


From structural constraints to hydraulic function in three *Vitis* rootstocks

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Abstract

Key message Narrow stem size in limiting *Vitis* rootstocks imposes a morphological constraint on the scion via reduced annual ring size, and thus reduces hydraulic conductivity and subsequently physiological performance and yield.

Abstract Graft is a union between two separate species or cultivars, which produces a chimera plant with new qualities—as rootstock affects scion growth, yield, and adaptability to different environmental conditions. In *Vitis*, it is possible to generate rootstock/scion combinations that produce a desired drought stress effect crucial for high-quality wine production, though the mechanisms for such interactions are complex and poorly understood. The current study was done on vines with an identical scion (*Vitis vinifera* ‘Cabernet Sauvignon’) grafted on three different rootstocks—either Riparia Gloire, Paulsen 1103 or 420A—in attempt to explain the differences in water status by examining the underlying anatomical constraints and calculated theoretical hydraulic conductivity. There was a significant difference in physiological responses and yield

between the grafts. Riparia Gloire grafts had the lowest water potentials and the highest quality grapes, together with low root, scion stem, and branch theoretical hydraulic conductivity. In scions grafted on Riparia Gloire, the annual growth rings were significantly narrower than in the other two grafts, causing a significantly lower theoretical hydraulic conductivity per annual ring. The narrow annual ring size in scion stem was imposed by the morphological constraint of the stem size. In hydraulically inferior Riparia Gloire grafts, the difference was disproportionately large, with a wide scion grafted on a very narrow rootstock, and Paulsen 1103 had the smoothest graft union. Our results indicate that the ability to develop stronger drought stress in *Vitis* grafts depends on rootstock-imposed morphological restriction of hydraulic conductivity.

Keywords Functional anatomy · Xylem · Hydraulic conductivity · Grafts · *Vitis vinifera*

Introduction

We thus see that although there is a clear and fundamental difference between the mere adhesion of grafted stocks and the union of the male and female elements in the act of reproduction, yet that there is a rude degree of parallelism in the results of grafting and of crossing distinct species (Charles Darwin 1859).

Graft is a union between two separate species or cultivars, which produces a chimera plant with new qualities—as rootstock affects scion growth, yield, and adaptability to different environmental conditions. Though rootstocks have been extensively used for fruit tree propagation for at

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least 2000 years (Webster 1995), their effects on scion physiology are still not fully understood. Over the years, several attempts of mechanistic explanations of this phenomenon were presented. Those can be roughly divided into three categories: (1) hormonal effects (*Lycopersicum*, Albacete et al. 2009; *Gossypium*, Dong et al. 2008; *Vitis*, Skene and Antcliff 1972), (2) assimilate and nutrient movement (*Vitis*, Tardaquila et al. 1995; *Malus*, Jones 1976), and (3) water status (*Malus*, Cohen and Naor 2002; *Olea*, Nardini et al. 2006; *Vitis*, Alsina et al. 2011; Tombesi et al. 2010a). The overall picture is quite complex and the data are sometimes contradictory.

Vitis grafting is a routine agricultural practice. *Vitis vinifera* is the commonly grown *Vitis* species in the Old World, while in North America, *Vitis riparia*, *Vitis labrusca*, and *Vitis rotundifolia* are frequently used for grape and wine production. Cross breeding among *V. berlandieri*, *V. riparia*, and *V. rupestris* has produced several widely used rootstocks. Currently, many commercial rootstocks are available, each having well-known characteristics. Rootstocks are selected on the basis of their performance in different soil types, water requirements, and disease susceptibility—while the scion is selected mainly on the basis of vigor and yield quality. In red grapevine cultivation, yield quality (rather than quantity) is the critical element, which depends mainly on the development of a mild drought stress during growth period—while excessive irrigation induces vigorous canopy growth that leads to both shading and reduced carbohydrate partitioning to reproductive organs (Bravdo and Hepner 1987). Drought stressed vines produce grapes with higher phenol and anthocyanin content that are essential for high-quality red wine production (Bravdo et al. 1985; Kennedy 2002; Castellarin et al. 2007). For instance, a mild drought stress of down to -1.2 MPa at midday for *V. vinifera* cv. Cabernet Sauvignon was shown to be the most effective threshold to optimize soil water availability, irrigation scheduling, yield, and grape quality (Acevedo-Opazo et al. 2010). Drought stress can be induced by reducing irrigation, but this is not always achievable due to either high precipitation or soil structure (terroir). It is possible to generate rootstock/scion combinations that produce a more desired wet soil tolerance (Christensen 2003), though the mechanisms for these beneficial effects are complex and poorly understood.

In the study reported here, significant differences in water status and subsequently yield parameters were found in vines with the identical scion (*V. vinifera* cv. ‘Cabernet Sauvignon’) grafted on three different rootstocks in clay soil with relatively high water content. The current study explains the differences in hydraulic performance by analyzing the underlying anatomical structure of the scion-graft combinations in roots, stems, and branches (shoots).

We undertook an indirect approach of assessing the hydraulic conductivity (calculated based on anatomical measurements)—as a direct measurement of hydraulic conductivity is not feasible in grapevine trunks. In grafted vines, the trunks are the most important structural element, which both combine the rootstock with the scion and integrate the multi-annual hydraulic history of the plant. Though theoretical calculated axial hydraulic conductivity is by no means fully comparable to measured values, they have been found to have a close correlation in *Vitis* branches (Lovisolo and Schubert 1998; Lovisolo et al. 2002).

Materials and methods

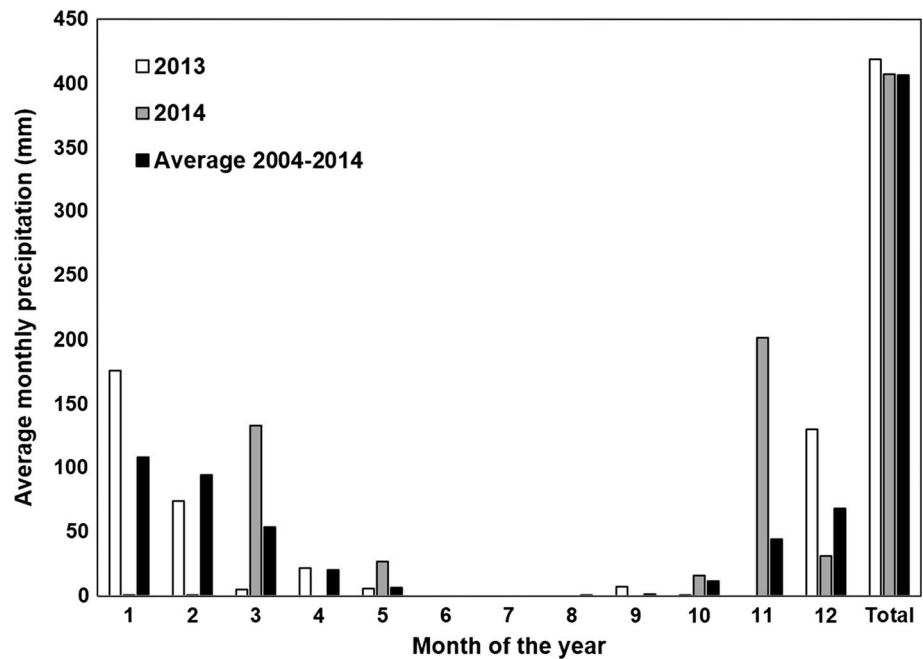
Experimental site

The vineyard examined in this study over two successive years (2013–2014) was planted in 2007. The experimental vineyard was a part of large commercial vineyard, located in the Judean mountain region, with deep clay soil with high stoniness and medium calcite (lime) content. Climatic data were obtained from regional meteorological station located 3 km from the experimental site. The region has semi-arid climate with winter dominant rainfall (Fig. 1). There was a large difference in monthly precipitation between 2013 and 2014, with 2014 being an unusual year (minimal mid-winter rains and pronounced late-winter rains), though the total annual precipitation was similar (Fig. 1).

Plants and agricultural practice

Three treatment groups were examined (graft combinations): *V. vinifera* L. ‘Cabernet Sauvignon’ was grafted on either (1) Riparia Gloire (*V. riparia*), (2) Paulsen 1103 (*Vitis berlandieri* × *Vitis rupestris*), or (3) 420A (*V. berlandieri* × *V. riparia*) rootstock. The grapevines were trained onto a two-wire vertical trellis system. Row direction was east/west, and vine and row spacing were 1.5 and 3 m, respectively (4.5 m² per vine). Winter pruning was conducted according to local practice (15 spurs per vine, two nodes per spur), thus rendering the plant height almost similar. Due to agricultural practice of uniform pruning, variance in canopy structure between the plants was negligible—which rather simplified the experimental design. Pest management and fertilization in the vineyard were performed according to local agricultural standard practice. Irrigation was minimal, 24–30 mm per season applied during August–September (12–18 mm before harvest during 2013 and 2014, respectively, and additional 12 mm after harvest). Irrigation was applied by an above-ground drip line positioned near the row. Harvest was conducted in October.

Fig. 1 Monthly precipitation during the years of the research, compared to a multiple years average of 2004–2014. Climatic data were obtained from regional meteorological station located 3 km from the experimental site



Experimental design

The experimental layout was a completely randomized block design with three treatments (rootstocks) each replicated six times, where each block consisted of one row. In each block, treatments comprised of 15 vines per plot with the outer two vines from each side being border vines and measurements conducted on the inner 11 vines (a total of 198 measurement vines, i.e., 11 vines \times 3 treatments \times 6 replicates). At each plot, three vines, representative of canopy size and trunk diameter, were marked and used for physiological measurements.

The growing season was divided into three phenological stages according to Kennedy (2002): stage I (from bloom to bunch closure), stage II (from bunch closure to veraison), and stage III (from veraison to harvest). The physiological parameters and leaf area index were measured at the end of each stage, yield parameters were measured at the end of stage III, and the anatomical parameters were measured 1 month after harvest.

Leaf area index (LAI)

Leaf area index (LAI) is the ratio of total green surfaces, including leaves, shoots, and fruit (when present), to unit of land area allocated for each vine. Leaf area of three representative vines per plot was determined at the end of each phenological stage using a non-destructive SunScan canopy analysis system (model SS1- R3-BF3, Delta-T Devices, Cambridge, UK). Eight radiation measurements were taken underneath each vine (spaced every 20 cm

covering the soil surface completely under a given vine (for details, see Netzer et al. 2009). To verify the LAI values obtained using this non-destructive method, a leaf area validation was performed using a destructive method. Direct measurement of leaf area was performed on different plants following leaf defoliation from 35 vines at different phenological stages for several different cultivars from different vineyards. Leaf area was then measured using an area meter (model LI-3100, Li-Cor, Lincoln, NE, USA). Estimated and measured LAI values were highly correlated with one another ($y = 0.663x + 0.17$, $r^2 = 0.911$, $p < 0.0001$, $n = 35$).

Physiological parameters

Midday stem water potential (Ψ_s) was measured at solar noon, using pressure chamber (Arimad-3000, MRC, Holon, Israel) according to Kramer and Boyer (1995). Two sunlit, mature, fully expanded leaves from each plot (12 leaves per treatment) were bagged 2 h prior to measurement in plastic bags covered with aluminum foil. The time passed between leaf excision and chamber pressurization was less than 30 s.

Leaf net CO_2 assimilation rate (A) and stomatal conductance (g_s) were measured on 3–4 leaves per plot (18 leaves per treatment), using a portable gas exchange system (LI-6400, Li-Cor, Lincoln, NE, USA). Gas exchange parameters were recorded, and at the same time, Ψ_s measurements were taken; leaves with similar characteristics were chosen for Ψ_s and gas exchange measurements. All physiological measurements were taken 1 day before irrigation.

Yield measurements

The yield measurements were done between the years 2011–2014, thus including two additional years prior to the start of the main research, as a part of a preliminary study. Each plot was harvested when the berry total soluble solids (TSS) reached 23.5°Brix. All 11 measuring vines within each plot were harvested, and number of clusters and yield per vine were recorded. A week before harvest, 36 bunches per plot, randomly chosen, were hand crushed for determination of total soluble solids and pH. At the end of stage III, 100 berries per plot were randomly sampled and weighted, and berry mass was determined.

Anatomy

Sampling

The samples for the anatomical observations were obtained 1 month after the end of stage III. Due to practical considerations, a destructive sampling was not possible, and the sampling was as unobtrusive as possible. *Roots*: Six root samples were sampled with a hoe from every treatment 20 cm from the plant stem, below the drip line. The roots were sampled as uniform as possible, without strong visible suberisation and of similar diameter. The root samples were immediately fixed in FAA (5:5:90, formalin:acetic acid:70% ethanol). *Branches*: Six 6–7-month-old branches (shoots) were sampled and put into polyethylene bags with wet paper towels until further processing in the lab. Branches were sampled with pruning shears; uniform branches were taken from between internodes two and three. *Stems*: Twelve stem cores per treatment were sampled and put into polyethylene bags with wet paper towels until further processing in the lab. Stem cores were taken both from rootstock and from scion. Trunk diameters at the drilling location were measured to calculate annual ring area later. Core samples were collected with 5 mm diameter increment borer (5.15 mm Core 3-Thread Increment Borer, 8", Hagölf, Sweden) and stored at 4 °C temperature in distilled water until sectioned. Rootstock stems were sampled 1 cm above the ground. Scion stems were sampled 50 cm above the ground. Two plants were sampled for each plot (1 scion + 1 rootstock sample per plant; 6 per treatment × 3 blocks, total 82 samples).

Histology

FAA fixed root fragments were dehydrated in a graded alcohol series and then embedded in paraffin wax (Paraplast plus, Leica). Cross sections (12 µm) were cut using a rotary microtome (Leica, Germany) and stained with Toluidine Blue O (O'Brien et al. 1964). Branch cross

sections were obtained by hand sectioning of fresh plant material. Stems were cross sectioned by sliding microtome (NR17800, Reichert, Austria) at thickness of 90 µm. Stem and branch sections were stained with phloroglucinol-HCl (Ruzin 1999). The sections were viewed and photographed under a stereo microscope (Olympus SZ2-ILST) equipped with a camera (Olympus LC20).

Image analysis

Image analysis was done using the ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, MD, USA, <http://imagej.nih.gov/ij/>, 1997–2015). Analysis of the roots and the branches was done on the whole section. Analysis of stem cross sections was performed by quantifying various parameters in the visible field separately for the three last annual growth rings (2012–2014). Thousands of vessels were measured simultaneously using Analyze Particles option, while the measured parameters were vessel area (A) and number (N). Vessel area (A) was later converted to diameter (d), as: $d = \sqrt{\frac{4A}{\pi}}$. Vessel number (N) was used to calculate density (D) as: $D = \frac{N}{A}$. Ring and bark diameter were measured in stem sections and used for ring area calculations. Vascular area (A_w) was measured in roots and branches—as the area excluding bark, cambium, and vascular cylinder/pith.

Specific hydraulic conductivity calculations

Theoretical specific hydraulic conductivity (k_s ; $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$) was calculated using the modified Hagen–Poiseuille's equation (Tyree and Ewers 1991):

$$k_s = (\pi\rho/128\eta A_w) \sum_{i=1}^n (d_i^4),$$

where k_s is the specific hydraulic conductivity, ρ is the density of the xylem sap (assumed to be 1000 kg m^{-3}), η is the dynamic viscosity of the xylem sap (assumed to be $1 \times 10^{-9} \text{ MPa s}^{-1}$), A_w is the area (m^2) of the xylem cross section measured, d is the diameter (m) of the i th vessel, and n is the total number of the vessels in the measured area. Hydraulic conductivity per annual ring ($\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$) was achieved by multiplying the theoretical xylem-specific hydraulic conductivity by annual growth ring area.

Statistical analysis

Analysis of variance (ANOVA) (JMP Pro 11 Statistical Software; SAS Institute Inc., Cary, NC, USA) was used to determine differences between treatments at $p \leq 0.05$. Tukey post hoc means comparisons were made to compare the significantly different treatments.

Results

Graft physiology and general observations

Visual observation of the different grafts revealed noticeable differences in stem morphology (Fig. 2a). There was a very large difference in stem diameter between Riparia Gloire rootstock and scion (almost 15 mm), a small but significant difference in 420A, while in Paulsen1103, rootstock and scion stem diameters were not significantly different (Fig. 2b). Paulsen1103 rootstock had the widest stem diameter and Riparia Gloire rootstock had the narrowest one. Interestingly, in Paulsen 1103 grafts, the scion diameter was also significantly the widest.

There were significant differences in various yield parameters between the grafts (Table 1). The yield parameters were taken over four consecutive years (2011–2014), including 2 years (2011–2012) of preliminary study. The general trends were preserved during the whole period. Plants grafted on 420A had the highest yield and cluster number, and Paulsen had the highest berry weight. 420A berries had the lowest sugar content (°Brix). Plants grafted on Riparia Gloire had lower yields, which yielded fewer berry clusters with smaller berries. Small berries are known to develop under drought stress (Bravdo et al. 1985) and are considered superior because of their higher skin-to-pulp ratio. In addition, Riparia Gloire berries had a significantly higher sugar content (°Brix) as compared to other two grafts—which is also an indicator of earlier ripening as a consequence of a drought stress (Munitz et al. 2016).

Different physiological parameters were examined during two consecutive years, 2013 and 2014 (Fig. 3). There were clear differences between the years, probably due to

high variation in precipitation distribution pattern. Though the physiological measurements in both years were made during the dry season (May–September), in 2014, the rains were unusually late and overlapped with the measuring season. In 2014, the leaf area index was higher as compared to 2013 (Fig. 3a, b). It is important to note that the decrease in leaf area index (LAI) during the measuring season was caused by routine green hedging (pruning) of the plant's canopy: the hedging was performed in July in 2013 (Fig. 3a), and in June in 2013 (Fig. 3b). The leaf area index was not significantly different between the rootstocks treatments in both years, though Riparia Gloire repeatedly showed the lowest values and Paulsen 1103 the highest ones. Leaf biomass was found to be an important factor in different hydraulic performance of grafted citrus trees (Rodríguez-Gamir et al. 2010), but in the current study, this parameter was equalized by canopy management agricultural practice.

In 2013, there were significant differences between the grafts in several physiological parameters. Riparia Gloire had significantly lower stem water potential (Fig. 3c), stomatal conductance (Fig. 3e), and CO₂ assimilation rate (Fig. 3g). On the other hand, in 2014, only stem water potential was significantly different between the grafts—when Riparia Gloire again had the lowest stem water potential of the three (Fig. 3d). In 2014, Riparia Gloire had lower values of stomatal conductance (Fig. 3f) and CO₂ assimilation rate (Fig. 3h), though the differences were not large and statistically insignificant. Paulsen 1103 had the highest physiological performance of the three grafts during 2013 (Fig. 3c, e, g). In 2014, the plants failed to develop a drought stress; all the water status values were higher than in 2013. In 2014, only in Riparia Gloire, the water potential approached -1 MPa, being much less

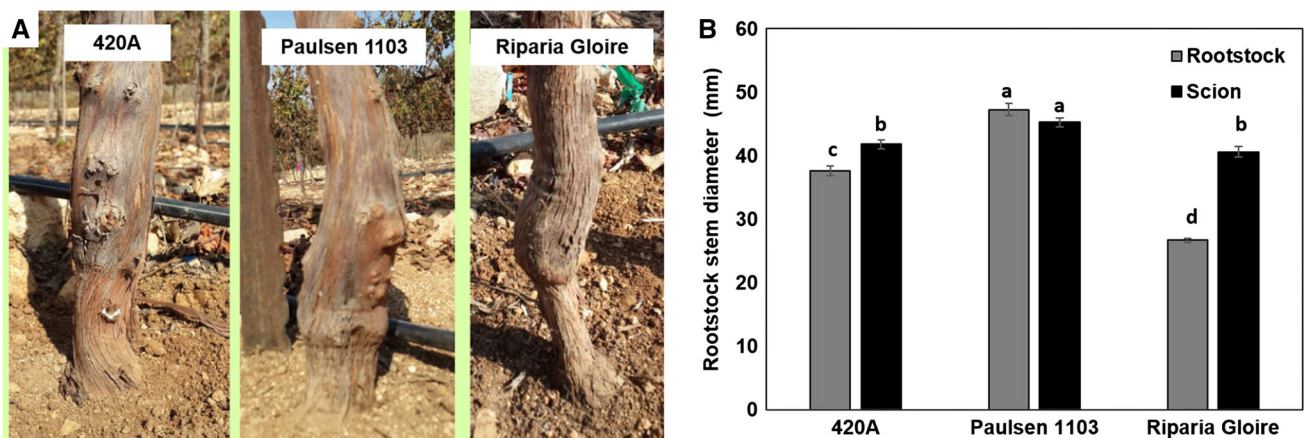


Fig. 2 Stem diameter in grafts of *Vitis vinifera* cv. Cabernet Sauvignon scions on three different rootstocks (420A, Paulsen 1103, and Riparia Gloire). **a** Examples of stem appearance at the graft union, **b** measured stem diameters. Each value is the mean of 12

vines of the same graft (12 rootstock stems + 12 scion stems) \pm standard error. Different letters indicate significant differences between rootstock versus scion at $p \leq 0.05$

Table 1 Yield parameters of *Vitis vinifera* cv. Cabernet Sauvignon scions grafted on three different rootstocks (420A, Paulsen 1103 and Riparia Gloire) in the years 2011–2014

	Year	Rootstocks		
		420A	Paulsen 1103	Riparia Gloire
Yield/plant (kg)	2011	9.90 a	9.40 ab	8.90 b
	2012	6.82 a	6.67 a	5.78 b
	2013	5.67 a	5.16 a	5.14 a
	2014	7.35 a	5.86 b	6.29 ab
Grape cluster N/plant	2011	52 a	49 a	47 a
	2012	37.5 a	38.3 a	35.5 a
	2013	26 a	25 a	25 a
	2014	35 a	30 ab	27 b
100 Berry weight (g)	2012	147 b	158 a	157 a
	2013	155 a	152 a	152 a
	2014	134 a	136 a	128 a
Grape must total soluble solids (°Brix)	2011	22.4 b	23.0 ab	23.8 a
	2012	25.0 b	25.7 a	25.9 a
	2013	24.42 b	24.96 ab	25.04 a
	2014	22.4 b	23.5 b	25.0 a
Grape must pH	2011	3.44 b	3.49 ab	3.51 a
	2012	3.56 a	3.53 ab	3.49 b
	2013	3.52 b	3.57 ab	3.58 a
	2014	3.71 b	3.79 a	3.64 c

Different letters indicate significant differences between rootstocks at $p \leq 0.05$

negative in Paulsen1103 and 420A—around -0.7 MPa (Fig. 3d). In comparison, during 2013, all the rootstocks reached a low water potential of about -1 MPa (Fig. 3c).

Anatomical characteristics and hydraulic conductivity

To understand the structure differences that underlay the variation in physiology, we examined the xylem anatomy of stems, roots, and branches. The rootstock stem vessel diameters were different, while the scion vessel diameters were very similar (Fig. 4). We analyzed both the average vessel diameter and separately large (over $100 \mu\text{m}$) vessel diameter—as large vessels have a significant impact on hydraulic conductivity (Tyree and Ewers 1991). Riparia Gloire rootstock stems had the widest vessel diameter (Fig. 4a) and the widest large ($>100 \mu\text{m}$) vessel diameter (Fig. 4b). Paulsen 1103 rootstock stems had the smallest maximal vessel diameter and fewer large vessels than the other rootstocks (Fig. 4c). Scion stems of all the grafts had similar vessel diameters (Fig. 4a, b). Similar trends could be seen in vessel size distribution frequencies (Fig. 4c, d). Rootstock stems had a large variation in vessel size frequency. Paulsen 1103 rootstock stems had less very small vessels (under $60 \mu\text{m}$) and less very large vessels (over $160 \mu\text{m}$) than the other two rootstocks, and the vessel distribution did not show the classical bimodal curve

(Fig. 4c). 420A and Riparia Gloire rootstock stems both showed a bimodal distribution, with Riparia Gloire having a larger percentage of both very small and very large vessels (Fig. 4c). Scion stem vessels were very similar in all the grafts (Fig. 4a, b, d), showing bimodal vessel distribution (Fig. 4d). Vessel distribution frequency in scions seemed most similar to 420A rootstocks. Vessel diameters in scion stems were larger than in the rootstock stems—vessels of over $280 \mu\text{m}$ were inexistent in rootstocks but comprised over 4% of the vessels in scions.

Stem hydraulic parameters showed very interesting tendencies (Fig. 5). Theoretical hydraulic conductivity per annual ring was significantly lower in the rootstocks as compared to scions, with 420A rootstock having the lowest conductivity (Fig. 5a). In scions, vines grafted on Riparia Gloire had the lowest conductivity. As hydraulic conductivity is influenced both by vessel size, vessel density, and growth ring area, it is important to inspect those parameters as well. The vessel density was similar between the rootstocks and the scions, with all the rootstocks having a slightly higher vessel density than the scions (Fig. 5b). This difference was highest in Riparia Gloire. Annual ring area was the smallest in Riparia Gloire as compared to other two grafts—both in rootstock and in scion (Fig. 5c). Paulsen 1103 rootstock had a significantly larger (though not by a large margin) annual ring area as compared to the Paulsen 1103 graft scion (Fig. 5c).

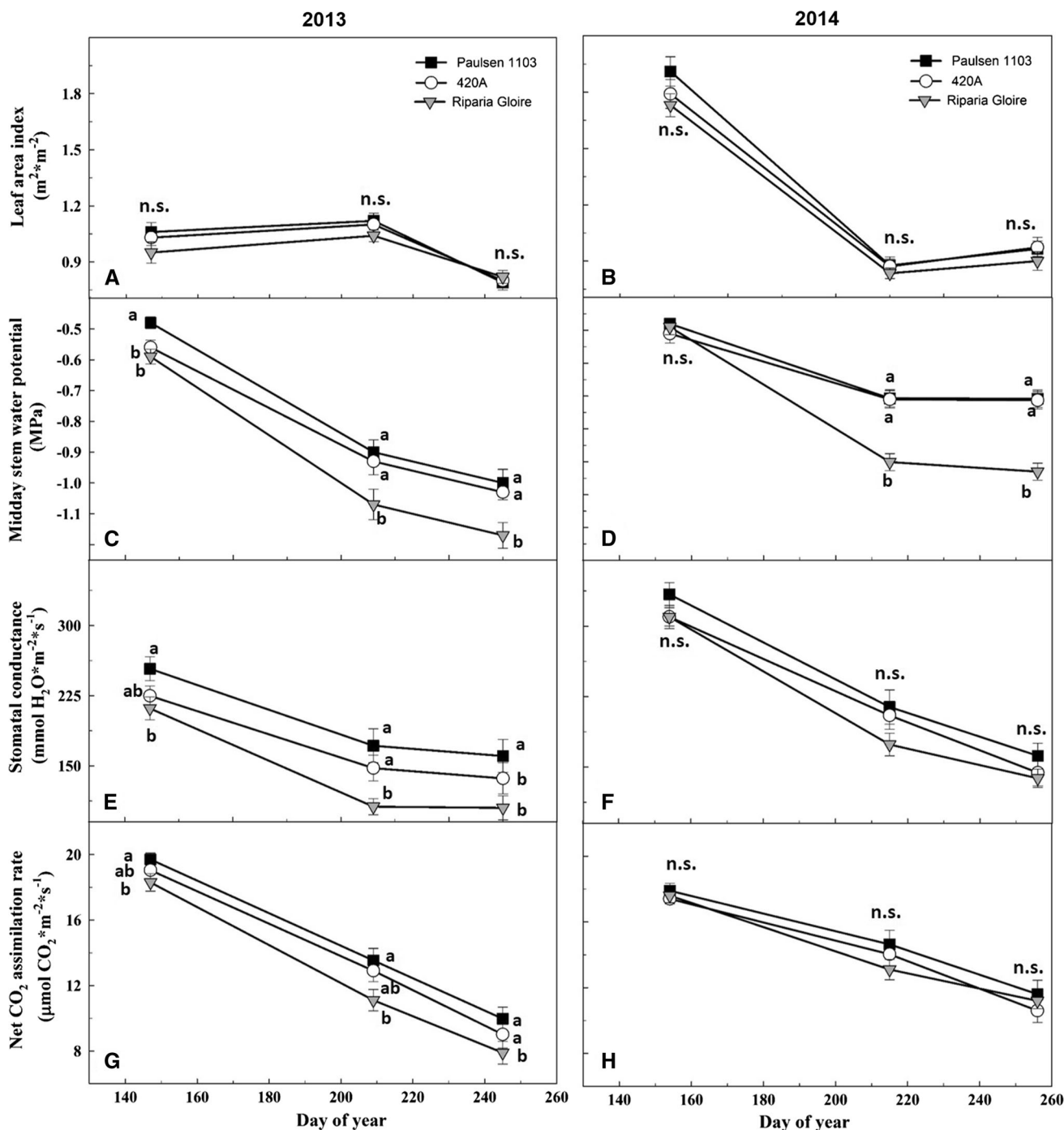


Fig. 3 Physiological parameters in grafts of *Vitis vinifera* cv. Cabernet Sauvignon scions on three different rootstocks (420A, Paulsen 1103 and Riparia Gloire) during the growth season in the years 2013 (a, c, e, g) and 2014 (b, d, f, h). Leaf area index (a, b), midday stem water potential (c, d), stomatal conductance (e, f), and

net CO₂ assimilation rate (g, h). The measurements were made during the dry season (May–September). Each value is the mean of 12 vines of the same graft ± standard error. Different letters indicate significant differences between rootstocks at $p \leq 0.05$

We also examined the vascular anatomy and calculated hydraulic conductivity of roots and branches (Fig. 6). In general, in all grafts roots had higher specific hydraulic conductivity than branches (Fig. 6). Such axial decrease in hydraulic conductivity is a known phenomenon, that was

reported for different woody species (Domec et al. 2010; Kotowska et al. 2015). Both roots and branches of plants grafted on Riparia Gloire had significantly lowest specific hydraulic conductivity, while those grafted on 420A had the highest ones (Fig. 6a, b). Accordingly, Riparia Gloire

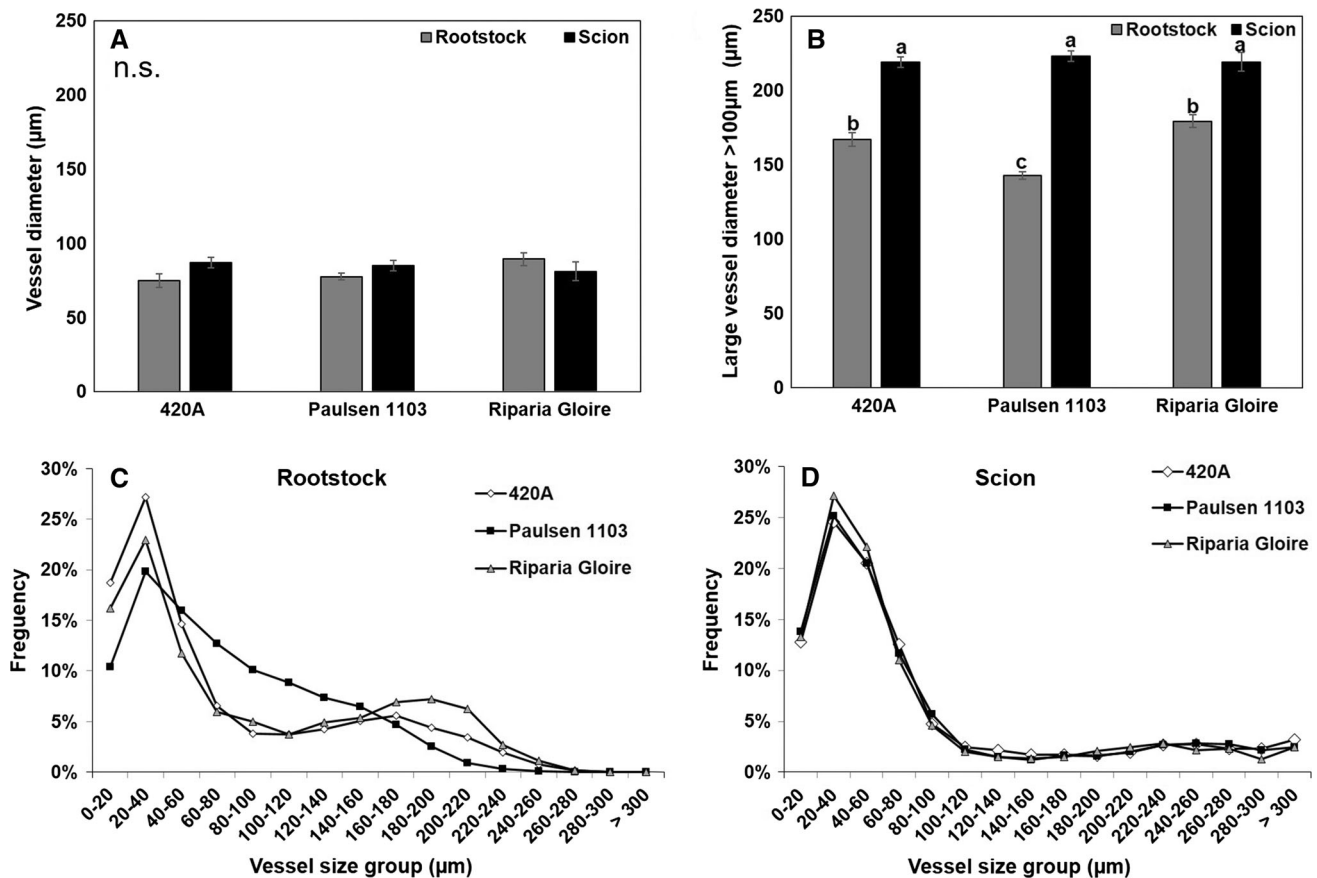


Fig. 4 Stem vessel characteristics in grafts of *Vitis vinifera* cv. Cabernet Sauvignon scions on three different rootstocks (420A, Paulsen 1103 and Riparia Gloire). **a** average vessel diameters, **b** average large (>100 µm) vessel diameters. Each value is the mean

of 12 vines of the same graft (12 rootstock stems + 12 scion stems) ± standard error. Different letters indicate significant differences between rootstocks at $p \leq 0.05$. **c, d** Vessel size distribution for all the vessels in rootstock and scion stem, respectively

grafts had the lowest vessel diameters (Fig. 6c, d). The vascular area showed an inconsistent trend—in roots, it was lowest in Paulsen 1103 and highest in 420A (Fig. 6e), and in branches was very similar in all three grafts (Fig. 6f).

Discussion

It is fascinating that scions of the same species and cultivar—*V. vinifera* cv. Cabernet Sauvignon—acquired very different structural and physiological characteristics when grafted on rootstocks of different *Vitis* species. When grown in clay soil with relatively high water content, Riparia Gloire (*V. riparia*) grafts were the most drought stressed (Fig. 3) and, therefore, had the highest yield quality and the lowest quantity (Table 1). The other two grafts were less drought stressed and even failed to reach the required low water potential threshold in the second trial year (Fig. 3d) and thus had an inferior yield quality. Such relationship between drought stress and yield quality

is well documented (Castellarin et al. 2007; Leeuwen et al. 2009; Acevedo-Opazo et al. 2010; Munitz et al. 2016); Riparia Gloire rootstock is, indeed, a restricting rootstock, suitable for inducing drought stress in water excess conditions. However, what is the mechanistic explanation of this phenomenon?

Riparia Gloire graft roots had the lowest specific hydraulic conductivity (Figs. 5a, 6a, b). Similarly, root xylem vessels' diameter was smaller in kiwifruit rootstocks of inferior quality (Wang et al. 1994). Reduced root conductivity was found in dwarfing rootstock in olive (Nardini et al. 2006) and apple (Atkinson et al. 2003); it was considered responsible for the restricting effect of these rootstocks. However, neither calculated nor measured root conductivity can represent the whole root system. We wish to emphasize that although the roots hydraulic parameters were significantly different, only a small sample of the whole root system was measured—six roots per treatment and we do not know the size of the whole root system. For instance, the aquaporins, radial conductivity, root pressure, total roots area, and xylem-to-phloem ratio should be

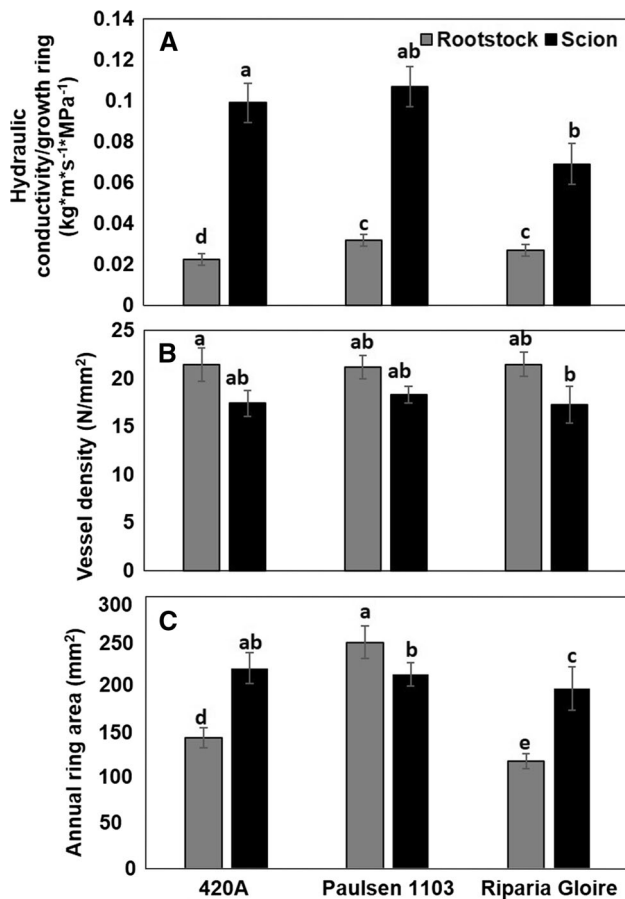


Fig. 5 Stem hydraulic parameters in grafts of *Vitis vinifera* cv. Cabernet Sauvignon scions on three different rootstocks (420A, Paulsen 1103 and Riparia Gloire). Each value is the mean of 12 vines of the same graft (12 rootstock stems + 12 scion stems) \pm standard error. Different letters indicate significant differences between rootstocks at $p \leq 0.05$

considered to describe the root systems complexity (Eshel 1998; Clearwater et al. 2007; Iwanami et al. 2009; Eshel and Grünzweig 2013; Gambetta et al. 2013). In addition, though 420A grafts were intermediate in their physiological performance (Fig. 3) and their quality parameters (Table 1), their roots had the highest hydraulic conductivity and vascular area (Fig. 6a), thus inconsistent with the theory that the roots are the major restricting factor.

It is important to note that all the stems (except Paulsen rootstocks) showed a bimodal vessel size distribution, with numerous small vessels and numerous large vessels, with a decrease in medium-sized vessels frequency. Such bimodal distribution is typical for vines and climbing plants (Carlquist 1985), and is thought to increase both conductivity and safety. The different rootstock stems had large variation in vessel size distribution (Fig. 4c), as they, indeed, belong to different species/hybrids. On the other hand, scion vessel size distribution was very similar between grafts (Fig. 4d), all of them being the same cultivar—*V.*

vinifera ‘Cabernet Sauvignon’. Similarly, in peach grafts, the rootstocks had very little effect on scion vascular anatomy (Tombesi et al. 2010a, 2012). The main significant anatomical difference in scion stems in different grafts was the growth ring area (Fig. 5c). In stems of scions grafted on Riparia Gloire rootstock, the growth rings were significantly narrower than in the other two grafts. Though the vessel frequency and size were not significantly different as compared to the other two grafts, narrow growth rings significantly reduced the water conducting area. Subsequently, in Riparia Gloire scion stems, the hydraulic conductivity per annual ring was the lowest (Fig. 5a). This low conductivity was preserved downstream in Riparia Gloire graft branches as well. It seems that the restricting action of Riparia Gloire rootstock is akin to the mechanism of dwarfing rootstocks, which induce a lower hydraulic conductivity in the scion (Cohen and Naor 2002; Tombesi et al. 2010b). Narrow growth rings were found to be an important anatomical parameter in slow-growing dwarf woody plants (Baas et al. 1984), and were reduced in water-deficient conditions in *Potentilla diversifolia* (Von Arx et al. 2012). Thus, the ability to develop drought stress in Riparia Gloire grafts was probably due to narrow annual rings that limited the downstream water flow capacity.

The narrow annual ring size in scion stem was imposed by the morphological constraint of the stem size. It seems that the hydraulic restriction point was basically the diameter difference between rootstock and scion stems (Fig. 2). In hydraulically inferior Riparia Gloire (*V. riparia*) grafts, the stem diameter difference was disproportionately high, with a wide scion growing on a very narrow rootstock. To adapt itself to growing on a narrow rootstock stem, the scion is forced to limit its stem size by narrowing the annual rings. Thus, such simple morphological constraint causes more elaborate anatomical and physiological changes. It is important to emphasize that Riparia rootstocks did not have a low hydraulic conductivity as compared to the other two rootstocks (Fig. 5); thus, the restricting effect was not due to reduced conductivity between the rootstock and scion, but rather due to structural constraint. Interestingly, (Webber 1948) considered the graft union shape in citrus as the definitive indicator of grafting success—while a smooth graft union was regarded as more successful. However, the major restricting effect might not be due to the decreased hydraulic conductivity in the scion stem itself. The axial portion of the stem pathway contributes a relatively small proportion of the total hydraulic resistance, while the most resistance typically resides in the fine roots, branches, and leaves. In Riparia grafts, the low conductivity was preserved downstream, in the branches (Fig. 6a), and it is possible that the overall restricting effect, while initiating at the morphological constraint at the graft junction, was due to increased

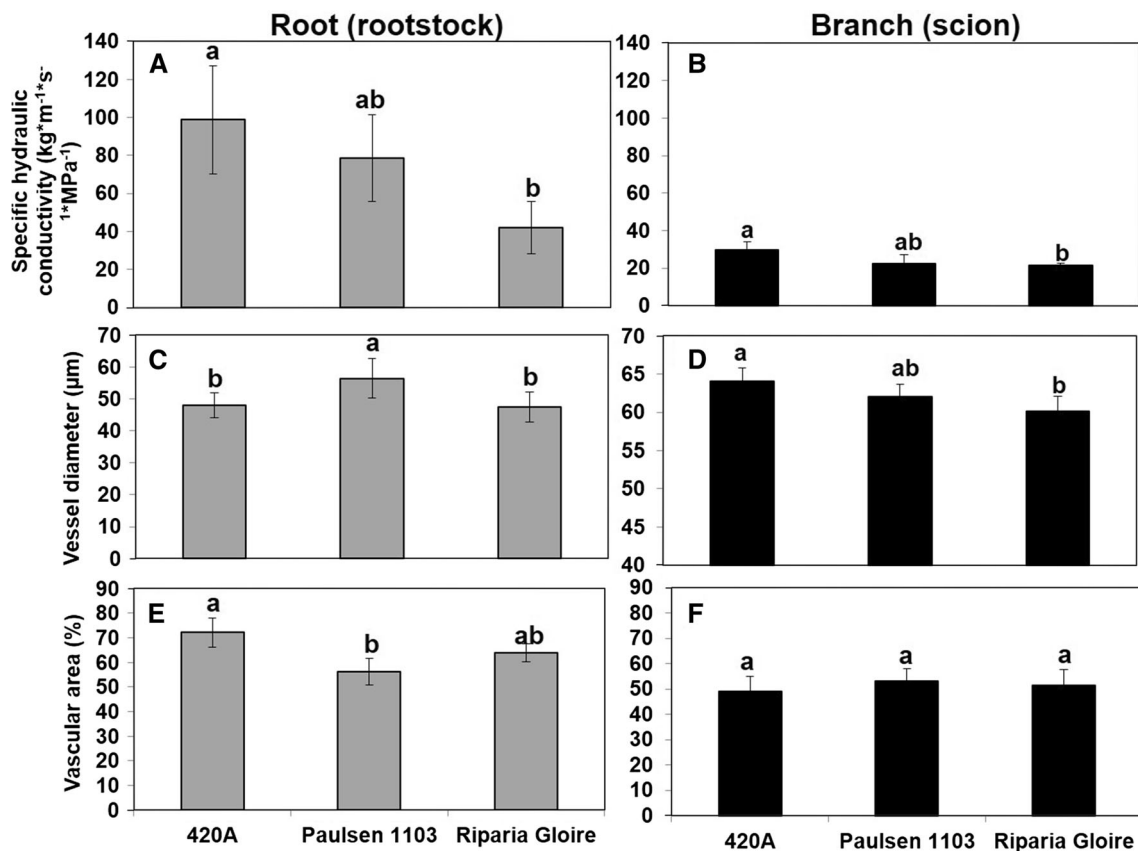


Fig. 6 Root (a, c, e) and branch (b, d, f) hydraulic parameters in grafts of *Vitis vinifera* cv. Cabernet Sauvignon scions on three different rootstocks (420A, Paulsen 1103 and Riparia Gloire). Each

value is the mean of 6 (roots) or 5 (branches) \pm standard error. Different letters indicate significant differences between rootstocks at $p \leq 0.05$

resistance in the end point of the soil to leaf pathway. Unfortunately, it is almost impossible to reliably measure transpiration of a whole plant in the field, and directly show the link between the xylem conductivity to water demand.

Graft is a union of two distinct species, which produces a chimera individual with combined qualities. Such close interaction between two organisms presents numerous difficulties, as they must adapt structurally to each other and function as a single whole. Our results demonstrate that the relationship between the graft and the scion requires compromises. The scion has to adapt structurally to the rootstock, and the extent of compromise needed possibly will determine the vitality of the grafted plant as a whole.

Author contribution statement IS—acquisition and analysis of data, writing, YH—acquisition and analysis of data, SM—acquisition and analysis of data, EH—design of the work, analysis of data, MA—acquisition of data, ED—analysis of data, AS—design of the work, YN—design of the work, acquisition and analysis of data, and writing.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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