instantaneous gas-exchange data indicated the rootstocks led to different water performances (Table 1). Evaluation of the scion WUE identified 161-49C and 41B as the most water-efficient rootstocks and 1103-P and 110-R as less water-efficient rootstocks.

Theoretically, a larger size of the root system maintains favourable plant water status, while a smaller size of the root system leads to a lower water transport capacity [15]. Therefore, chemical signals, transported to the leaves in the transpiration stream, may reduce stomatal conductance and/or growth, and thus increase water-use efficiency (WUE).

4.3. Rootstocks Influence Mineral Nutrition. Vines grafted on more vigorous rootstocks, such as 1103-P or 110-R, which are both classified as rootstocks with high drought tolerance [5, 15], maintained higher root water uptake during the growing season, probably due to differences in root quantity, distribution, and/or apparent efficiency of water uptake and transport (Marin et al. 2021). Consequently, these rootstocks were able to exploit soil water resources more efficiently, and this increased transport capacity was reflected by higher leaf blade and petiole nutrient contents. Indeed, leaf and petiole mineral compositions were significantly affected by both the rootstock and the vine phenological stage (Figures 3 and 4). The concentrations of each element follow different trends throughout the season and, despite the fact that the evolution of the nutrient concentrations was similar in the blade and petiole, the concentrations of the elements are generally significantly different between the leaf blade and petiole at each phenological stage [59, 60]. Indeed, the nutrient concentrations at each phenological stage, specifically flowering and veraison, differ so significantly that specific references for nutritional diagnosis have been proposed for each element at each phenological stage [61].

In general, rootstocks exhibit different root architectures, root cation exchange capacities, and root exudates and, in turn, these factors influence the leaf nutrient concentrations [62]. Thereby, it may be possible to select the rootstocks that most efficiently capture and translocate

mineral elements in the soil, which would allow the use of fertilizers to be reduced [63]. Thus, we performed principal component analysis (PCA) to classify the influence of the rootstocks on mineral composition. In general, the PCA differentiated the most invigorated rootstocks (1103-P and 110-R) from the least vigorous rootstocks (41B and 161-49C), which suggests that different rootstocks, essentially their root water absorption capacity, significantly influence mineral nutrition in the scion. Indeed, recent studies by Gautier et al. [64] and Gautier et al. [14] confirmed the existence of a significant relationship between the genetic background of a rootstock and its ability to modify concentrations of phosphorus, magnesium, and sulfur in the petioles of the scion. In these studies, rootstocks with a *Vitis riparia* genetic background (i.e., 161-49C) conferred lower petiole P concentration compared with other rootstocks with *V. rupestris* or *V. berlandieri* genetic backgrounds (i.e., 1103-P and 110-R), which increased petiole P concentration.

Accordingly, in the present study, 1103-P, which centered on all axes in the PCA, led to the best position with respect to macro and micronutrients, i.e., led to higher N, P, K, Ca, Mg, Na, Cu, Fe, B, and Mn concentrations in the petioles and leaf blades compared to the other rootstocks. 161-49C and 41B, with the lowest mineral concentrations, were in the most opposite position to the positive axes, while 110-R was more closely related to 1103-P (Figures 3 and 4). Moreover, the petiole, 1103-P was dominated by N, Cu, and Zn while 110-R was dominated by B. Within the leaf blade, the concentrations of N, P, Ca, Mn, and Cu were explained by PC1 and the concentrations of K and Mg were explained by PC2. In this case, 1103-P was dominated by the concentrations of Ca and Mn, N, Cu, P, K, and Zn while 110-R was dominated by the concentration of B.

The rootstock 1103-P stands out due to its high capacity to absorb Mg [65]. High levels of potassium in the soil solution can limit the absorption of magnesium, and thus reduce the availability of magnesium to the plant [66]. Thus, in high-potassium soils, selecting a “magnesium-absorbing” rootstock, such as 1103-P, may represent a simple strategy to avoid a deficiency of this nutrient. In this regard, the genetic diversity within *Vitis* spp. can provide new functional abilities to match specific scion/rootstock/site combinations. Furthermore, we also highlight the inefficiency, or maybe inability, of 161-49C to absorb and translocate Na. Thus, this rootstock may be a good candidate for high-salinity soils, although we cannot ignore the fact that salt tolerance can also be conferred by Cl uptake, which we did not analyse in this study. Rootstocks are considered as one important way to improve the salt tolerance of grapevines [67, 68], which represents another example of how specific scion/rootstock/site combinations may contribute to better vineyard management.

4.4. Nondestructive Diagnostic Testing of Mineral Nutrition of Grapevine Based on DUALEX® Measurements and its Applicability in Detecting Leaf N Content in Different Grapevine Rootstocks. We also assessed the ability of optical sensors to characterize the influence of the rootstock on scion leaf composition and plant vegetation indices over three years

(Table 5). Our results indicate the universality of these indices, at least for the Tempranillo cultivar.

Overall, Pearson’s correlation tests confirmed a strong association between the chlorophyll abundance and N content within the leaf, with the chlorophyll (Chl) index better related to N than flavonols (Flav) or the nitrogen balance index (NBI).

In general, chlorophyll meter leaf Clips are the most precise optical technique for assessing N levels [69]. In that study, significant correlations were also observed between Chl and the N content ($P < 0.01$) in wheat [69]. Cartelat et al. [31] proposed the NBI, which is the ratio of Chl to epidermal Flav leaf content, for the evaluation of nitrogen nutrition in wheat in the context of precision agriculture. The NBI index is proposed to be more sensitive to phenology and therefore reflects N availability better than either of the other two indicators (Chl and Flav) individually [69–71] because leaf Chl and Flav contents on a surface area basis are both dependent on the age of the leaf and light exposure during growth, especially during the first part of the season [72, 73]. During the second part of the season, Chl tends to decrease while Flav remains constantly high [72]. However, in our study, the Chl index was the most robust optical index for N estimation in the leaf scion as a diagnostic method. This divergence from previous results might be either due to the study of varied crops with different performances or because the turnover between Chl and Flav had not yet been established at the time the measurements were taken.

Less significant correlations were also observed between the optical indices and nutrients other than N, such as P, K, Ca, Mg, Fe, and Mn (Table 5). Similarly to N, the concentrations of these nutrients tended to decrease throughout the season and showed significant negative correlations with the determined optical indices (Table 5). On the other hand, nutrients that increase in concentration [59] positively correlated with the three optical indices (NBI, Chl, and Flav) (Table 5). This is because many essential elements are involved in photosynthetic processes and are therefore related to the chlorophyll and flavonoid contents of leaves, including elements from leaf structures such as Mg or N, those involved in chlorophyll synthesis or elements that play a role in maintaining the structure of chloroplasts, such as Mg, Mn, and Fe.

Thus, this study showed the potential of optical indices to predict leaf N content independently of the phenological stage in four rootstocks. However, the potential of DUALEX to discriminate between rootstocks was not clear (Table 6). Although a clear positive correlation between leaf Chl and vine vigour had already been reported (Sampaio 2007 and Blank et al. 2018), in this study, the more vigorous rootstocks did not always result in a higher N status in the scion. Indeed, positive correlations between vine vigour and the N concentration were only found at flowering in 2019 and 2020, but not at flowering in 2018 or veraison in any year (Table 6).

Cartelat et al. [31] reported that Chl values to increase with N concentration in wheat, irrespective of the growth stage, cultivar, or year. However, in grapevines, the relationship between the optical indices and leaf N content varied depending on the year and phenological stage, thus these indices did not adequately discriminate between the rootstocks.

4.5. Rootstock Performance and Grape Quality Parameters.

Compared to rootstocks commonly considered to have low-to-moderate vigour (161-49C and 41B), the higher soil water uptake by the high-vigour rootstocks (1103-P and 110-R), probably explained by its deeper root proliferation during the hottest and driest part of the season [15], was reflected in a higher pruning mass (Table 2) and higher yield response (Table 3). Thus, the significant effects of the rootstock on the scion yield provide evidence that selection and classification of rootstocks based on conferred vigour may help to control the productivity of Tempranillo cv. Rives [74] found that both, the inherent vigour of the scion (own vigour) and that conferred by the rootstock were contributing factors to yield performance. Furthermore, detailed studies of crop development, including the assessment of shoot fruitfulness, flower number, and fruit set, are required to further elucidate the different scion responses.

In this study, the bunch mass, but not the number of bunches, mainly contributed to the yield variability between the rootstocks, with 1103-P and 110-R being the most productive and 41B and 161-49C being the least productive. Traditionally, high vine vigour and yields are associated with grapes and wines of low-quality [7, 75–77]. Herein, the grape yield was higher on the rootstock 1103-P than on the low-moderate vigour rootstocks. The higher vine vigour and grape yield of the 1103-P rootstocks usually corresponds with low-quality grape parameters such as a higher pH, higher malic acid, higher potassium, and lower polyphenol content. The higher potassium uptake and malate levels observed for this rootstock may require the addition of higher amounts of tartaric acid during winemaking to adjust the pH [78]. For all rootstocks, YAN was present at acceptable concentrations for successful fermentation (i.e., >150 mg/L) [79]. Interestingly, of the low vigour rootstocks (161-49C and 41B), rootstock 41B strongly reduced vegetative development of the scion in comparison with 161-49C (Table 2), while the yield was similarly affected by both of this rootstock (Table 3). Therefore, we observed higher Ravaz index values for Tempranillo vines grafted onto the 41B rootstock than for 161-49C rootstock (Table 2). The Ravaz index (yield/pruning mass), often referred to as the vine balance, ranges from five to ten for the balanced vine in warm climates, whereas from 3 to 6 may be more appropriate for cool climates [80]. Thus, we assume that the 41B rootstock contributes to excessive crop yield (more fruit and smaller vines), and, therefore contributes to the unbalanced development of the vine and thus negatively affects the grape quality. In contrast, compared to 41B, Tempranillo vines grafted onto 161-49C had optimal Ravaz index ratios and may be considered well balanced, with higher fruit quality, indicated by higher TSS content, total acidity, and IPT.

Overall, these results indicate the potential for the selection of an appropriate rootstock to modulate fruit composition, with the rootstock significantly affecting TSS (Brix), pH, TA, malate, potassium, YAN, anthocyanin, and TPI.

5. Conclusions

The current study aimed to investigate the influence of four different rootstocks on the performance of 30-year-old Tempranillo cv. vines. The varied effects of these well-established 30-year rootstocks in the field were consistent throughout the three years of the study, although interactive effects between year and rootstock existed for most parameters. Overall, the less vigorous rootstocks (41B and 161-49C), as determined by NDVI, conferred drought adaptability traits and influenced the capacity for water-saving, thereby increasing WUE. In contrast, the more vigorous rootstocks (1103-P and 110-R) increased water transport capacity, which was related to higher nutrient uptake efficiency.

Moreover, yield increases were generally associated with increased cluster mass, likely due to increased water uptake in vines grafted to a particular rootstock. Thus, this study provides evidence that appropriate selection and classification of rootstocks based on their conferred vigour may help to improve productivity. Indeed, Tempranillo vines grafted onto the rootstock 161-49C may be considered well balanced, as they had an optimal Ravaz index ratio, which favours better fruit quality such as a higher TSS content, total acidity, and TPI.

Finally, correlations were observed between the leaf optical indices (Chl index, Flav index, and NBI) and the concentrations of nutrients such as N, P, K, Ca, Mg, Fe, and Mn, independently of the phenological stage, in all four rootstocks. However, these indices did not clearly discriminate between the rootstocks. 110-R.

Overall, this study demonstrates that the appropriate selection of rootstocks is crucial for grape growers seeking to improve vine performance and wine quality.

Data Availability

The data analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Hereby, all authors declare that they have contributed to the development of this field trial and that they are all in agreement with the manuscript.

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Supplementary Materials

Supplementary Table 1: properties of the soil of the vineyard used in the experiment, Supplementary Table 2: mean values of predawn and midday leaf water potential (leaf, MPa) measured at flowering and veraison onto each rootstock and year, and their interactions, Supplementary Table 3: mean values of NDVI at flowering and veraison onto each rootstock and year, and their interactions, and Supplementary Table 4: correlation between NDVI values at flowering and veraison and several vegetative developments and productive parameters (NMS: number of main shoots·vine⁻¹, PW: pruning weight Kg·vine⁻¹, yield: Kg·vine⁻¹, clusters: number·vine⁻¹, cluster weight: g·clusters⁻¹, and 100-berry weight, g) in Tempranillo scions grafted onto four selected rootstocks at three seasons for NDVI values ($n = 12$) and two seasons for vegetative growth parameters ($n = 8$). (*Supplementary Materials*)

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