Rootstock effect on tree-ring traits in grapevine under a climate change scenario

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ABSTRACT

Projected changes in drought occurrence in the Mediterranean region are raising concerns about the adaptive capability of rainfed crops, such as grapevine, to increasing aridity. Cultivation management, especially the techniques influencing the hydraulic pathway, can play a role in plant adaptation to drought for the consequent changes in wood anatomical functional traits. The aim of this study was to assess the effect of grafting on wood anatomy in tree-ring series of Vitis vinifera L. ‘Piedirosso’ grapevine cultivated in a volcanic area in Southern Italy. Tree-ring anatomy was analysed in vines grown on their own roots or grafted onto 420A rootstock. Results showed that grafted vines had a higher occurrence of wood traits linked with safety of water transport if compared with non-grafted vines. Grafting induced the formation of tree rings with higher incidence of latewood also characterised by narrower and more frequent vessels if compared with non-grafted vines. This study suggested a different regulation of water flow in the grafted and non-grafted vines. Such findings support the analysis of wood anatomy as a tool to drive decisions linked with plant cultivation management. In this specific case, our results encourage to further explore the change from a traditional cultivation with own-rooted grapevines towards grafted models inducing better xylem adaptation to increasing drought.

Keywords: Drought, functional wood traits, rootstock, tree rings, Vitis vinifera, water use efficiency.

INTRODUCTION

Scenarios of environmental changes in the Mediterranean basin indicate an increase in drought, which is raising concerns for resource management in both natural environments and agriculture (WWAP 2014; IPCC 2017). At the same time, there is increasing awareness about the need to create synergies among disciplines to reach an integrated management of forests and cultivated areas for regulating water use efficiency.
Plant adaptation to drought and crop productivity surely rely on plant hydraulic traits and on their plasticity that are affected not only by complex interactions among multiple environmental factors, but are also influenced by cultivation techniques (De Micco & Aronne 2012; Cirillo et al. 2014). Recently, wood anatomy has been recalled as a discipline capable of responding to the increasing demand of trait-based ecology, forestry and agro-ecology to understand plants’ responses to stress (Beeckman 2016). Indeed, plant vascular architecture and hydraulics are affected by plant habit that in cultivation is severely influenced by training techniques, whose importance in the control of water use efficiency has been recently recognised under regimes of deficit irrigation (Cirillo et al. 2017).

The grapevine (Vitis vinifera L.) is particularly interesting from a crop management perspective because, being mostly rainfed-cultivated in Italy, there is a demand for cultivation techniques that improve water stress tolerance. Grapevine shows a ring porous, sometimes semi-ring porous, wood characterised by the occurrence of very large solitary vessels in earlywood and very narrow latewood vessels in radial files or small groups (Schweingruber 1990). Earlywood vessels promote efficient water transport, but have low safety against embolism under conditions of water deficit (Hacke et al. 2006). Several drivers have been proposed as guiding vessel size, including environmental factors and plant architecture (De Micco et al. 2008; Olson et al. 2014; Pfautsch et al. 2016). Cultivation techniques (i.e. training system and pruning) affect the crown structure, consequently light interception, and water flow resistances throughout the plant, and therefore ultimately influence water transport, carbon assimilation and partitioning of resources (Willaume et al. 2004; Souza et al. 2011; Cirillo et al. 2017). Grafting is a well-known system that can be appropriately managed to modify plant growth, water use efficiency, photosynthetic performance and finally fruit (berry) composition and quality as well as wine characteristics (Düring 1994; Stevens et al. 2016). In viticulture there is an interest in choosing the appropriate rootstock to induce specific properties in the obtained combined/chimera plant. Shtein et al. (2017) have recently shown how different rootstocks can affect water transport properties also helping to induce and control water deficit through morpho-anatomical restriction of hydraulic conductivity. The rootstock-induced water deficit can improve wine quality due to higher phenol and anthocyanin content in those areas where such water stress is not easily obtained due to either high precipitation or soil structure (Christensen 2003). In some geographic areas of the Mediterranean basin, viticulture is facing the opposite problem because of rising temperatures and changes in precipitation frequency; therefore, in these areas, the choice of rootstock and cultivation techniques needs to be addressed to enhance plant physiological adaptation to drought and to increase water use efficiency. Drought tolerance can be a crucial factor in grapevine cultivation, especially in arid and semi-arid regions where irrigation is limited, unavailable, or not permitted. This need is particularly important in some areas of Italy such as the Campania region, considered one of the most appreciated districts for wine production thanks to its peculiar climatic conditions, fertile soils and presence of several autochthonous varieties (Muccillo et al. 2014). Among these, Piedirosso, red grape, has very ancient origins and it was often exalted as excellent nectar by Plinius the Elder in the “Naturalis Historiae”. In some
volcanic areas of the Campania Region, where Piedirosso is widely grown, grapevine cultivation is traditionally performed by avoiding the use of rootstocks, thanks to the soil type that is inhospitable to *Phylloxera* pest.

Starting from the above considerations, here we present a pilot study to explore possible effects of grafting on vine adaptation to drought in a scion/graft combination of *V. vinifera*. We analysed the main functional wood traits of Piedirosso grapevines grown on their own roots or grafted onto 420A rootstock (*Vitis berlandieri* × *Vitis riparia*). The improvement of grapevine adaptation to drought through grafting onto rootstock is important for cultivation management in a context where the legislation requires that vineyards are rainfed in order to achieve quality and/or geographical indication labels.

**MATERIALS AND METHODS**

**Study site and plant material**

The study was conducted at the vineyard Vigna Jossa (40° 52' 10.0" N, 14° 10' 10.2" E – 200 m asl), located within the farm Cantine degli Astroni, in the Campi Flegrei area (Naples, Southern Italy). The climate is Mediterranean with hot dry summer and wet mild winters. The average summer and winter temperatures are 24.6 and 9.7 °C, respectively; the cumulative annual precipitation is 758.5 mm, with precipitation mainly concentrated in autumn and winter (monthly average from September to February is 84.5 mm). Data are from the period 2000–2015 (Fig. 1; from CRU TS4.00 gridded dataset at 0.5° resolution).

Two sampling sites were selected on two terracings of about 1200 m², comparable in their environmental and physiographic conditions (slope, elevation, S-exposition). Plants of *Vitis vinifera* L. ‘Piedirosso’ were planted with NNW-SSE row orientation and 0.9 × 1.7 m spacing (≈ 6.500 vines/ha), and trained to the double arched Guyot system, on two terracings. One of the terracings hosted non-grafted (NG) vines (rooted cuttings), while the other held plants grafted (G) onto 420A rootstock (*Vitis berlandieri* × *Vitis riparia*). Analyses were performed on five-year-old vines.

![Figure 1. Location of the study site and climate diagram from the CRU TS4.00 gridded dataset at 0.5° resolution data of the period 2000–2015 (Kahle & Wickham 2013).](image)
The soil consists of incoherent volcanic products, proximal facies of pyroclastic-flow, having different characteristics due to the primary magma composition, mechanisms and environment of formation, and alteration processes undergone after deposition that are arranged in complex structural dishomogeneities, overlying a formation of neapolitan yellow tuff (Nunziata et al. 1999).

The vineyards were not irrigated and conventional technical practices, including pruning and mineral nutrition, were applied with the same management regime to both NG and G vines.

**Biometry**

Biometric analyses were performed at harvest on 10 vines per treatment. Two one-year-old shoots (those holding the production of the year) were selected per plant to measure their length and basal diameter.

According to Caccavello et al. (2017), the lamina area (LA) of each leaf was estimated by measuring the lamina width (LaW) in the median position and applying the following functions, respectively for NG and G vines:

\[
\begin{align*}
\text{LANG} &= 0.823 \text{LaW}^2_{\text{NG}} - 1.758 \text{LaW}_{\text{NG}} + 4.479 \\
\text{LAG} &= 1.013 \text{LaW}^2_{\text{G}} - 5.875 \text{LaW}_{\text{G}} + 11.099
\end{align*}
\]

Such functions were calculated by measuring LaW and LA with a leaf area meter (LI-3100, LI-COR Inc., Lincoln, Nebraska, USA) on a subsample of leaves (n = 100) for NG and G vines, respectively. The presence of different relations between LA and LaW indicated a different lamina shape in NG and G vines. Leaves from secondary shoots were not considered since they are generally removed during cultivation.

**Tree-ring data**

We applied common dendroecological techniques to build tree-ring series. Core sampling was carried out in December 2016 passing the stem from side to side by means of an increment borer (diameter 5 mm) at a height of 30 cm. We were allowed to sample 5 vines per treatment for tree-ring analysis. Although this number is lower than the minimum number of sampling (10 plants) of standard dendrochronology to produce a climate reconstruction, it was enough for our specific aims of comparing the two hydraulic systems.

The cores were seasoned in a fresh-air dry store and sanded with different grain-size paper. Tree-ring width (TRW) measurements were made at a resolution of 0.01 mm, using LINTAB measurement equipment fitted with a stereoscope and equipped with TSAP Win software (Frank Rinn, Heidelberg, Germany). Tree-ring series were visually cross-dated and compared using standard dendrochronological techniques (Stokes & Smiley 1968). The cross-dating accuracy was then checked using the program COFECHA (Holmes 1983). Mean chronologies of the sites and chronology quality assessment were implemented with the software R using the Dendrochronology Program Library (dplR; Bunn 2008). Chronology quality was assessed calculating the total correlation between trees (Rbt) as a measure of common signal strength and the Expressed Population Signal (EPS; Wigley et al. 1984). EPS is an absolute measure...
of chronology error that determines the level of coherence of the constructed chronology and how it estimates the theoretical population chronology. EPS quantifies the degree to which each particular sample chronology portrays the theoretical population chronology and 0.85 is considered the threshold value. Wood growth was assessed by calculating the Cumulative Basal Area Increment (Cumulative BAI).

**Wood anatomical traits**

The cores were observed under a reflected light microscope (SZX16, Olympus, Hamburg, Germany). Microphotographs were obtained with a digital camera (XC50, Olympus) at different magnifications to obtain digital images of whole tree rings and details from earlywood and latewood. Images were analysed with the Cell F 3.4 (Olympus) software program. The following anatomical features were quantified in the last four rings: width of earlywood and of latewood (along 5 rays); lumen diameter of vessels in earlywood and in latewood (20 vessels per ring); vessel frequency (the number of vessels per mm²) in both earlywood and latewood, determined by counting the vessels present in a known area, according to the IAWA List of microscopic features for hardwood identification (IAWA Committee 1989). Relative conductivity was calculated according to Van den Oever et al. (1981) and Zimmermann (1983) as the fourth power of the vessel diameter.

Anatomical results were subjected to statistical analysis (ANOVA) using the SPSS 13.0 statistical package (SPSS Inc., Chicago, IL, USA). Shapiro–Wilk and Kolmogorov–Smirnov tests were performed to check for normality.

**RESULTS**

Grafted (G) and non-grafted (NG) vines, trained to double arched Guyot system, formed a similar number of shoots (17.6 ± 0.49). NG vines showed shoots significantly longer than G vines, while no significant differences were found in shoot basal diameter (Table 1). Shoots held a similar number of leaves, which had larger lamina in NG than in G vines (Table 1).

Average ring-width chronologies using raw data and mean Cumulative BAI chronologies are shown in Figure 2. It was possible to recognise, measure and cross-date the rings and build robust mean chronologies in both G and NG plants (Fig. 2a). Mean Basal Area Increment (BAI) chronologies showed high EPS and Rbt values (Fig. 2b, Table 2), with the EPS higher than the 0.85 threshold for robust series in both G and NG vines.

<table>
<thead>
<tr>
<th></th>
<th>G</th>
<th>NG</th>
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<tbody>
<tr>
<td>Shoot length (cm)</td>
<td>79.3 ± 2.48 b</td>
<td>89.6 ± 4.01 a</td>
</tr>
<tr>
<td>Shoot basal diameter (mm)</td>
<td>12.9 ± 0.28 a</td>
<td>11.8 ± 0.43 a</td>
</tr>
<tr>
<td>Leaf area (cm²)</td>
<td>122.3 ± 5.11 b</td>
<td>144.3 ± 7.53 a</td>
</tr>
</tbody>
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Table 1. – Effect of grafting on length and basal diameter of shoots and on the area of leaf lamina in grafted (G) and non-grafted (NG) vines of *Vitis vinifera* L. ‘Piedirosso’. Mean values ± standard errors are shown. Different letters correspond to significantly different values (p < 0.05).
NG vines. The overall mean wood growth was not significantly different between the two sites (Table 2). Cumulative BAI chronologies showed an increase of wood growth during 2015 and 2016 in the NG plants, compared to G ones, while the previous two years of growth showed a similar trend (Fig. 2b).

![Cumulative BAI chronologies](image)

**Figure 2.** Tree-ring series (raw data, mean values and standard errors) (a) and Cumulative Basal Area Increment (BAI, mean values) (b) in grafted (G) and non-grafted (NG) vines of *Vitis vinifera* L. ‘Piedirosso’.

<table>
<thead>
<tr>
<th>Year</th>
<th>G</th>
<th>NG</th>
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<tbody>
<tr>
<td>2013</td>
<td>0.87</td>
<td>0.95</td>
</tr>
<tr>
<td>2014</td>
<td>0.58</td>
<td>0.8</td>
</tr>
<tr>
<td>2015</td>
<td>40.89 ± 4.31 a</td>
<td>47.61 ± 8.63 a</td>
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<tr>
<td>2016</td>
<td>1292 ± 74.6 b</td>
<td>1530 ± 62.5 a</td>
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</tbody>
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Table 2. Quality assessment indexes of Basal Area Increment (BAI) mean annual chronologies, mean BAI, EW- and LW-width in grafted (G) and non-grafted (NG) plants of *Vitis vinifera* L. ‘Piedirosso’. Mean values ± the standard error; different letters correspond to significantly different values (p < 0.05).
Microscopy observations showed that G and NG vines were characterised by similar tree-ring anatomy (Fig. 3a, b). Tree-ring boundaries were evident and wood was ring- or semi-ring porous. Vessels in earlywood were solitary but in ordered radial rows, delimited by wide parenchymatous rays, while in latewood they were organised in small clusters. Vessel size and grouping helped to distinguish between earlywood (EW) and latewood (LW). EW width was significantly higher in NG than in G vines (Table 2). On the contrary, latewood (LW) was significantly wider in G than in NG vines (Table 2). The LW/EW ratio (expressed in %) was significantly higher in G (26.5 ± 0.15) than in NG (12.7 ± 0.01) vines. Vessel lumen area in earlywood did not show significant differences between the two training systems, whereas conduit size in latewood was significantly narrower in grafted than in non-grafted plants (Fig. 3c). Relative conductivity was higher in earlywood of G vines (6.23 × 10⁹ μm⁴) than NG ones (5.39 × 10⁹ μm⁴), while in latewood the opposite trend was found with values higher in NG (2.69 × 10⁷ μm⁴) than in G plants (1.90 × 10⁷ μm⁴). Vessel frequency in earlywood did not show significant differences between the two training systems, whereas vessels were more frequent in G than in NG vines in latewood (Fig. 3d).
DISCUSSION

*Vitis vinifera*, the most widely cultivated and economically important fruit crop in the world, is expected to suffer from climate change in Mediterranean semi-arid areas, also given that berry development and ripening occur in the hottest and driest season (Vivier & Pretorius 2002; Jones *et al.* 2005; Poni *et al.* 2013). It is well known that the application of grafting, based on the union between hybrids/cultivars, leads to organisms holding combined qualities. Grafting onto specific rootstocks is used in viticulture to induce specific traits linked to plant growth performance (*e.g.* tolerance to pests and pathogens, pedo-climatic adaptability), yield and berry quality, thus wine composition and score (Christensen 2003; Stevens *et al.* 2016). Specific rootstocks have been mainly selected for their “restricting role” as they determine constrictions in water flow, inducing stress due to water deficit which is considered positive to improve fruit colour and content of antioxidant and phenolic compounds (Stevens *et al.* 2016). However, the mechanistic explanation of this phenomenon has not been completely unravelled yet, but recently it has been related to changes in theoretical calculated axial hydraulic conductivity, due to modification in wood anatomical traits (Shtein *et al.* 2017). In our study on Piedirosso grapevine, the grafting induced more compact habit, confirmed by reductions in some biometrical traits of the canopy, namely shoot length and leaf area; such changes were accompanied by modifications in wood anatomical traits in tree rings. Interestingly, in grafted plants there was a reduced wood biomass accumulation (cumulative BAI) and a general modification in tree-ring structure with tree rings presenting higher incidence of latwood over earlywood, if compared to non-grafted plants. The occurrence of tree rings with ring- or semi-ring porous structure, accompanied by peculiar anatomical traits differing between earlywood, specialised for high conductivity when water is available, and latwood specialised for safety during drought periods, is a strategy adopted by many species to cope with Mediterranean environmental fluctuations (Carlquist 1989; De Micco *et al.* 2008; De Micco & Aronne 2009). In the Piedirosso vines, earlywood anatomical traits were not significantly affected by grafting, meaning that hydraulic conductivity is maintained similar in the two types of plants (grafted and non-grafted), since large earlywood vessels account for most of the water transport of the whole ring following Poiseuille’s law (Zimmermann 1983). On the other hand, the presence of the rootstock induced changes in latewood towards a safer hydraulic system made of more frequent and narrower vessels than in own-rooted vines. The tendencies of variation found in the latewood of grafted vines reflect the general trends of dwarf growth which is associated with high vessel frequency and low vessel size (Baas *et al.* 1984). The occurrence of frequent and very narrow vessels, although inefficient when there is large availability of water in the soil, would furnish a competitive advantage in the grafted vines under conditions of water deficit since they are less prone to drought-induced embolism than large ones; moreover, they guarantee a sort of redundancy of the conducting elements when part of the xylem is de-activated due to embolism (Carlquist 1989; Wheeler *et al.* 2005; Sperry *et al.* 2006; De Micco & Aronne 2009). Changes in vessel frequency and size have been recorded as response to grafting also in other species, such as peach, cherry and apple, and are considered...
responsible for modifications in axial hydraulic conductivity and used for controlling the final plant size (Olmstead et al. 2006, Goncalves et al. 2007, Tombesi et al. 2010). It has also been reported that various combinations of scion/graft can show different xylem plasticity, resulting in different hydraulic resistance under variable conditions of water availability (Bauerle et al. 2011).

Our finding that the use of a rootstock of another variety induces changes in wood anatomical properties indicates high plasticity which is fundamental for adaptation, especially in a context of climate change, and is of practical interest for cultivation management. However, plasticity is species-specific and shows different tendencies of variations as reported by Shtein et al. (2017), who found significant differences in the growth ring area of the scion, but absence of rootstock-induced variations in vessel density and frequency.

In conclusion, our results suggest that the use of rootstock in autochthonous grape varieties, where cultivation is traditionally performed by avoiding the use of rootstocks and where irrigation is not allowed by legislation, can induce the formation of a wood in the scion characterised by traits of higher safety in water transport if compared with non-grafted plants. In the sight of increasing drought, such findings seem to make sense to evaluate options to move from a traditional cultivation with own-rooted grapevines towards grafted models which maintain wood anatomical traits of high efficiency in water transport in earlywood, while improving the traits linked to safety in latewood. Wood anatomy can thus be a promising tool in decision-making during cultivation management in a context of water saving strategies.

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REFERENCES


