OENO One
VINE AND WINE OPEN ACCESS JOURNAL
By IVES International Viticulture & Enology Society

DOI:10.20870/oeno-one.2022.56.1.4459
OPEN ACCESS

Grapevine rootstock genotypes influences berry and wine phenolic composition (*Vitis vinifera* L. cv. Pinot noir)

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Associate editor:
Nathalie Ollat

Received:
12 August 2020

Accepted:
15 January 2022

Published:
7 February 2022

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ABSTRACT

Grapevine rootstocks can affect the nitrogen (N) status of the grafted plant due to discrepancies in their nutrient uptake and their efficiency in the allocation of assimilates. When N becomes a limiting factor, the production of phenolic compounds in grapes is enhanced as a result of a down-regulation of the flavonoid production pathway. However, it is still not fully understood if the impact of rootstocks on fruit and wine composition is mediated by their effect on the vegetative growth and N status of the scion. The main objective of the study was to test if rootstock influence on Pinot noir berry and wine phenolic composition could be related to the N status of the scion. An investigation was carried out on Pinot noir (*Vitis vinifera* L.) vines grafted onto six rootstocks over three vintages (2012–2014). A micro-scale fermentation technique was used to produce wines from each field replicate. Scions grafted onto SO4, a high vigour rootstock, were characterised by a 15 % higher tannin concentration in berry seed and skin compared to those grafted onto the low vigour Riparia Gloire de Montpellier, while final tannin concentration in wines depended on the rootstock. Anthocyanin concentration was higher in berries of Pinot noir grafted onto R110 compared to 125AA, which was also reflected in the wines. A Multiple Linear Regression analysis suggested that rootstock influence on berry anthocyanins was linked to the N status of scion leaves (higher Leaf NBI_R). Understanding the interaction between the N uptake efficiency of rootstocks and scion berry/wine phenolic composition will help improve the selection of suitable rootstocks that match the desired wine profile.

KEYWORDS: grapevine rootstock, phenolic composition, micro-scale winemaking, *Vitis vinifera*

INTRODUCTION

Phylloxera-tolerant rootstocks have been required in European vineyards since the 19th century, representingthe most prolonged use of a biological control strategy against a pest. Rootstocks provide a root system onto which a scion of a chosen cultivar is grafted. Thus, rootstocks are responsible for water and nutrient uptake, serve as storage organs and are sources for chemical signals to the scion. However,the influence of rootstocks on the uptake, translocation and partitioning of nitrogen (N) compounds in grapevines has not been well characterised (Delrot *et al.*, 2020). Some studies have suggested that rootstocks could affect N uptake and CO_2 assimilation (Candolfi-Vasconcelos *et al.,* 1994; Lecourt *et al.*, 2015). It has also been shown that different rootstock genotypes may regulate root system growth and architecture differently to optimise nutrient uptake in response to N availability (Cochetel *et al.,* 2019).

When N becomes a limiting factor, the production of phenolic compounds is enhanced (Hilbert *et al.,* 2003; Delgado *et al.,* 2004; Schreiner *et al.,* 2014). Therefore, vine N status has a direct influence on grape phenolic composition together with an indirect influence on growth and vigour (Cortell *et al.,* 2005). The flavonoid production pathway is down-regulated by high N availability (Soubeyrand *et al.,* 2014). However, it seems that this inhibition depends on the rootstock genotype (Habran *et al.,* 2016). Thus, rootstocks could be a powerful tool for managing phenolics in fruits. Some studies have evaluated the impact of rootstock on anthocyanins in berries for *Vitis vinifera* L. cv. Pinot noir (Sampaio*,* 2007; Berdeja *et al.,* 2014), Cabernet-Sauvignon (Koundouras *et al.,* 2009; Wang *et al.,* 2019), Zinfandel (Nelson *et al.,* 2016), Regent (Mijowska *et al.,* 2017), Merlot (Gutiérrez-Gamboa *et al.,* 2019) and Greco nero n. (Suriano *et al.,* 2016). Only a few studies have integrated the analysis of tannins in fruits (Sampaio*,* 2007; Koundouras *et al.,* 2009; Wang *et al.,* 2019; Nelson *et al.,* 2016; Mijowska *et al.,* 2017), finding that the rootstock had an overall low impact on fruit phenolics. Some studies have included winemaking over several vintages for Shiraz (Walker *et al.,* 2010; Harbertson and Keller*,* 2012; Olarte Mantilla *et al.,* 2018), Merlot (Harbertson and Keller, 2012) and Greco nero n. (Suriano *et al.,* 2016).However, wine phenolic composition has been evaluated only once (Harbertson and Keller, 2012). Therefore, it is the objective of the present study to investigate the rootstock impacts on berry and wine phenolic composition in relation to scion N status. A companion analysis reported the rootstock effects on some parameters describing scion vigour and leaf N status (Blank *et al.,* 2018). The current study provides comprehensive information about the interaction between rootstock vine N status and scion berry/wine phenolic composition, which will help improve the selection of suitable rootstocks that match the desired wine profile.

MATERIALS AND METHODS

1. Growth conditions and experimental setup

A rootstock trial was established in 2003 in a vineyard of the Hochschule Geisenheim University in the Rheingau Region (Germany; 49°98'77.9"N 7°93'98.5"E). Further information about the experimental setup is provided in Blank *et al.* (2018). *Vitis vinifera* L. cv. Pinot noir, clone Gm1-1 was used as scion and grafted onto six rootstocks: Kober 125AA (125AA), Selection Oppenheim 4 (SO4), Riparia Gloire de Montpellier (Riparia), 110 Richter (R110), 101-14 Millardet et de Grasset (101-14MGt) and Schwarzmann. A randomised block design was applied, with 14 vines per replicate and four replicates per treatment. The weather conditions were recorded daily and used for the modelling of vine and soil water balance, as described by Blank *et al.* (2019). In general, the years were quite similar in terms of ΣGDD10 °C over the growing season (2012-1187, 2013-1148 and 2014-1155). However, 2013 was extremely 'warm and dry' during the period flowering to *véraison* (supplementary Table S1, supplementary Figure S1).

2. Description of vigour induced by the rootstock

The pruning mass, fruit yield, cross sectional trunk area and shoot length were recorded for each of the four replicates per treatment during the experiment (2012, 2013 and 2014) and reported in Blank *et al.* (2018).

3. Must composition at harvest

At harvest, ten bunches were selected from each replicate to determine cluster weight, number of berries per cluster and average berry weight. After pressing by hand, total soluble solids (TSS; °Brix) was determined using a handheld refractometer (Leo Kübler GmbH, Karlsruhe, Germany), and pH and titratable acidity using a titration system (Titrino 719S, Metrohm AG, Herisau, Switzerland). The juices were also analysed with Fourier-transform infrared spectroscopy (FTIR; FT2 Winescan Flex (Foss, Hilleroed, Denmark)) for malic and tartaric acid concentration.

4. Vine and berry nitrogen status

The N status of grapevine leaves was estimated at pea size on 16 leaves per replicate using a chlorophyll fluorescence based sensor Multiplex MXH (FORCE-A, Orsay, France) (Blank *et al.,* 2018). The chlorophyll index Leaf_SFR_R, the flavonol index Leaf_FLAV related to the epidermal flavonol concentration and the N balance index Leaf_NBI_R which corresponds to the ratio between Leaf_SFR_R and Leaf_FLAV were determined.

Juice Yeast Assimilable Nitrogen (YAN) was determined on the must samples collected at harvest (described in Section 3) with an Amino Acid Analyzer S 433 (Sykam GmbH, Eresing, Germany). Briefly, the amino acids were separated according to their gradient on an ion exchange column and the primary amino groups marked with ninhydrin. The absorption of the different peaks was determined quantitatively with a

two-channel detector at 440 nm and 570 nm using an internal standard (200 nMol/mL Norleucine).

5. Berry sampling

One hundred berry samples (50 berries per canopy side) were collected from each field replicate on the same harvest date for all rootstocks (26.09.2012, 01.10.2013 and 20.09.2014) at around 21 °Brix and they were then frozen at -20 °C. A 20-berry sub-sample was later selected for the analysis of phenolic compounds in the berries and the residual 80-berry sub-sample was used for micro-scale winemaking.

6. Grape skin and seed extracts

After the samples had been weighed, each berry was sliced with a scalpel to separate the skin and seeds. The grape skin was grounded in liquid nitrogen and the resulting frozen powder suspended in 20 mL of a 70 % (v/v) acetone/water solution. The seeds were extracted in the same way as the skin without grinding. After a 24 h extraction period at room temperature in an orbital shaker (KL2, Edmund Bühler GmbH, Hechingen, Germany), the solution was collected by vacuum filtration and the volume was adjusted to 25 mL with 70 % (v/v) acetone/water. The acetone was removed at 35 °C using a rotary evaporator at reduced pressure equipped with a 12-well rack for 50-mL tubes (Multivaporsystem P 12 related to a Rotavapor R-210, BÜCHI Labortechnik GmbH, Essen, Germany).

7. Micro-scale winemaking

Fermentors were created from 250 mL jam jars with lids fitted with air-locks and a stainless steel disc and handle for crushing the pomace (Figure 1). The repeatability of the method was between 4 and 12 % for tannins and 5 and 9 % for anthocyanins (Blank *et al.*, 2021). The concentration of tannins obtained after micro-scale fermentations was linearly correlated to that obtained after higher scale fermentation (R^2 = 0.86), which was also the case for anthocyanins (R^2 = 0.89) (Blank *et al.*, 2021). After three months at -20 °C, the 80 berry subsamples were thawed at room temperature (around 20 °C) for three hours and crushed by hand. The grape material was transferred to the fermentor and 50 mg/kg of SO_2 was added. The must was inoculated with 250 mg/kg Oenoferm® Klosterneuburg yeast (*Saccharomyces cerevisiae*; Erbslöh AG, Geisenheim, Germany). The wines were fermented at approximately 20 °C for six days on skins and the cap was manually plunged down three times a day at approximately six hour intervals. Weight and temperature were recorded daily. The pomace was pressed for 10 min after a two-week post-fermentation period in a pressure-controlled ice-machine at 1 bar (Longarone 85, QS System GmbH, Norderstedt, Germany). Phenolic analyses were performed according to the "Harbertson-Adams" assay on samples of the resulting wines. Standard wine parameters were determined by FTIR spectroscopy (OenoFoss™, Foss, Hilleroed, Denmark).

8. The "Harbertson-Adams" assay

The analysis of tannins was performed according to the "Harbertson-Adams" assay (Harbertson *et al.,* 2002). A protein solution for tannin precipitation was prepared by dissolving BSA (Bovine Serum Albumin) in buffer A, resulting in a concentration of 1 mg BSA m/L buffer A solution. Skin/seed extracts or wine were diluted in a model wine solution and 1 mL of BSA protein solution was dispensed to react with the tannin. After incubation, the samples were centrifuged (Minispin® Plus, Eppendorf AG, Germany). The supernatant was discarded, 875 µL of the TEA/SDS buffer (containing 5 % TEA (v/v) and 10 % SDS (w/v) adjusted to pH 7.9) was added and the tube was vortexed (Reax-Top, Heidolph Instruments GmbH, Schwabach, Switzerland) to dissolve the pellet. Background absorbance of the solution was read at 510 nm with VIS spectrophotometer (Odyssey, Hach Lange GmbH, Düsseldorf, Germany), and again after the addition of 125 µL ferric chloride reagent (10 mM $FeCl_3$ in 0.01 N HCl). Tannin concentration was calculated from a standard curve as mg CE (Catechin Equivalent). The repeatability of the method was evaluated at 4 % (coefficient of variation) using a commercial Pinot noir wine from the Rheingau region. For the analysis of anthocyanins, skin extracts or wine samples were diluted in the model wine solution and 1 mL of maleic buffer was added (Heredia *et al.,* 2006). After 5 min, the absorbance at was read at 520 nm. The anthocyanin concentration was calculated as Malvidin-3-O-glucoside (M3OG) equivalents.

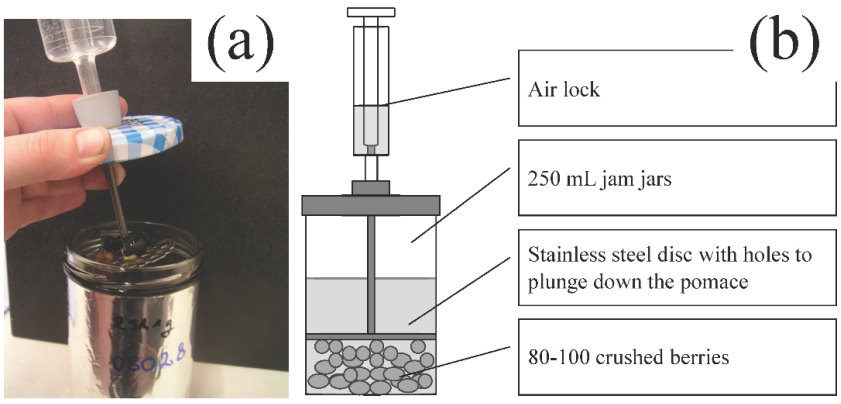


FIGURE 1. Micro-scale fermentor represented in a photo (a) and a diagram (b).

9. Statistical analysis

The open source R 3.3.1 statistical computing environment (R Foundation for Statistical Computing, Vienna, Austria) was used for all ANOVA and graphs with the platform Rstudio (Version 1.3.1093). Differences between treatment means were compared using the Tukey HSD test ($P = 0.05$). Multiple linear regressions (MLR) and model reduction through a stepwise selection of predictor variables were conducted using the R package MASS and its function stepAIC (Venables and Ripley, 2002).

RESULTS

1. Grape maturity parameters

All the primary compound parameters of Pinot noir juice (Total soluble solids (TSS), total titratable acidity (TA), malic and tartaric acids) were affected by both the rootstock genotype and the year (Table 2). TA was high with 10 g/L for the 2014 season, extremely cool during the maturation period and on average 3 g/L higher than the 2012 season.

Those differences were reflected in the concentration of malic and tartaric acid. Pinot noir grafted onto Riparia and Schwarzmann had a significantly lower berry weight than vines grafted onto SO4 and 125AA. TSS was comparable for the rootstocks, but TA in musts of Pinot noir grafted onto SO4 was significantly higher by 2 g/L than that of vines grafted onto all the other rootstocks in the trial.

2. Nitrogen grape juice status

Overall, 22 free amino acids (FAAs) were identified and quantified in Pinot noir juices for 2012-2014 seasons, and the amino acids profile was dominated by Glutamine (GLN), Proline (PRO), Alanine (ALA) and Arginine (ARG) (supplementary Table S1). Year and rootstock genotype altered the concentration of most amino acids significantly in Pinot noir juice, and the interaction between both factors was generally not significant. The amino acids in juices most strongly affected by the rootstock were ARG, GLN, PRO and HIS. Both year ($F = 4.75^{***}$) and rootstock ($F = 7.29^{***}$) had a significant influence on the yeast assimilable nitrogen content (YAN) of Pinot noir juices, while the interaction between the two factors was not significant ($F = 0.91$, $p = 0.528$) (Table 1).

The YAN in Pinot noir juice was 20 % higher for the 2013 season than for the 2012 season, mainly due to an enhanced accumulation of free assimilable nitrogen (FAN- ΣAAS) rather than of ammonia. The YAN content of the juice of Pinot noir grafted onto R110 and Schwarzmann rootstock was 25 % lower than when the vines were grafted

TABLE 1. Juice primary compounds for Pinot noir grafted onto six rootstocks for three seasons.

	Berry weight	TSS	TA	Malic acid	Tartaric acid
	[g berry^{-1}]	[°Brix]	[g L^{-1}]	[g L^{-1}]	[g L^{-1}]
Year (Y)					
2012	1.86 ± 0.19^{a}	21.51 ± 0.63^{b}	7.45 ± 0.81^{c}	2.42 ± 0.43^{c}	5.43 ± 0.95^{b}
2013	1.63 ± 0.18^{b}	22.53 ± 0.49^{a}	8.93 ± 1.21^{b}	3.33 ± 0.82^{b}	6.02 ± 0.48^{a}
2014	1.73 ± 0.17^{b}	19.10 ± 0.71^{c}	10.18 ± 1.83^{a}	4.15 ± 1.21^{a}	6.07 ± 0.81^{a}
Rootstock (R)					
Riparia	1.69 ± 0.14^{bc}	20.52 ± 1.94	7.92 ± 0.87^{b}	2.64 ± 0.49^{b}	5.49 ± 0.58^{b}
Schwarzmann	1.61 ± 0.12^{c}	21.24 ± 1.40	8.45 ± 1.91^{ab}	3.21 ± 1.41^{ab}	5.48 ± 0.59^{b}
101-14	1.81 ± 0.21^{ab}	21.22 ± 1.51	8.58 ± 1.36^{ab}	3.09 ± 0.93^{ab}	5.76 ± 0.71^{b}
R110	1.64 ± 0.24^{bc}	21.24 ± 1.40	8.82 ± 1.87^{ab}	3.31 ± 1.09^{ab}	5.61 ± 0.62^{b}
SO4	1.83 ± 0.18^{ab}	20.88 ± 1.71	10.24 ± 1.70^{a}	4.11 ± 1.03^{a}	6.88 ± 0.74^{a}
125AA	1.87 ± 0.18^{a}	21.16 ± 1.57	9.12 ± 1.87^{ab}	3.47 ± 1.19^{ab}	5.82 ± 0.83^{b}
ANOVA factors and interactions (F* values)					
Year (Y)	11.40***	233.87***	33.12***	30.93***	10.52***
Rootstock (R)	4.94***	2.35^{ns}	5.53***	4.86***	11.57***
Interaction (Y*R)	0.38^{ns}	1.31^{ns}	1.10^{ns}	1.22^{ns}	2.01^{ns}

A sample of five bunches per replicate was pressed and analysed to evaluate the concentration of primary compounds in Pinot noir juices including TSS and TA (expressed as g/L tartaric acid). Values represent the mean as mg/L ± standard deviation at harvest, for the four replicates per rootstock within one year. Rootstock (R) and year (Y) effects were evaluated using two-way ANOVA, the F* values are reported for each factor. Main effects and interactions significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ or ns not significant. Values with the same letter within one column are not significantly different at $p < 0.05$ using the LSD Post hoc test.

onto 125AA. This was mainly due to a lower FAN content, rather than ammonia content, as confirmed by the lower concentrations of FAN in the juice of Pinot noir grafted onto R110 rootstock and Schwarzmann when compared to 125AA.

The YAN in Pinot noir musts was not significantly correlated to pruning weight ($R^2 = 0.062^{ns}$), yield ($R^2 = 0.001^{ns}$) and leaf chlorophyll index (Leaf_SFR_R, $R^2 = 0.002^{ns}$), reported in Blank *et al*. (2018). When considering the N balance index in leaves (Leaf_NBI_R), the correlation between Leaf_NBI_R, YAN ($R^2 = 0.191^{***}$) and FAN ($R^2 = 0.192^{***}$) was weak but significant: two groups could be distinguished, with Riparia and Schwarzmann being significantly lower than the four other rootstocks.

3. Tannin concentration in berries and wines

3.1. Tannin composition in berries

Although Pinot noir grafted onto Riparia and Schwarzmann had smaller berries, the proportion of whole-berry fresh mass represented by seeds and skin was not affected by the rootstock (supplementary Table S2). Seed number per berry did not differ according to the treatments, but average seed weight was lower for Pinot noir grafted onto Riparia. Total tannin concentration in berries was strongly affected by the year ($F = 135.6^{***}$) and, to a lesser extent, by the rootstock genotype ($F^*value = 3.94^{**}$) (Table 3). Indeed, the 2013 growing season was characterised by a 35 % higher tannin concentration in both skin and seeds in comparison to 2012 and 2014, irrespective of the rootstock. Independently of the year, Pinot noir berries from vines grafted on SO4 had a significantly ($p < 0.001$) higher tannin concentration compared to 101-14, 125AA and Riparia, as a result of an accumulation in both seeds and skins. The difference in berry tannin composition between rootstock genotypes was mostly preserved when data were analysed as content (on a per berry basis) (supplementary Table S3). Furthermore, tannin concentration in seeds was significantly higher by 25 % in berries of vines grafted onto Schwarzmann compared to 125AA. This was due to an increase in tannins *per se* when expressed on a weight basis (mg per g seed) (supplementary Table S3). Tannin concentration in berries was not significantly correlated with pruning weight ($R^2 = 0.031^{ns}$) and yield ($R^2 = 0.009^{ns}$).

A multiple linear regression (MLR) analysis was performed (supplementary Table S4). 88.1 % of the variation in tannin concentration in berries ($R^2 = 0.881^{***}$) could be explained, with a positive contribution of average seed weight, seed number per berry, and TSS (°Brix), but a negative contribution of leaf chlorophyll index SFR_R, berry weight and pH.

TABLE 2. Nitrogen grape juice status for Pinot noir grafted onto six rootstocks for three seasons.

	YAN	FAN	Ammonia
	ΣAAS+$NH4^+$	ΣAAS	$NH4^+$
	[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]
Year (Y)			
2012	343 ± 78.16^b	291 ± 66.23^b	51.8 ± 13.64^a
2013	406 ± 55.82^a	355 ± 59.15^a	50.6 ± 17.68^{ab}
2014	369 ± 56.56^{ab}	327 ± 52.61^{ab}	42.3 ± 5.66^b
Rootstock (R)			
Riparia	380 ± 55.92^{ab}	336 ± 57.52^{ab}	44.3 ± 10.91^{ab}
Schwarzmann	322 ± 74.09^b	284 ± 70.01^b	38.6 ± 10.06^b
101-14	384 ± 49.56^{ab}	337 ± 51.82^{ab}	47.6 ± 10.57^{ab}
R110	346 ± 74.91^b	300 ± 64.12^b	46.2 ± 14.01^{ab}
SO4	375 ± 58.97^{ab}	318 ± 57.69^{ab}	57.1 ± 15.49^a
125AA	428 ± 55.45^a	372 ± 56.03^a	55.9 ± 13.74^a
ANOVA factors and interactions (F* values)			
Year (Y)	4.75^{**}	3.86^{**}	3.92^{**}
Rootstock (R)	7.29^{***}	8.19^{**}	4.26^{*}
Interaction (Y*R)	0.91^{ns}	0.77^{ns}	0.671^{ns}

A sample of five bunches per replicate was pressed and analysed to evaluate the concentration of single amino acids. YAN = yeast assimilable nitrogen content, FAN = free assimilable nitrogen. Values represent the mean as mg/L ± standard deviation at harvest, for the four replicates per rootstock within one year. Rootstock (R) and year (Y) effects were evaluated using a two-way ANOVA, the F* values are reported for each factor. Main effects and interactions significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ or ns not significant. Values with the same letter within one column are not significantly different at $p < 0.05$ using the LSD Post hoc test.

3.2. Tannin composition in wines

As the wines were produced using micro-scale fermentation, each field replicate could be separately investigated under similar conditions and a two-way ANOVA was applied. The main wine characteristics are given in Supplementary Table S5. Both year ($F = 5.45^{***}$) and rootstock genotype ($F = 3.90^{***}$) had a significant influence on tannin concentration in Pinot noir wines. In 2013, the season characterised with the higher tannin concentration in berries, tannin in wines reached 20 % higher concentrations compared to 2014 (Table 4).

The tannin concentration in wines of Pinot noir grafted onto Schwarzmann was 30 % higher than when grafted onto Riparia or 125AA, regardless of differences in alcohol content (supplementary Table S5). Total iron reactive phenolics concentration (TP) was significantly correlated to tannins, but the correlation was higher in 2012 and 2013 ($R^2 = 0.836^{***}$ and $R^2 = 0.873^{***}$ respectively) than in 2014 ($R^2 = 0.532^{***}$).

The relationship between the tannin concentration in berries and in wines was loose, but the correlation was highly significant ($n = 72$, $R^2 = 0.213^{***}$) (Figure 2a) and clearly depended on the season (R^2 was between 0.035 and 0.398). Furthermore, while the concentration of tannin in wines depended on its concentration in skins ($p = 0.014$*) (Figure 2b), it mostly depended on its concentration in the seeds. When considering the relationship between the tannin concentration in wines to that in seeds (Figure 2c), a clear seasonal effect on the regression parameters was observed. The slope of the regression was similar ($p = 0.867$) for 2012 (457) and 2013 (382), and these were much higher ($p = 0.031$) than that of 2014 (37). The intercept was comparable for 2012 (7.17) and 2013 (-27.97), whose intercepts were significantly lower than that of 2014 (460).

The prediction of tannin concentration in wines by the MLR ($R^2 = 0.621^{***}$) (supplementary Table S6) showed a positive contribution of seed number per berry, seed tannin concentration and skin anthocyanins concentration, but a negative contribution of average berry weight, skin weight

TABLE 3. Berry phenolics for Pinot noir grafted onto six rootstocks for three seasons.

	Seed tannins	Skin tannins	Total tannins	Skin Anths
	[mg CE g^{-1} BFW]	[mg CE g^{-1} BFW]	[mg CE g^{-1} BFW]	[mg M3OG g^{-1} BFW]
		Year (Y)		
2012	1.27 ± 0.30^{c}	1.22 ± 0.17^{b}	2.49 ± 0.28^{c}	0.76 ± 0.09^{b}
2013	1.71 ± 0.25^{a}	2.03 ± 0.16^{a}	3.75 ± 0.37^{a}	0.87 ± 0.12^{a}
2014	1.52 ± 0.19^{b}	1.25 ± 0.13^{b}	2.77 ± 0.23^{b}	0.67 ± 0.10^{c}
		Rootstock (R)		
Riparia	1.40 ± 0.27^{bc}	1.42 ± 0.38^{cd}	2.82 ± 0.59^{b}	0.79 ± 0.14^{ab}
Schwarzmann	1.74 ± 0.24^{a}	1.34 ± 0.51^{d}	3.08 ± 0.59^{ab}	0.78 ± 0.12^{ab}
101-14	1.43 ± 0.25^{bc}	1.47 ± 0.34^{bcd}	2.90 ± 0.55^{b}	0.74 ± 0.12^{ab}
R110	1.46 ± 0.33^{bc}	1.60 ± 0.41^{ab}	3.06 ± 0.70^{ab}	0.86 ± 0.16^{a}
SO4	1.62 ± 0.29^{ab}	1.63 ± 0.39^{a}	3.25 ± 0.63^{a}	0.76 ± 0.08^{ab}
125AA	1.36 ± 0.32^{c}	1.52 ± 0.40^{abc}	2.88 ± 0.65^{b}	0.67 ± 0.12^{b}
		ANOVA factors and interactions (F* values)		
Year (Y)	21.51^{***}	332.48^{***}	135.6^{***}	24.80^{***}
Rootstock (R)	5.97^{***}	8.81^{***}	3.94^{**}	4.30^{**}
Interaction (Y*R)	1.82^{ns}	1.55^{ns}	0.71^{ns}	0.77^{ns}

At harvest, a sample of 20 berries per replicate was analysed to evaluate the concentration of phenolic compounds in skins and seeds according to Harbertson *et al.* (2002). CE = Catechin Equivalent; BFW = Berry fresh weight; Anths = Anthocyanins, M3OG = Malvidin 3-O-Glucoside. Values represent the mean as mg/g BFW ± standard deviation at harvest, for the four replicates per rootstock within one year. Rootstock (R) and year (Y) effects were evaluated using a two-way ANOVA, the F* values are reported for each factor. Main effects and interactions significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ or ns not significant. Values with the same letter within one column are not significantly different at $p < 0.05$ using the LSD Post hoc test.

per berry and pH together with yield and leaf chlorophyll index SFR_R.

4. Anthocyanins concentration in berries and wine

4.1. Anthocyanin composition in berries

The anthocyanin concentration in skins (expressed on a fresh weight basis) was significantly affected by both year ($F = 24.80^{***}$) and rootstock genotype ($F = 4.30^{**}$) while the interaction was not significant ($F = 0.77$, $p = 0.73$) (Table 3). 2013 resulted in a significantly higher concentration of anthocyanins in comparison to 2012 and 2014 (15 and 35 % higher respectively). Independently of the weather conditions, the anthocyanin concentration was higher in berries of vines grafted onto R110 than those grafted onto 125AA. An MLR analysis was performed on the dataset (supplementary Table S7) and 76.8 % of the variation in anthocyanin concentration in berries was found to be partly explained by differences in wood weight, yield, berry and skin weight, all contributing negatively to the model. Enhanced concentrations were found for treatments with an overall higher maturity, corresponding to higher TSS together with a lower TA and malic acid concentration. Lastly, a higher N status of the scion (higher Leaf NBI_R) led to lower concentrations of anthocyanins in the skins.

4.2. Anthocyanin composition in wines

When considering the wines, the concentration of anthocyanins depended both on year ($F = 22.27^{***}$) and rootstock genotype ($F = 3.55^{**}$), with both factors interacting significantly ($F = 3.49^{**}$). Anthocyanin concentrations were found to be up to 10 % higher in wines from the 2013 season compared to the 2014 season, following the trend already observed in berries. Interestingly, the lowest concentrations were observed for wines from the 2012 season - as much as 30 % lower than those from 2013. Anthocyanin concentrations were significant higher in wines of Pinot noir grafted onto R110 compared to 125AA, regardless of differences in vigour (Table 4). For the other rootstocks, the concentration was intermediate and not significantly different from R110 and 125AA

TABLE 4. Wines phenolics for Pinot noir grafted onto six rootstocks for three seasons.

	Wine tannins	Wine TP	Wine Anths
	[mg CE L^{-1}]	[mg CE L^{-1}]	[mg M3OG L^{-1}]
Year (Y)			
2012	566 ± 127^{ab}	2403 ± 349^{a}	177 ± 26.0^{c}
2013	629 ± 160^{a}	1968 ± 252^{b}	233 ± 38.8^{a}
2014	517 ± 112^{b}	2103 ± 403^{b}	212 ± 42.9^{b}
Rootstock (R)			
Riparia	496 ± 115.3^{b}	1823 ± 295^{c}	215 ± 38.1^{ab}
Schwarzmann	660 ± 145.1^{a}	2302 ± 457^{ab}	206 ± 45.2^{ab}
101-14	599 ± 100.4^{ab}	2223 ± 324^{ab}	196 ± 39.3^{ab}
R110	624 ± 183.9^{ab}	2213 ± 254^{ab}	227 ± 48.5^{a}
SO4	539 ± 86.6^{ab}	2369 ± 432^{a}	216 ± 33.5^{ab}
125AA	505 ± 129.8^{b}	2017 ± 261^{bc}	182 ± 44.7^{b}
ANOVA factors and interactions (F* values)			
Year (Y)	5.45^{***}	19.67^{***}	22.27^{***}
Rootstock (R)	3.90^{***}	8.10^{***}	3.55^{**}
Interaction (Y*R)	1.65^{ns}	3.73^{***}	3.49^{**}

The wines were made by micro-scale winemaking using 80 berries per replicate taken at harvest. TP = Total iron reactive phenolics, Anths = Anthocyanins, CE = Catechin Equivalent, M3OG: Malvidin 3-O-Glucoside. Values represent the mean as mg/L ± standard deviation at harvest, for the four replicates per rootstock within one year. Rootstock (R) and year (Y) effects were evaluated using a two-way ANOVA, the F* values are reported for each factor. Main effects and interactions significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ or ns not significant. Values with the same letter within one column are not significantly different at $p < 0.05$ using the LSD Post hoc test.

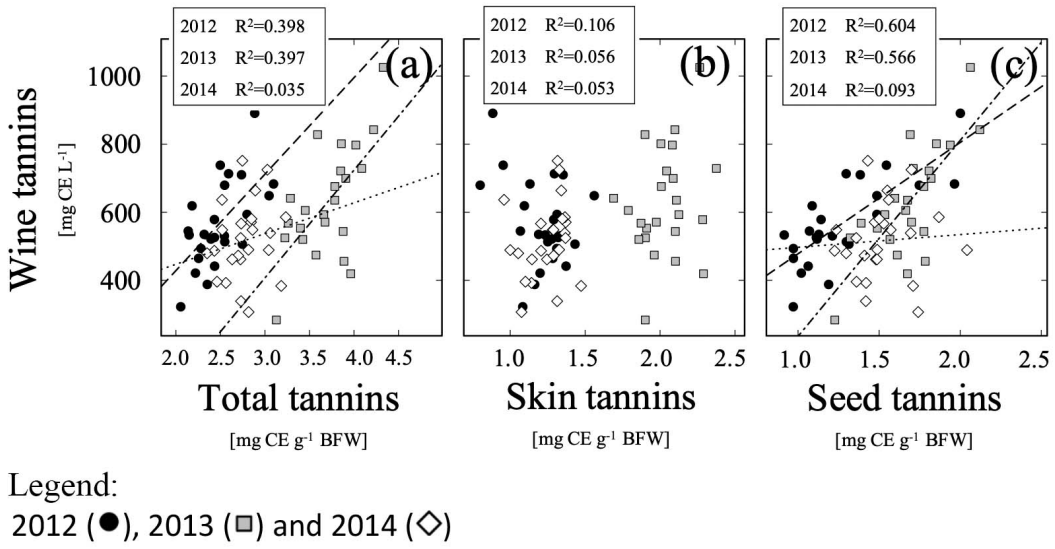


FIGURE 2. Relationships between fruit and wine tannins.

Relationships between tannin in wines of Pinot noir grafted onto six different rootstocks against (a) tannins in seeds, (b) tannins in skins and (c) total tannins [as the sum of skin and seed tannins]. Concentrations are expressed in mg catechin equivalent (CE)/g BFW (berry fresh weight) for berry composition or mg CE/L for wine. Correlation coefficients (R^2) have been calculated for each year separately.

The concentrations of anthocyanins in skins were a significant predictor of their concentrations in wine. The slope depended on the season: it was similar for 2013 (238.94) and 2014 (287.72), being significantly higher for these two seasons than for 2012 (140.89). Meanwhile, the intercept was lower for 2013 (23.21) and 2014 (17.83) than for 2012 (69.33) (Figure 3). However, the concentration of anthocyanins in the skins was not the only significant predictor for the concentration in the wine. Indeed, the results of the MLR (supplementary Table S8) showed that anthocyanin concentrations in the skins contributed positively to wine anthocyanins, together with skin weight per berry and the negative contribution of berry weight, while higher Brix and lower malic acid concentration led to increased anthocyanins into the wine. However, the most important contributor to predicting anthocyanin in wine was the leaf nitrogen index of the scion (Leaf NBI_R). A higher leaf N status of the scion (e.g. higher NBI_R), which was induced by the rootstock, resulted in wine with a reduced colour intensity.

DISCUSSION

The influence of six rootstock genotypes on berry and wine phenolic composition with Pinot noir vines was investigated over three consecutive years. The aim of the study was to examine whether the results could be related to the scion N status. To accomplish this, the N status of Pinot noir leaves was monitored at pea size using chlorophyll fluorescence technics in a previous study (Blank *et al*., 2018) and the concentration of nitrogen compounds in juice and wines are reported in the present study.

1. Seasonal differences on tannin balance

In our study, the YAN of Pinot noir juice was enhanced by 20 % for the 2013 season in comparison to the 2012 season. We observed that tannins in berries were also significantly higher by 40 % in seeds and 65 % in skins for the 2013 season, with the higher sum of GDD10 °C between flowering and *véraison.* This resulted in higher concentrations in wines by 20 %, irrespective of the rootstock. Results over a decade showed that higher ΣGDD10 °C before the *véraison* period was the most relevant for tannin production, causing an increase in tannin concentration in fruit, with an enhanced accumulation in skins compared to seeds (Blank *et al.,* 2019). The extraction rate of tannins from grapes to wines, calculated by comparing the concentration in wine (as mg g BFW) to the initial (total) fruit tannin concentration, was lower for the 2013 (11.17 %) and 2014 (12.96 %) seasons than the 2012 season (15.54 %). We observed a clear seasonal effect on the extractability of tannin compounds into wine. Indeed, the relationship between grape and wine tannins clearly depended on the year and obviously mostly depended on differences in tannin concentration in the seeds. A large variability in the tannin extraction rate between years ranging from 5.75 % to 13.85 % had already been reported, and a negative correlation between the extraction rate and the $\Sigma GDD_{10°C}$ between flowering and *véraison* had been established (Blank *et al.,* 2019). This corroborates the results of Casassa *et al.* (2015), which suggest that warmer conditions could affect the proportion of tannin extracted from seeds. More in-depth investigations will be required to determine the origin of extracted tannins in wine; i.e., whether they are skin-derived or seed-derived (Harbertson *et al.,* 2009).

2. Rootstock influenced Pinot noir leaf N status

In our experiment, we observed that the two rootstocks of V. *berlandieri* × V. *riparia* crosses, 125AA and SO4, conferred an overall higher vigour to the Pinot noir scion compared to Riparia and Schwarzmann rootstocks, with no differences in yield (Blank *et al.*, 2018). Furthermore, we had previously established that there is a positive correlation between the chlorophyll index (Leaf_SFR_R) and the pruning mass ($R^2 = 0.874$); the leaves of Pinot noir on 125AA and SO4 had higher nitrogen balance index compared to when on Riparia and Schwarzmann (Blank *et al.*, 2018).

3. Pinot noir juice N status depended on the rootstock onto which it was grafted

In our study, the YAN in the Pinot noir juice was 20 % higher for the 2013 season than for the 2012 season, mainly due to an enhanced accumulation of free assimilable nitrogen (FAN- ΣAAS) rather than of ammonia. N availability for grapevines is related to water supply, and a weak mineralisation of soil N was probably induced by water deficit that occurred in 2012, thus leading to an N deficiency in the vines. The YAN in juice of Pinot noir grafted onto R110 and Schwarzmann rootstock was 25 % lower than that of Pinot noir grafted onto 125AA. Furthermore, the composition of some individual amino acids (ARG, GLN, and PRO) was also significantly altered by rootstock in concordance with previous studies (Ough *et al.*, 1968; Treeby *et al.,* 1998). Indeed, Treeby *et al.* (1998) found that PRO, ARG, GLU and ALA concentration in the juice of cv. Chardonnay were significantly affected by rootstock. It has been suggested that scion fruit grown on low vigour rootstocks tend to have lower N status than fruit grown on higher vigour rootstocks (Ough *et al.*, 1968; Treeby *et al.,* 1998; Clingeleffer *et al.*, 2019). In our experiment, 125AA and SO4 conferred an overall higher vigour to the Pinot noir scion and higher YAN than the low vigour rootstocks Riparia and Schwarzmann. However, YAN in Pinot noir musts was not significantly correlated to pruning weight ($R^2 = 0.062$).

4. Concordance between leaf and juice N status

The correlation between leaf N status (leaf NBI_R) and juice N status (YAN) was significant, though weak ($R^2 = 0.190$). It appeared that the rootstock R110 showed the most discrepancies with high leaf N status, but the lowest juice N status. When R110 was discarded from the model, the correlation was greatly improved ($R^2 = 0.467$). In fact, N absorption and/or assimilation is largely determined by the genotype of the plant to which it is related (Tomasi *et al.,* 2015). Our results suggest there may be some differences in terms of N allocation for the R110 rootstock.

5. Rootstock influenced tannin concentration in berries and wines

Total tannin concentration in berries was strongly affected by the year and the 2013 growing season, which was extremely warm and dry during the period flowering to veraison, resulted in a 35 % increase in tannins in both skin and seeds irrespective of the rootstock. Warmer conditions before veraison are likely to favour the accumulation of tannins in berries (Blank *et al.*, 2019). Regardless of the year, tannin concentration in Pinot noir berries from vines grafted onto

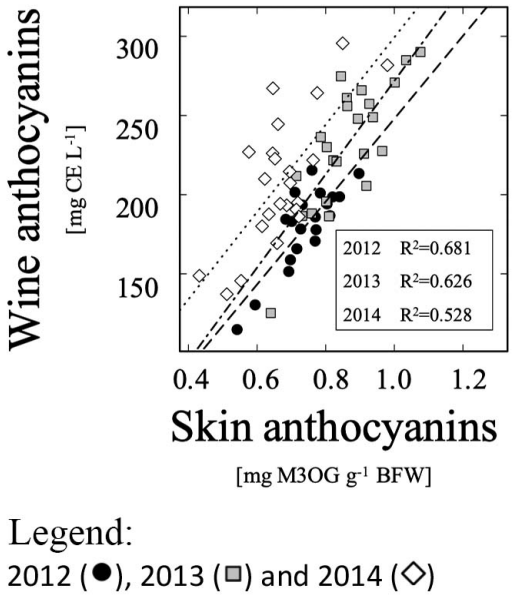


FIGURE 3. Relationships between fruit and wine anthocyanins.

Relationships between anthocyanins in berries and in micro-scale wines (Figure 1) for Pinot noir grafted onto six different rootstocks. Concentrations are expressed in mg Malvidin 3-O-Glucoside (M3OG) g^{-1} BFW (berry fresh weight) for berry or mg M3OG/L for wine. Correlation coefficients (R^2) have been calculated for each year.

SO4 was 15 % higher than that in Pinot noir berries from vines grafted onto 101-14, 125AA and Riparia, as a result of tannin accumulation in both seeds and skins. Some authors have found minor differences in terms of rootstock influence on the concentration of tannin in scion skin (in Cabernet-Sauvignon: Koundouras *et al.,* 2009; Wang *et al.,* 2019, in Merlot or Syrah: Olarte Mantilla *et al.*, 2018, and in Greco nero n.: Suriano *et al.*, 2016). An experiment performed in Oregon with Pinot noir showed that, in contrast to our results, fruit from vines grafted onto R110 and 125AA had higher amounts of skin tannins than other rootstocks, among which SO4 (Sampaio, 2007). The discrepancies between these two studies may be due to differences in rootstock impact on vine vigour. Other authors have suggested that rootstock may not have any influence on seed composition (Sampaio, 2007) and it is only recently that some results have been published (for Cabernet-Sauvignon: Koundouras *et al.*, 2009, and for Merlot and Syrah: Olarte Mantilla *et al.*, 2018); they show that rootstocks which induce moderate to high vigour, like 1103 Paulsen, result in an increased concentration of seed tannins (Olarte Mantilla *et al.*, 2018).

6. Rootstock influenced tannin concentration in wines

We observed that the concentration of tannins in wines from Pinot noir grafted onto Schwarzmann was significantly higher by 30 % than that in wines from Pinot noir grafted onto 125AA; this result seems to be independent of any differences in vigour. Some results available for Pinot noir have also shown a 10 % increase in wine when the same scion was grafted onto Schwarzmann (Sampaio, 2007); but the data had only been collected from a single year of vinification. While the more vigorous rootstock (St. George) yielded fruit with higher concentrations of tannin (Ough *et al.*, 1968), the tannin level in the resulting wines remained similar. A few recent studies have involved winemaking (Shiraz: Olarte Mantilla *et al.*, 2018, Greco nero n.: Suriano *et al.*, 2016, Merlot: Nelson *et al.*, 2016 Cabernet-Sauvignon: Wang *et al.,* 2019): it was shown that the concentrations of tannin in Shiraz wines were higher for R110 and Schwarzmann than for 1103 Paulsen, but the lowest for its own roots (Olarte Mantilla *et al.*, 2018), which is similar to our results.

Most critics of micro-scale winemaking focus on the fact that the extraction process is not representative of larger-scale fermentations. We have found the phenolic concentrations in micro-scale wines to be highly correlated ($R^2 = 0.86$ for tannins, $R^2 = 0.89$ for anthocyanins) with concentrations in wine from larger scale (50 kg and 400kg pomace) fermentations (Blank *et al*., 2021). However, it should be noted that the extraction of tannins was 35 % higher when the berries were fermented using the micro-scale winemaking technique. One explanation may be that the berries for micro-scale fermentation had been stored at -20 °C for three months prior to fermentation. We found that freezing the berries prior to extraction led to a 20 % increase in the extraction of tannins compared to berries processed fresh using this micro-scale winemaking method. In fact, it has been shown that freezing the must before fermentation damages the cell membranes and seems to be an effective technique for releasing both anthocyanins and tannins.

7. Tannin concentrations were limited when CHL in leaves was high

In the present study the results show that leaf CHL index (Leaf SFR_R) is negatively correlated to the concentration of tannins in berries. As a consequence, the leaf CHL index is also a negative predictor of the concentration of tannins in wines, with lower concentrations. Negative correlations between the normalised difference vegetation index (NDVI) and total phenols and anthocyanins have previously been reported for Cabernet-Sauvignon (Lamb *et al.,* 2004), and Pinot noir (Ledderhof *et al*., 2016), which supports our findings. The results of the MLR showed that the concentration of berry anthocyanins is a significant predictor of the concentration of tannin in wines. Indeed, a recent study on Shiraz found that fruit anthocyanin concentrations correlated with wine tannin concentrations, wine colour and polymeric pigment formation (Kilmister *et al.*, 2014).

8. Anthocyanins in Pinot noir berries were limited for rootstock with a higher N status

The concentrations of anthocyanins in berries were reduced in the cool and wet year of 2014, resulting in 35 % lower concentrations compared to the 2013 season. The yield of the "cool and extremely wet" 2014 season, which had high precipitations during the growing season, was significantly higher than the other seasons (Blank *et al*., 2018). Regardless of the weather conditions, the anthocyanin concentrations of berries were significantly higher when the Pinot noir was grafted onto R110 than when it was grafted onto 125AA. A study conducted on a nearby vineyard gave similar results (Berdeja *et al.*, 2014): anthocyanin concentrations were also increased by water stress, probably due to the up-regulation of genes of the phenylpropanoid pathway and some transcription factors involved in the regulation of this biosynthetic pathway (Berdeja *et al.,* 2015). Under drought stress a limited effect of the rootstock on berry anthocyanin concentrations was reported for Cabernet-Sauvignon (Koundouras *et al.,* 2009), Merlot and Shiraz (Harbertson and Keller, 2012). Differences in berry anthocyanins induced by the rootstock were not found to depend on the water status for Zinfandel in central California in a hot climate (Nelson *et al.,* 2016) or for Pinot noir in Germany in a temperate climate (Berdeja *et al.*, 2014). We found that for the two seasons with a moderate water supply, the accumulation of anthocyanins in Pinot noir berries was limited for a rootstock with a higher leaf N status, as determined by the NBI_R index. Indeed, a limited N supply will stimulate the processes of the phenylpropanoid pathway and anthocyanin production (Hilbert *et al.,* 2003). It has been shown that N controls a coordinated regulation of both positive (MYB) and negative (LBD) regulators of the flavonoid pathway in grapevine (Soubeyrand *et al.*, 2014).

9. Anthocyanins in berries were a significant predictor of the concentration of anthocyanins in wine

The concentration of anthocyanin in skins was a significant predictor of the concentration of anthocyanin in wine, suggesting that there is a simple diffusion mechanism. However, the slope of the linear relationship between berry and wine anthocyanin concentrations depended clearly on the year, with much lower values for 2012 compared to the other two seasons. Indeed, it seems that factors other than only concentration may play a role in anthocyanin (Kilmister *et al.*, 2014) and tannin extraction (Bindon *et al.*, 2012). This may be due to interactions between phenolics and cell-wall components of yeast lees. However, in a study by Guchu *et al.* (2015), up to 30 % of the original mass of anthocyanins was found to remain bound in lees, while 35 % was extracted into wine and 10 % remained in the pomace. Our results show that anthocyanin concentration clearly depended on seasonal variations and rootstock genotype, while the concentrations found the wines paralleled those found in the berries. The concentration was significantly higher in wines from Pinot noir grafted onto R110 compared to 125AA Similar results with enhanced anthocyanin concentrations for R110 are available for Pinot noir in Oregon (Sampaio, 2007) and Shiraz in South Australia (Olarte Mantilla *et al.,* 2018).

CONCLUSIONS

The main objective of the study was to test if rootstock has any influence on Pinot noir berry and wine phenolic composition, and, if so, whether it could be related to the N status of the scion. The rootstock indeed showed a significant impact on Pinot noir wine tannin levels and its extraction rate under moderate climatic conditions. Vintage differences were high for overall tannin levels, but rootstock impact on phenolic components seemed to be independent of weather conditions. Anthocyanin concentration was higher in berries from Pinot noir grafted onto R110 than from Pinot noir grafted onto 125AA, which was transferred to wines, reflected inversely by the grape juice N status. We showed that differences in plant vigour or N status was not the only factor explaining the rootstock impact on grape phenolics, specifically when considering tannin compounds. The micro-scale fermentation technique developed to produce wines from each individual field replicate is a strong tool for the analytical determination of rootstock impact on the phenolic composition of wines. Differences in extraction rates clearly indicate the involvement of additional parameters which influence tannin extractability.

ACKNOWLEDGEMENTS

The authors would like to thank the technical staff of the Department of General and Organic Viticulture for its support in vineyard management operations, as well as the laboratory team for its help with the sampling and analysis of juices. Lastly, we would like to thank the Department of Soil Science and Plant Nutrition for the analysis of amino-acids in juices.

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