Connection matters: exploring the implications of scion–rootstock alignment in grafted grapevines

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Abstract

Background and Aims: Grafting in viticulture has been essential since the advent of the phylloxera crisis at the end of the 19th century, but relatively little is known about the relevance of the quality of the connection at the grafting junction on the behaviour of grafted grapevines.

Methods and Results: An experimental procedure comparing omega grafted vines with complete alignment of the scion and the rootstock (CA) and vines with partial alignment (PA) was developed. Three complementary trials were carried out in the nursery, in the field and under controlled conditions. The CA vines increased the success rate in the nursery. Vine growth was significantly affected by the alignment between the rootstock and scion both in the nursery and in their first year of establishment in a commercial vineyard and in a potted trial, although these differences dissipated in years 2 and 3 under commercial vineyard conditions. The CA graft also maintained a higher level of leaf gas exchange, but such differences did not correspond to those in the hydraulic conductivity across the graft union itself.

Conclusions: The degree of alignment of the grafted vine material affected vine development, but a better understanding of the role of vascular connections in different graft types and qualities is needed.

Significance of the Study: To the best of our knowledge, this is the first scientific study that developed an experimental procedure for assessing the implication of the alignment at the grafting point on grapevine physiology and water use.

Keywords: *graft quality, hydraulic conductivity, omega graft,* Vitis vinifera *L., water use*

Introduction

Grafting in different plant species has a long history and its potential benefits are mentioned as early as the fourth century BC in some texts such as *Parva naturalia* by Aristotle or *De causis plantarum* by his disciple Teophrastus (Labajos and Morales 2007). Columella, an agriculture writer during the Roman empire, focused an important part of his work on the study of grafting on the grapevine (Álvarez de Sotomayor and Rubio 1824, Labajos and Morales 2007). This long history highlights the importance of grafting techniques for plant propagation. In viticulture, grafting was originally used to change cultivars, but it became essential at the end of the 19th century because of the phylloxera crisis in Europe. Today, European and even worldwide viticulture is inconceivable without this technique (Ollat et al. 2016).

Verified information on the evolution of grafting practices in approximately the last 150 years is scarce; however, it is known that initially grafting was carried out manually in the field, requiring much time and effort and skilful labour. Later, the nursery sector flourished, and bench grafting dormant plants became increasingly popular. Since then, both field and bench grafting have coexisted. The former method, however, has gradually lost presence in many wine regions, as it requires much more time and skilled labour (Alley 1980), while bench grafting in nurseries has become more common (Waite et al. 2015). Bench grafting in nurseries has evolved from the earlier production schemes, and the omega type grafting has become the most widely used grafting technique (Mary et al. 2017). This technique has superseded other grafting methods because of its high productivity, relatively low skill requirement and the ease of semi-mechanisation. Producing plants at a high speed, however, with relatively unskilled labour can result in poorly performing plants. In this regard, it has been hypothesised that the decrease in vineyard longevity observed in most of the

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world's winegrowing regions where bench grafted plants are used (Gramaje and Armengol 2011, Waite et al. 2015, Mondello et al. 2018) might be partially explained by the low quality of the material produced in nurseries (Stamp 2001, Smart et al. 2012, Waite et al. 2015), as the decline of vines is frequently attributed anecdotally to a poor connection between the scion and the rootstock.

Graft union development is a relatively complex process that begins with the proliferation of a mass of undifferentiated parenchymatous cells at both the scion and the rootstock, known as callus, that initiates the healing process of the graft area as a wound response (Pina et al. 2012, Assunção et al. 2019). The callus acts as a bridge between the scion and the rootstock until xylem and phloem tissues differentiate, enabling the vascular connection between the two plant individuals (Melnyk 2017). This process involves a complex signalling between the scion and the rootstock that is conditioned by the specific graft combination (Tedesco et al. 2021).

The success of the graft requires the development of a functional vascular system between both individuals (Pina and Errea 2005, Milien et al. 2012, Loupit and Cookson 2020). Previous studies have shown that vascular connection development was dependent on the interaction between rootstock and scion (Santarosa et al. 2016). Thus, the combination of the more vigorous rootstock–scion presented a greater proportion of larger diameter vessels (≥ 50 μm) and xylem areas in anatomical analysis (Santarosa et al. 2016). In contrast, Clemente Moreno et al. (2014) concluded that there was a need to improve the understanding of how grafted plants communicate after finding no histological changes in the graft interface.

There are several factors that contribute to graft success in grapevine: (i) genetic compatibility between rootstock and scion; (ii) presence of pathogenic organisms; and (iii) what nurseries call 'graft quality', associated with the goodness of the connection between the rootstock and scion. Genetic incompatibility in grapevine has been widely studied, including how the combination of different scions and rootstocks affects growth and berry quality, the response to biotic and abiotic stresses and the molecular dialogue between scions and rootstocks such as the upregulation of the transcription factors related to the cambium, phloem and xylem development (e.g. Cookson et al. 2013, Canas et al. 2015, Assunção et al. 2016, 2019, Loupit and Cookson 2020, Tedesco et al. 2020). Similarly, the role of the genetic background of the scion and the rootstock on the defence against pathogens has been increasingly studied highlighting variable resilience against pathogens within different combinations (Rego et al. 2000, Aroca et al. 2010, Agustí-Brisach et al. 2013, Mary et al. 2017, Cui et al. 2019, Aigoun-Mouhous et al. 2021). Nonetheless, there is barely any research aimed to evaluate the implications of graft quality, that is goodness of the connection, on the behaviour of grafted plants in the vineyard (Milien et al. 2012). Graft quality is affected by the hardwood cutting characteristics (i.e. carbohydrate storage, good preservation prior to grafting), the conditions during the callusing process and in the rooting fields, as well as the grafting technique and the skilfulness and care of the nursery operators (Gramaje and Armengol 2011). Thus, Waite et al. (2015) stressed the need of a detailed description of practices for obtaining high-quality plants given that one of the most significant challenges for nurseries is the maintenance of a consistent supply of vines that are sound, healthy and uniform.

In this study we focused on assessing the implications that the connection at the grafting junction have for grapevine performance. To the best of our knowledge, this is the first time that a systematic protocol for obtaining plants with different levels of scion–rootstock connection has been used as an experimental model to evaluate its implications on nursery and field performance of grafted grapevines. For this purpose, the degree of alignment between the scion and rootstock cuttings was modified to obtain plants where the alignment was optimum (CA, complete alignment) and others for which it was suboptimum (PA, partial alignment). This is a realistic approach because it mirrors the range of alignment positions that can be found in commercial nurseries. In general, plant producers know that alignment between the vascular cambium of the scion and rootstock is a key criterion for a good connection (Gambetta et al. 2009, Hartmann et al. 2011, Santarosa et al. 2016), but it is frequently overlooked in order to increase production rate. Therefore, the results obtained with this approach may have implications for both nurseries and grapegrowers.

The aims of this study were to assess the impact of the connection between the scion and rootstock hardwood cuttings on: (i) the success rate (SR) and the vegetative growth in the nursery; (ii) the vegetative growth in field-grown vines during the first 3 years after planting; and (iii) the water use and hydraulic behaviour of potted vines.

Materials and methods

Evaluation of performance at the nursery

Plant material and grafting conditions. To evaluate the effect of the two different levels of connection associated to the degree of alignment (CA vs PA) on the performance of omega grafted plants in the nursery, three batches of plants were produced as detailed in Table 1. The Tempranillo cultivar was chosen as the scion as it is the most widely grown red cultivar in Spain. 110 Richter (110 R) (*Vitis berlandieri* × *Vitis rupestris*) and RG8 (41 B × 110 R) were chosen as rootstocks, the former accounting for approximately 60% of the

Table 1. Detailed information on each of the three batches of plants omega grafted with Tempranillo cultivar on 110 R and RG8 rootstocks by the Vitis Navarra nursery facilities during 2018 and 2020.

Year of grafting	Rootstock	Batch number	Degree of alignment	Number of grafts
2017	110 R	Batch 1	CA	360
			PA	240
	RG8	Batch 2	CA	360
			PA	226
2020	110 R	Batch 3	CA	247
			PA	246

CA, grafted vines with complete alignment of the scion and the rootstock; PA, grafted vines with partial alignment of the scion and the rootstock.

grafted plants produced currently in Spain (Marín et al. 2019). The latter, obtained by the collaborating nursery, is a newly developed rootstock which has recently been authorised as a commercial rootstock, and the Community Plant Variety Office recently granted to it Community plant variety right (Marín et al., pers. comm., 2022). To obtain CA plants, the plant material was carefully selected prior to grafting, so that the scion and the rootstock canes were of similar diameter, while for PA plants a moderate lack of diameter match was intentionally sought. Scion and rootstock canes in PA plants were aligned at one of their sides to guarantee contact between the cambium of both canes (Figure S1a,b). All grafting tasks were performed in the Vitis Navarra nursery facilities, and both the acquisition of plant material and the grafting process itself were carried out according to the protocol commercially used in the nursery. Namely, plant material was collected from certified virus-free mother plants during winter. Prior to grafting, one-bud (approximately 4–5 cm) hardwood dormant cuttings were prepared for scions and 30 cm manually de-budded canes were prepared for rootstocks. The material was not heat treated prior to grafting, although in 2020 representative samples of the plant material were tested for the presence of the major wood fungal species or major viruses, and found to be free of these pathogens. The different scion/rootstock combinations were prepared by omega bench grafting with dormant plant material, within the same day and by the same nursery operator. Grafted plants were immediately sprayed with fungicide and waxed to avoid dehydration, and placed in a callusing chamber (TARRE, Noáin, Navarra, Spain) at controlled temperature (26–28°C) and humidity (92–95%) for 22 days. After the callusing period, grafted plants were again sprayed with fungicide and rooting hormone (indole-3-butyric acid) and re-waxed before being planted in the rooting field following a randomised complete block design, where they remained growing until uprooted when dormant in December. Grafted plants were mechanically uprooted with a John Deere 6620 tractor adapted to this task (John Deere, Moline, IL, USA) and immediately carried to the nursery for their evaluation.

Evaluation of the success rate and growth after uprooting. Grafting success rate (SR) was determined by individually evaluating first the resistance and robustness of the graft union (Waite et al. 2015), and then the general aspect of the plant and the characteristics of their root system. To assess the robustness of the union, each graft was manually subjected to the 'thumb test', by firmly pushing the scion with the thumb finger (Figure S1c). All grafts that broke when performing the thumb test were counted as unsuccessful. Then, the plants that had passed the thumb test were visually evaluated, classifying as unsuccessful those plants that had bad aspect (crooked or broken plants mainly) and those that had less than three well-developed and well-distributed roots. After the evaluation process, SR (%) was calculated as the ratio of successful plants, which were used to perform different growth measurements.

The effect of the two different connections on plant growth was evaluated at the root and shoot level. Root development was evaluated by measuring the thickness of all roots with a Mitutoyo CD67-S15PP digital calliper (Mitutoyo, Kanagawa, Japan), at a distance of 5 cm from the base of the plant, on a sample of 30 plants randomly selected from each degree of alignment of each combination of cv. Tempranillo grafted onto 110 R and RG8 rootstocks. Then, the number of roots and the sum of their cross-sectional area (in mm^2) were calculated. Growth of the aerial part was evaluated by measuring the diameter of all the shoots produced by the scion in Tempranillo/110 R plants, and then the sum of their cross-sectional area (in mm^2) was calculated. Last, in order to have a surrogate measure of total plant development, the growth of the rootstock cane was determined in Tempranillo/110 R plants. To do so, cane diameter was measured in the middle of the first internode below the grafting point prior to callusing and after uprooting. Diameter was used to calculate cross-sectional area values, and their difference considered as an estimator of total growth (cane growth, in mm^2).

Evaluation of field performance in the vineyard

Plant material and experimental design. To evaluate the implication of the two different levels of connection on the behaviour of grafted plants in a commercial vineyard, a field trial was established in 2018 with part of the successful Tempranillo/110 R and Tempranillo/RG8 plants, and their growth was monitored for the first three seasons (2018–2020). The experimental plot was part of a vineyard planted for commercial purposes in Murieta (Navarra, Spain, 42.663 N, −2.157 W) belonging to Quaderna Via winery. The experimental design consisted of four completely randomised blocks, with ten plants per block and combination degree of alignment/rootstock, therefore accounting for 160 plants. The vineyard was planted at a spacing of 3 m × 1.20 m, and grapevines were intended to be trained as a bilateral cordon Royat, located at a height of 75 cm above the soil. The vineyard had a training wire at 75 cm, two more to embrace vegetation at 1.1 m, and a last wire at a height of 1.5 m. The shoots were trained vertically. The soil at the site was a glacis-type quaternary sedimentary soil, with a loamy texture and 9% of active lime, highly calcareous and of high fertility (2.74% organic matter). The climate was classified as Continental–Mediterranean, with cool summers and cold winters. Average temperature varies between 5°C in winter and 22°C in summer, and the average rainfall was about 600–750 mm/year (Sistema de Información Agroclimática para el Riego 2021). The vineyard was drip irrigated through 4 L/h pressure compensated emitters placed 0.75 m along a single drip line under the vines. Depending on the weather, three irrigations of 12 h each were applied per year, accounting for approximately 150 L per vine per year [0.41 ML/(ha · year)].

Evaluation of vegetative growth. To evaluate vegetative growth, the trunk cross-sectional area (TCSA) was measured for each plant at the end of the three seasons (2018–2020) by using a Mitutoyo CD67-S15PP digital calliper. In 2018, since there was still no single trunk formed, the trunk cross-sectional area was estimated through the sum of the cross-sectional area of all the shoots that had grown during the season. For the other two seasons, the trunk cross-sectional area was estimated by measuring the diameter of the main trunk. In addition, at the end of the last season considered, the growth of all the shoots grown during the season was also measured per plant, to obtain the total shoot cross-sectional area (SCSA). During the spring of 2019, a frost affected the trial causing burns on some of the shoots, which had sprouted 1 or 2 weeks earlier. A few days later, an evaluation of frost damage was made, evaluating all the shoots of each plant and assigning a level of damage ranging from 0 to 100%, with no significant difference in damage observed between treatments (data not shown).

Evaluation of hydraulic performance and water use

Plant material and experimental design. To evaluate the implication of the two different levels of connection between the scion and the rootstock on hydraulic behaviour and water use, a trial under controlled conditions was carried out in a high throughput-phenotyping platform at the ISVV-INRAE Bordeaux (France) in 2020. Three-year-old plants were uprooted in winter 2020 from a plot established in 2018 in Spain with successful CA and PA plants from Tempranillo grafted onto RG8 combination described previously. Specifically, five CA plants and five of PA plants were uprooted during dormancy, in order to minimise stress, and transferred to Bordeaux (France) to place them in 12 L pots with a substrate mixture of gravel, sand and commercial potting medium. After planting in February, the plants were grown in a greenhouse under well-watered conditions for 5 months before the experiment started, letting two main shoots grow on each plant. The plants were drip irrigated with a nutritive solution [$NH_4H_2PO_4$ 0.1 mmol/L; NH_4NO_3 0.187 mmol/L; KNO_3 0.255 mmol/L; $MgSO_4$ 0.025 mmol/L; 0.002 mmol/L Fe, and microelements (B, Zn, Mn, Cu and Mo)] to avoid any deficiency during their development, and the surface of the pots was covered with a plastic bag to prevent water loss by soil evaporation.

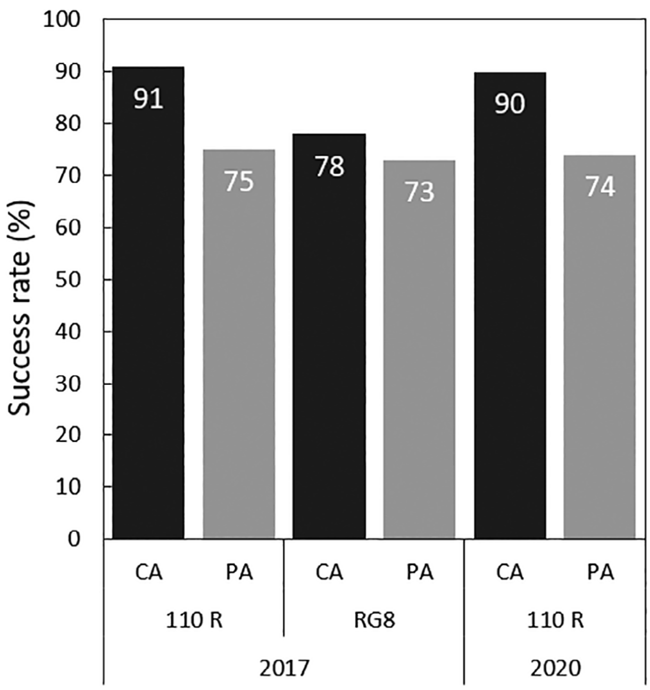

Figure 1. Success rate for the combination of Tempranillo scion on 110 R and RG8 rootstocks of grafted plants in the 2017 and 2020 seasons. There was a significant difference according to Pearson's chi-squared test in the success rate between grafted vines with complete alignment of the scion and the rootstock (CA) and grafted vines with partial alignment of the scion and the rootstock (PA) for 110 R rootstock in 2017 and 2020 ($P \leq 0.0001$) but not for RG8 ($P = 0.273$).

Mini-lysimeter phenotyping platform experiment. On 1 July, the ten plants were transferred to an automated mini-lysimeter greenhouse phenotyping platform and their transpiration rates monitored during the month of July (28 days). Following the procedure detailed in Dayer et al. (2020), pots were continuously weighed on OHAUS CHAMP CH15R11 individual scales (OHAUS CHAMP, Nänikon, Switzerland) and watered daily based on the plant mass loss by transpiration. The day before the experiment started, all the plants were irrigated up to their pot capacity and allowed to drain overnight. Air temperature, RH, and radiation conditions were automatically monitored with a Campbell weather station (Campbell Scientific, Logan, UT, USA) every 15 min at three different positions in the greenhouse. Air temperature was maintained approximately below 25°C by the cooling system of the greenhouse to avoid any heat stress.

Analysis of grapevine growth and water balance data. Leaf area (LA) was estimated through the relationship obtained between the leaf midrib length and the LA (measured with a Model LI-3000 leaf area meter (LI-COR Biosciences, Lincoln, NE, USA) using about 150 leaves of Tempranillo. The leaf midrib length was measured weekly on all the leaves of each plant, and the total LA per plant was estimated by applying the obtained formula.

The transpiration per LA [E in mmol/($m^2 \cdot s$)] was calculated as (Equation 1):

$$E = \Delta_w / LA / MW_w \quad (1)$$

where Δ_w is the change in mass within the considered period (g/s), LA is the leaf area (m^2) and MW_w the molecular mass of water (18 g/mol).

Table 2. Evaluation in the nursery of plant growth of Tempranillo plants grafted on 110 R and RG8 rootstocks.

		Root development		Aerial development	
Rootstock	**Degree of alignment**	**Root no.**[†]	**Root cross-sectional area (mm^2)**[†]	**Shoot cross-sectional area (mm^2)**[‡]	**Cane growth (mm^2)**[§]
110 R[¶]	CA	29.2 ± 1.9	30.7 ± 1.5	38.67 ± 1.23	0.12 ± 0.01
	PA	27.0 ± 2.6	30.9 ± 1.3	33.52 ± 1.44	0.09 ± 0.01
Significance		n.s.	n.s.	**	**
RG8	CA	38.9 ± 3.4	47.3 ± 2.4	–	–
	PA	33.8 ± 3.6	46.8 ± 1.7	–	–
Significance		n.s.	n.s.		

Significant differences regarding the degree of alignment according to two-sample *t* test are indicated by: **, $P < 0.01$; n.s., non-significant differences according to the two-sample *t* test for differences of means ($P < 0.05$); –, aerial development was not assessed for rootstock RG8. [†]Total root cross-sectional area and total root number were measured on a sample of 30 plants randomly selected from each combination. [‡]Shoot cross-sectional area was measured in all the plants after they uprooted from the rooting field at the end of the season. [§]Cane growth was estimated as the difference between the initial and final values of the cane cross-sectional area. [¶]For rootstock 110 R, root development parameters were evaluated in batch 1 whereas aerial parameters were evaluated in batch 3. CA, grafted vines with complete alignment of the scion and the rootstock; PA, grafted vines with partial alignment of the scion and the rootstock.

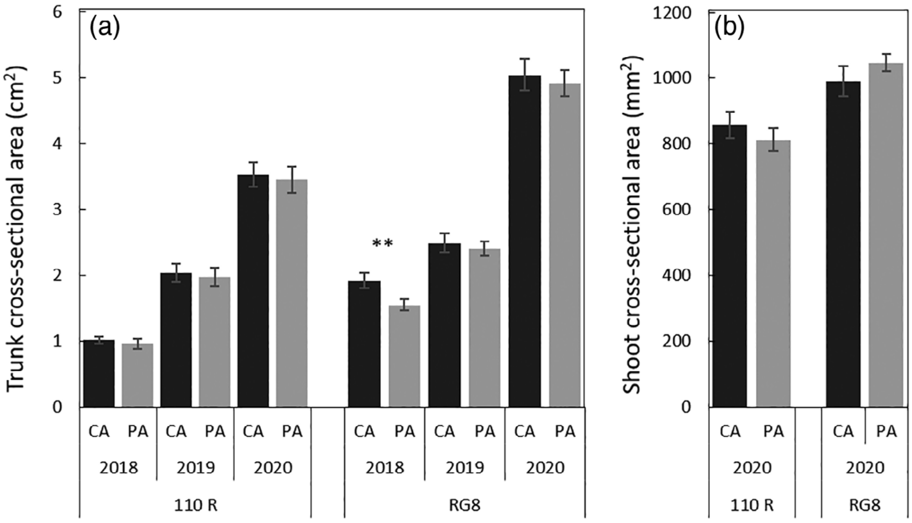

Figure 2. Effect of grafted vines with complete alignment of the scion and the rootstock (CA) and grafted vines with partial alignment of the scion and the rootstock (PA) on (a) trunk cross-sectional area and (b) shoot cross-sectional area in the field trial during 2018–2020. Each bar represents the mean value of all the plants of a combination ± SE. Sample size = 40 vines. Significant differences regarding the degree of alignment according to two-sample *t* test are indicated by asterisks symbols: **, $P \leq 0.01$.

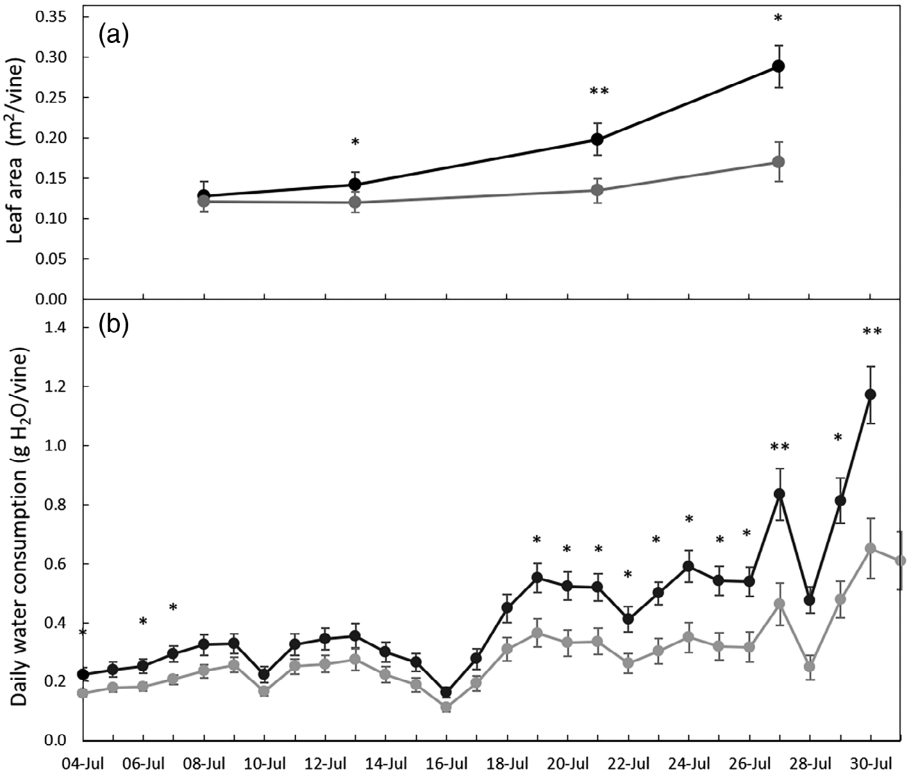

Figure 3. Effect of pot vines of Tempranillo grafted onto RG8 rootstocks with complete alignment of the scion and the rootstock (●) and vines with partial alignment of the scion and the rootstock (●) on the evolution of (a) leaf area and (b) daily water consumption. The vines were grown in a mini-lysimeter greenhouse phenotyping platform. Each point represents the mean value of all plants for each degree of alignment ± SE, for a specific day of measurement. Significant differences regarding the degree of alignment according to two-sample *t* test are indicated by asterisks symbols: *, $P \leq 0.05$; **, $P \leq 0.01$.

The canopy stomatal conductance g_c [mmol/(m^2 · s)] was calculated using the simplification suggested by Charrier et al. (2018) and Dayer et al. (2020) (Equation 2):

$$g_c = K_G\,(\mathrm{T}) \times E/\mathrm{VPD} \qquad (2)$$

where K_G (T) is the conductance coefficient in (kPa · m^3)/kg (Ewers et al. 2001) accounting for temperature effects on the psychrometric constant, latent heat of vaporisation, specific heat of air at constant pressure, and the density of air (Phillips and Oren 1998), E is the plant transpiration [mmol/(m^2 · s)], and VPD is the vapour pressure deficit (kPa) calculated from the air temperature and RH data (from the greenhouse weather station), using the formula proposed in the infrared gas analyser manual WALZ GFS-3000.

Water status of the potted grapevines during the experiment was monitored by measuring the pre-dawn leaf water potential (ψ_{PD}) on three plants per treatment. Measurements were performed six times throughout the trial (every 3–4 days), in a basal fully expanded leaf prior any light exposure (between 0500 and 0600) using a Scholander pressure chamber (SAM Precis 2000, Gradignan, France).

Measurement of xylem hydraulic conductivity. After the end of the mini-lysimeter experiment, plants were maintained under controlled conditions throughout the summer. The last week of September 2020 hydraulic conductivity was measured in the ten plants used in the experiment, by applying the gravimetric method proposed by Torres-Ruiz et al. (2012) in a plant portion containing the graft union. To prepare the samples, a cut was made first in the rootstock part to discard the root system, after submerging the plant in water to avoid the entry of air when cutting. Then, both shoots were gently cut until reached a proper length to be conveniently transported to the laboratory, always submerged in water. Once in the laboratory, and just before carrying out the measurement of one plant, it was recut until obtaining a piece of plant with a rootstock length of 10 cm and a length of 4.5 cm for each shoot, to minimise size differences between samples. Then, both shoots were connected via a tubing system to a tank containing a 20 mmol/L KCl solution, and a flow was allowed to pass through the sample to a Sartorius CPA225D precision electronic balance (Sartorius, Göttingen, Germany)

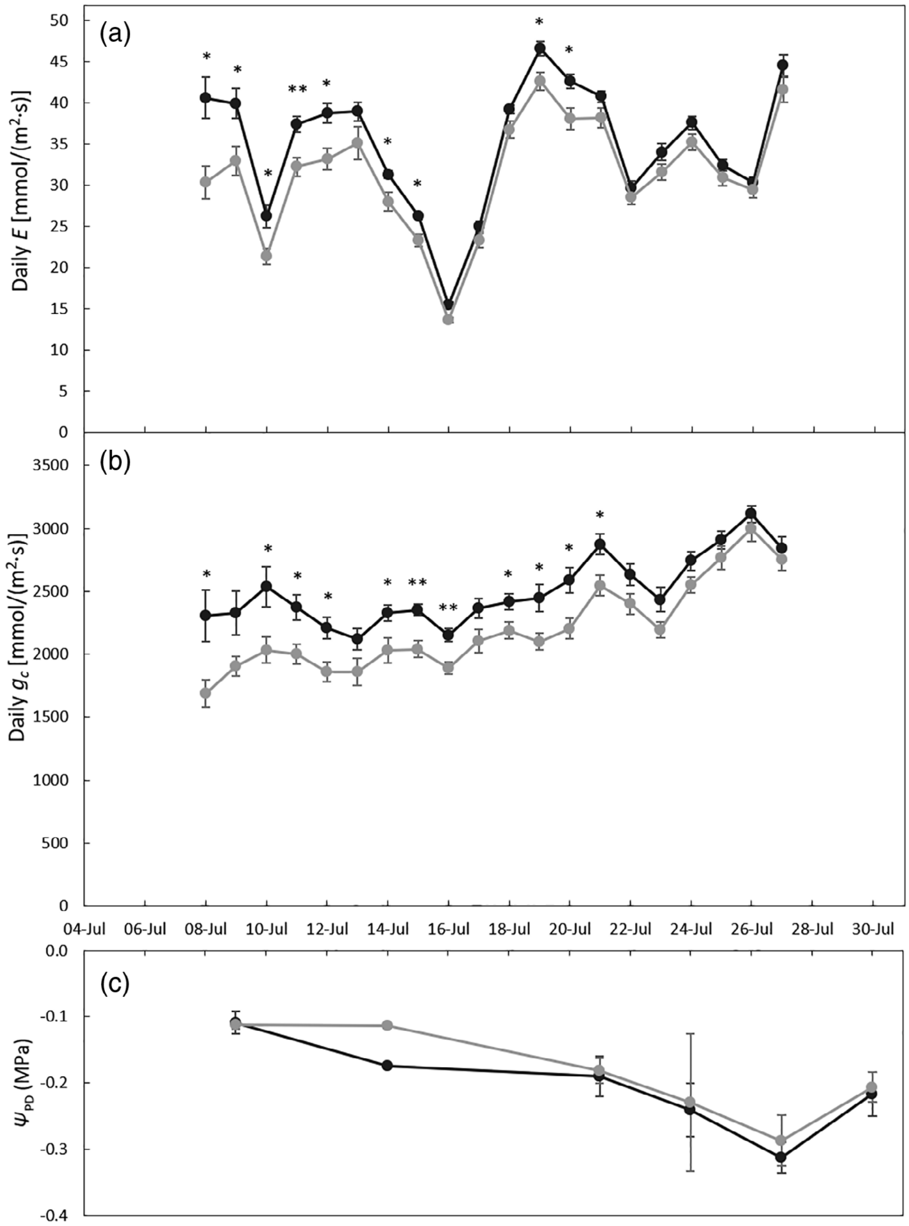

Figure 4. Effect of pot vines of Tempranillo grafted onto RG8 rootstocks with complete alignment of the scion and the rootstock (●) and vines with partial alignment of the scion and the rootstock (●) on the evolution of (a) daily transpiration rate (E), (b) daily canopy stomatal conductance (g_c) and (c) pre-dawn leaf water potential (ψ_{PD} measured between 0500 and 0600) expressed per vine. The vines were grown in a mini-lysimeter greenhouse phenotyping platform. Each point represents the mean value of all plants for each degree of alignment ± SE, for a specific day of measurement (daily sum). Significant differences regarding the degree of alignment according to two-sample t test are indicated by asterisks symbols: *, $P \leq 0.05$; **, $P \leq 0.01$.

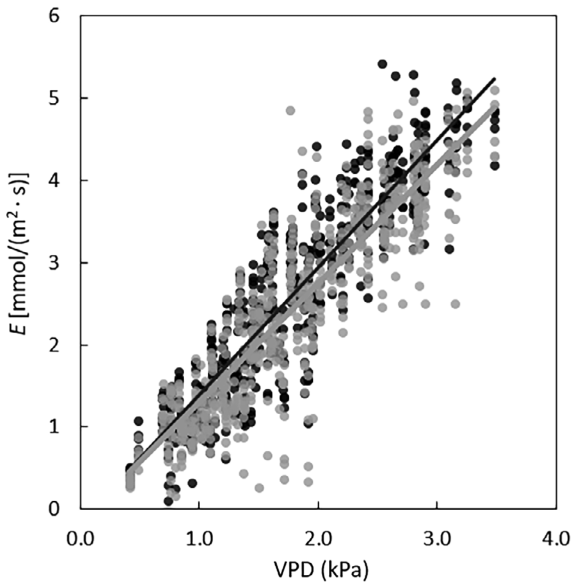

Figure 5. Effect of pot vines of Tempranillo grafted onto RG8 rootstocks with complete alignment of the scion and the rootstock (●) and with partial alignment of the scion and the rootstock (●) on the relationship between hourly plant transpiration rate (E) and vapour pressure deficit (VPD) registered between 1000 and 1500. The vines were grown in a mini-lysimeter greenhouse phenotyping platform, during 20 days of the trial (8 July to 27 July). Significant differences were found for the slope ($P < 0.0001$) of the linear regression lines. Complete alignment $y = 1.5478x - 0.1595$, $R^2 = 0.8296$; partial alignment $y = 1.4462x - 0.1522$, $R^2 = 0.7795$.

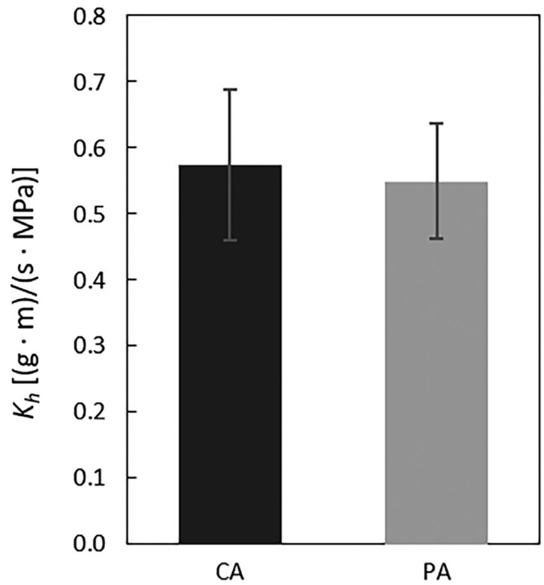

Figure 6. Effect of pot vines of Tempranillo grafted onto RG8 rootstocks with complete alignment of the scion and the rootstock (CA) (■) and vines with partial alignment of the scion and the rootstock (PA) (■) on the hydraulic conductivity (K_h). The vines were grown in a mini-lysimeter greenhouse phenotyping platform. Bars represent the mean values for each degree of alignment ± SE.

recording the mass every 5 s through WinWedge v3 5.0 Standard Edition (TAL Technologies, Philadelphia, PA, USA) at seven increasing pressures. The pressure was enhanced by raising the source height and determined by Newton gravitational law, where 1 m height corresponded to 9806.65 Pa. The average flow for each pressure step was determined when flux stabilised as the average of 10–15 measures. Hydraulic conductance, k [g/(s · MPa)] was obtained by the linear coefficient of the slope generated by flux and the corresponding pressure gradient. For all the samples, the linear relationship between flux and pressure obtained was characterised by $R^2 > 0.95$.

The length of the rootstock and shoot portions was measured with an electronic calliper in order to standardised data. Finally, hydraulic conductivity [(g · m)/(s · MPa)] was calculated as (Equation 3):

$$K_h = k \times L\,(\mathrm{m}) \tag{3}$$

where L was the sum of the length of the rootstock and of the two shoots.

Statistical analysis

Statistical analysis was carried out with R version 4.1.0. (R Core Team 2020) and Rstudio (version 1.4.1103) software (RStudio Team 2021). For all the trials, outliers were removed before carrying out the different analyses, using the identify_outliers tool from the *rstatix* package (Kassambara 2020). The number of outliers removed did not exceed 5% of the values in any case. The dependence between the degree of alignment and SR results was evaluated by means of a Pearson's chi-square test for each batch. Growth data obtained from the nursery and the field experiments were expressed as means ± SE. After confirming their normality with the Shapiro–Wilk's normality test, growth and gas exchange data from nursery, field and greenhouse plants were subjected to Student's t test to assess the statistical difference ($P \leq 0.05$) between the two levels of connection developed in the model at each time point. Linear regression between E and VPD was calculated for each degree of alignment, and the slope was compared at $P < 0.05$ using analysis of covariance in R.

Results

Nursery trials: evaluation of success rate and growth measurements

A complete alignment between the scion and the rootstock cuttings (CA) resulted in a higher SR than for PA, ranging from 5 to 16%, this effect being significant in batches 1 and 3 grafted with 110 R rootstock (Figure 1). Above-ground growth was significantly different between alignments with CA vines showing higher SCSA (Table 2). Similarly, the total growth estimated through the increase of rootstock cane cross-sectional area was greater in CA vines (Table 2). There was no difference in root development, with no change for either root number or size (Table 2).

Field trial: effect of the degree of alignment on agronomic parameters

Trunk growth values from the field trial showed higher growth of CA vines with respect to PA vines for the RG8 rootstock in 2018, but these differences dissipated over the years. For 110 R, field growth differences did not reach a significance level in any of the seasons (Figure 2a). There was also no significant difference in shoot development during 2020 between the alignments (Figure 2b). Therefore, we can state that, over the years evaluated, there was no effect of treatment on growth in the field once the vines were fully established.

Mini-lysimeter phenotyping platform trial: effect of the degree of alignment on water use and hydraulic behaviour

We characterised the water use for each degree of alignment of the grafted material by continuously monitoring water use of CA and PA vines grafted onto RG8 under semi-controlled conditions. From the beginning of the trial, the two different connection levels showed a differentiated behaviour, partly related to the difference in the LA evolution throughout the trial (Figure 3a). The CA and PA vines had a similar LA value (about 0.12 m^2/vine) when the trial started, but CA vines significantly increased LA compared to PA vines along the season, resulting in twofold LA growth rate. The CA vines exhibited higher water consumption than PA vines over the duration of the experiment, even prior to the establishment of differences in LA (Figure 3b). Such a difference became more pronounced, however, from 19 July to the end of the experimentation coinciding with an increasing difference in LA.

The CA vines generally showed higher whole-vine transpiration (E) and canopy stomatal conductance (g_c) values, especially at the beginning of the experiment when there was no difference between CA and PA LA (Figure 4a,b). The vine water status decreased throughout the season in CA and PA vines (Figure 4c). Despite the differences observed between the two treatments for transpiration and stomatal conductance parameters, there was no significant effect on water potential values (ψ_{PD}) throughout the trial. A significant positive relationship between E and VPD was observed for both CA and PA vines (Figure 5). Therefore, increased E values were associated with increased VPD values across a range of 0.5–3.5 kPa, finding a significant difference between the slope of the regression lines at $P < 0.0001$.

Finally, we determined the hydraulic conductivity (K_h) across the grafts at the end of the trial. We found that the mean K_h values measured in PA vines were 4% lower than those from CA vines, although these differences were not statistically significant ($P \leq 0.05$) (Figure 6).

Discussion

To date, grafting knowledge and techniques are essentially based on the practical experience of nursery operators, rather than on scientific study (Milien et al. 2012). To the best of our knowledge, this is the first scientific study which aims to increase the understanding of the effect of an adequate alignment of plant material during omega type bench grafting on the agronomic behaviour, physiology and hydraulic conductance of grafted grapevines, both in the nursery and in the vineyard.

Nursery success rate

In our study CA grafts had considerably higher SR for all the combinations analysed, differences being significant for the 110 R rootstock for both years of the study. Previous studies evaluating different grafting techniques also showed a clear effect of the graft characteristics on SR. Cui et al. (2019), in their study of micrografting, green grafting and omega bench grafting as possible methodologies for evaluating graft incompatibility of grafting virus-infected scions onto healthy grape rootstocks, found the best SR for the omega technique. Çelik (2000) evaluated the grafting success of three cut manual grafting units and found the best results for chip budding, followed by the cleft graft and finally the omega graft. Cangi and Etker (2018) claimed that some characteristics of the plant material, such as its health status, preservation conditions and rooting capacity, nutrition and lignification of the rootstocks, are key factors influencing the success of grafted grapevine plants. Our results provide evidence of the importance of the alignment of the material during grafting for guaranteeing vine success in addition to the abovementioned intrinsic factors. Similarly, Cangi and Etker (2018) quoted that, prior to grafting, rootstocks and scions should be classified according to their diameter to improve grafting productivity, and the reason is that there is a need for contact between the cambial tissues of both individuals to achieve a successful grafting (Sabir 2011).

Grapevine growth

Vine growth was remarkably affected by the alignment between the rootstock and scion, highlighting the relevance of a good connection for vine performance. This effect could be observed at the three experimental levels considered: (i) nursery, where CA vines showed an increased shoot and total vine growth; (ii) field, where CA had increased trunk growth in the first season; and (iii) greenhouse, where CA vines developed a LA considerably higher than that of PA vines. This behaviour could be linked to the differences in xylem vessel development that arise when rootstock and scion cuttings are not properly aligned at the time of grafting (Milien et al. 2012). These authors, using X-ray tomography observations on a single vine, reported that when alignment was adequate the surface of new xylem developed at the scion area in the first 8 months (nursery stage) accounted for a threefold increase compared to the xylem area at grafting, whereas with severely forced misalignment, the newly developed xylem area was even smaller than the xylem area at grafting. Regarding root development, no differences were observed in root number and total cross-sectional root area developed in the rooting field. Similarly, Tedesco et al. (2020) found that the overall graft takes correlated better with the scion growth and with the proliferation of callus tissue around the union rather than with the root length and the stem diameter below the union.

Nevertheless, the differences in vine growth initially observed gradually disappeared in the field (years 2 and 3). This fact could be indicating that the development of new xylem and phloem allows the initial connection limitation to be overcome, in agreement with Pratt (1974), who indicated that two to three annual increments of functional phloem and xylem are enough to sustain growth and production in adult plants. For the establishment of our experimental field, we used only vines that had overcome quality control in the nursery trial, removing the most limiting connections, which has probably contributed to the smaller difference between batches.

The implications of the differences in the connection between the rootstock and scion hardwood cuttings apparently fade as the vine age, however, it is necessary to keep in mind that it could have other negative consequences on the future development of the vines. Misalignment results in an increase of necrotic wood tissue at the graft junction (Milien et al. 2012) that could favour the development of wood fungi inside the vine (Gambetta et al. 2009, Foglia et al. 2022) and might have a negative effect on vineyard performance in the mid-term and even reduce its longevity.

Water use and hydraulic conductivity

Based on the differences in SR and initial growth parameters observed between CA and PA vines, we hypothesised that differences in rootstock and scion cane alignment could affect gas exchange and plant water use. The CA vines exhibited a higher level of transpiration and canopy conductance which likely contributed to their increased growth rate (i.e. greater LA). The differences in water consumption are clearly marked by variations in LA development; however, when water consumption is expressed per unit LA (i.e. transpiration rate), significant differences are still visible, especially at the beginning of the trial when the differences in LA were not so noticeable. Therefore, CA vines exhibited significantly higher vigour than PA vines and, consequently, higher water consumption rates which was likely explained by the different degree of alignment. Similarly, Torres et al. (2021) found that increased transpiration, stomatal conductance and stem water potential were associated with the increment of leaf biomass of adult Cabernet Sauvignon grapevines, accounting for a higher carbon assimilation rate and intrinsic water use efficiency.

Under our experimental conditions, a linear relationship between E and VPD was revealed in accordance with previous studies conducted under well-watered conditions that found that VPD is a main driver of transpiration (Oren et al. 1999, Schultz and Stoll 2010, Devi and Reddy 2018, Dayer et al. 2020). Additionally, CA vines had a slightly higher water use over a wide range of VPD compared to that of PA vines which is consistent with the results outlined above.

Our experimental set-up did not reveal differences in hydraulic conductivity across the graft at the end of the experiment. Based on the results reported earlier, it could have been expected that the CA vines would have higher K_h values across the graft; however, our study suggested that the graft itself did not limit water use in the PA. Previous researchers reported that the graft interface itself is unlikely to affect the scion development directly when connections are totally formed because the graft union offers little resistance to water movement [reviewed by Gautier et al. (2019)]. In this sense, Alsina et al. (2011) found that changes in hydraulic conductance were not explained by the xylem anatomy, while Gambetta et al. (2009) observed a well-integrated xylem network across the graft union despite the presence of shorter open-conduit lengths in grafted vines. Additionally, it is well established that the cambium becomes mitotically active and forms a considerable body of secondary vascular tissue during the first growing season, and after that, small increments of vascular tissues are required for sustaining vegetative growth (Pratt 1974) as could happen under our experimental conditions minimising growth differences between CA and PA vines.

Conclusion

Our results show that the connection at the grafting point has a clear effect on the success rate in the nursery, plant development during the first year in potted and field vines, and on plant water relations in potted vines. The implications of graft connection in the field are still to be elucidated since, although in our trial differences in vine growth disappeared over the years, yield and general performance data are still to be obtained, and other negative aspects associated to poor alignment causing more necrotic wood in the graft area might arise.

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Author contributions

L.G. Santesteban conceived and designed the trials conducted in Spain and acquired the funding. S. Dayer, G.A. Gambetta and J.M. Torres-Ruiz designed the trial in pots conducted in Bordeaux. D. Marín, S. Dayer, A. Villa-Llop and F.J. Abad performed the experiments, measurements and trial evaluation. L.G. Santesteban and G.A. Gambetta supervised the trials. D. Marín and N. Torres conducted the statistical analysis, and wrote the first version of the manuscript. D. Marín, N. Torres, L.G. Santesteban, S. Dayer, G.A. Gambetta and J.M. Torres-Ruiz revised and edited the manuscript. All authors approved the final version of the manuscript.

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Supporting information

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Figure S1. Examples of (a) completely aligned scion and rootstock cuttings (CA) and (b) partially aligned scion and rootstock cuttings (PA). (c) Nursery operator manually performing the thumb test to assess the robustness of the union between the scion and the rootstock.