IMPACT ON AGRONOMIC PARAMETERS IN VINES AND WINE QUALITY OF FOLIAR TREATMENTS WITH SPECIFIC FRACTIONS OF YEAST DERIVATIVES

*Any mention of yeast derivative is referring to the product: LalVigne® Foliar Spray.

Javier TÉLLEZ, Elisa GARCÍA, Emilio PEIRO, Vanesa GONZÁLEZ, José Ramón LISSARRAGUE

Grupo de Investigación en Viticultura, Universidad Politécnica de Madrid, Spain

gi.viticultura@upm.es

1. Introduction

Winemakers and grape growers are constantly looking for opportunities to improve wine quality. Producing high quality fruit for wine production is challenging and dependent on many factors, including the regional climate, seasonal variations in precipitation and the temperatures in the growing season. As a result, wineries are often required to work with unbalanced grapes, overcoming such obstacles as poorly ripened fruit, high pH, low acidity and wines that will lose quality quickly. High quality wine production in the cellar starts with high quality grape production in the vineyard.

Many producers would like to get grapes with moderate sugar content in the pulp, good acidity, low pH and good skin maturity, skin that is both phenolic and aromatic and, if possible, mature seeds.

To try to meet these challenges to wine quality, Lallemand has developed a series of products that stimulate phenolic maturity and increase the synthesis of grape aroma precursors through the foliar application of yeast derivatives. These yeast derivatives are designed for use as per WO/2014/024039 patent-pending foliar application technology.

Two different yeast derivative compositions, RD-LM for red grape varietals and RD-LA for white grape varietals, were tested to evaluate their ability to stimulate phenolic maturity and enhance the synthesis of grape aroma precursors during ripening. Evaluation of wine tasting was done by a trained expert tasting panel.

The concept of phenolic maturity in grapes is linked to the concentration of anthocyanins in the skin, their extractability and the tannin content, as well as the concentration of condensed tannins in the seeds.

Compounds of a phenolic nature greatly impact the colour, aroma, taste and organoleptic characteristics of the must, and therefore, of the wine (de la Fuente et al. 2007). Anthocyanins and tannins are the main representatives of the phenolic compounds present in grapes and red wines. Anthocyanins are the main colour components in red wines (Jackson and Lombard 1993), and tannins impart bitterness and astringency (Harborne 1984). The union of anthocyanins and tannins forms polymers that provide stable pigments necessary to maintain the stability of the colour of red wines over the long term.

During the early stages of berry ripening, the amount of anthocyanins increases. At ripening, the accumulated amount of anthocyanins reaches a maximum then remains relatively constant or declines. Several studies have shown a decrease in anthocyanin content in the later stages of ripening (e.g., Somers 1976, Keller and Hrazdina 1998, and Holt et al. 2010).

Meanwhile, it appears that tannin synthesis occurs early in berry development (Downey et al. 2003). Studies by Bogs et al. (2005) suggest that tannin is being synthesized even before the berry has set. Tannins increase at a steady rate from fruitset to a peak around veraison. Some studies show a decrease – for example for Shiraz, a 60% decrease over a range of 6° to 30°Brix (Downey et al. 2003) – while others have shown that tannin content remained essen-

tially constant from veraison to harvest, for Cabernet Sauvignon and Pinot Noir, for example, which are essentially constant from veraison to approximately 24°Brix (Harbertson et al. 2002). In addition, cultural practices and climatic conditions are likely to influence the metabolism of tannins in skins (Iland et al. 2011).

From this, we can take away that the harvest moment is a key factor in the degree of phenolic maturity. Grapes that have not reached phenolic maturity will produce wines that are not so balanced and have a strong sensation of astringency.

Regarding aroma, clearly aroma is one of the factors that most determines the quality of a wine. Aroma compounds, which come from the secondary metabolism of the vine, include terpenes, norisoprenoids, thiol precursors and methoxypyrazines. These compounds contribute to the varietal character that typifies the wine. This varietal character comprises, on one hand, the free volatile compounds of direct aromatic expression, which are few in number, and on the other hand, the aroma precursors, which are non-volatile components freed during aging or thanks to the metabolic activity of yeasts (Jackson 2008). Some research shows that glycosidic aroma precursors are not present in all grape varieties, and when they are present they are not in the same concentrations (Reyero et al. 2000, Garcia-Moruno et al. 2000, and Reynolds and Wardle 1997).

During the period of berry formation, the concentration of the free monoterpenes and most of the bound ones fell to low levels. From veraison onwards, only the linalool, diendiol and the pyran ring linalool oxides accumulated to any significant extent. These continued to a maximum of about 24°Brix, and then declined.

In grape berries, norisoprenoid levels are low prior to veraison and increase after veraison (Razungles et al. 1993 and 1996, Marais et al. 1991, Baumes et al. 2002, Bindon 2004, and Bindon et al. 2007). The pattern of change in these compounds and carotenoids led researchers to propose that the formation of norisoprenoids is linked to the degradation of carotenoids.

Methoxypyrazine content increases during berry formation, reaches a peak around veraison, then decreases during berry ripening (Boss et al. 2008, Ryona et al. 2008, and Reynolds 2010). Typically, levels of methoxypyrazines are higher in berries from vines grown in cool climates than those from warm and hot climates (Boss et al. 2008).

There are many factors that affect phenol synthesis and aroma precursors in the vine, including light, temperature, altitude, soil type, water, nutritional state, microbial interactions, pathogens, growth regulators and defoliation.

Accordingly, many techniques have been applied to improve the content of these factors in the berries. The most common techniques are related to agricultural techniques, as thorough studies have been conducted on the impact of such factors as the optimal exposure of clusters (Carbonneau and Costanza 2004), the canopy microclimate (Jackson and Lombard 1993), the optimization of leaf surface areas (Bonnisseau and Dufourcq 2004), moderate hydric stress (Seguin 1975, Bravdo et al. 1985, and Carbonneau 1987) and the effect of nitrogen fertilizer (Bell and Henschke 2005).

In an attempt to improve the phenolic content in the grape, other mechanisms have recently garnered attention, such as the use of elicitors – phytochemicals that do not kill pathogens per se but rather trigger mechanisms that improve plant resistance to pathogens, including an increase in phenolic compound levels (Vitalini et al. 2011).

The concentration and quality of phenolic compounds, as well as aroma precursors, are parameters that depend on the variety being studied. Therefore, these trials have been done on two common viticultural varieties with great oenological potential: Shiraz and Sauvignon Blanc.

Our studies have focused on trying to determine the behaviour and response mechanisms of vines to the application of yeast derivatives, in order to verify and quantify agronomical and oenological interest, showing that the studied applications have an impact on the quality of the final wine. This project aimed to study the effect of yeast derivatives on the vines – evaluating responses in varieties with great oenological potential, verifying the effect of the yeast derivative composition, doses and timing, studying the relationship between the response and the applied dose – then come to a conclusion regarding cause–effect relationships. We also verified whether the effect of yeast derivative products on the grapes and wine is manifested according to the time of harvest and/or the ripeness of the grapes.

Yeast derivative applications tested in the vineyard were effective for defining different wine qualities, without affecting yield or other agronomical parameters. The wine descriptors were evaluated as more important than the control plots.

2. Material and methods

2.1 Vineyard Location. Plant Material and Experimental Design

This experiment was performed in Finca Constancia (Toledo, Spain) in 2013. The area presents a warm climate with marked seasonal variation and a period of noticeable summertime drought. In 2013, there were 2,135 growing

degree days (GDD) and 384 mm of rain. Vineyard soils are inceptisols and alfisols, according to the USDA.

The study was carried out on grapevines planted in 2002, with a distance of 2.4 m between rows and 1.2 m between plants within the row. The rows are orientated North–South, and the trellis system is Vertical Shoot Positioned with bilateral cordon and spur pruning.

The plant material used to perform the trials was made up of vines from two different cultivars, the Shiraz clone 470 grafted on 1103-P and the Sauvignon Blanc clone 700 grafted on 110-R.

All treatments were applied in four repetitions arranged in a completely randomized design. Vines were sprayed with a water solution of yeast derivatives at the beginning of veraison (5 to 10% of veraison then again 10 days later). The solutions applied were the RD-LM product at a rate of 1 kg/ha on Shiraz vines, and the RD-LA product at 3 kg/ha on Sauvignon Blanc. In both cases, 300 L/ha was applied with a 16-litre capacity Matabi model E+ electric motor sprayer, which ensures constant pressure.

2.2 Measurements

Vegetative Growth

Pruning Weight and Shoot Weight

The pruning weight (PW) was measured in five plants of average vigour at each repetition. The number of shoots per vine was counted, and the shoots removed were weighed with a 5 kg maximum weight and 50 g precision Pesola model 8.004 dynamometer. Two average-sized shoots were taken in each repetition, then weighed on a scale with a sensitivity of 0.01 g (OHAUS Adventurer™ Pro model AP AV412) and were kept in separate labeled bags. These bags were placed on a stove at 70°C until a constant weight was reached.

Vegetative/Productive Balance

Dry Weight

Two representative vine shoots of average vigour were chosen from each repetition in each of the experimental plots for a total of eight per treatment. Stems, leaves and bunches were weighed on a scale with a sensitivity of 0.01 g (OHAUS Adventurer™ Pro model AP AV412), to obtain their fresh weight. Each part was then kept in a separate labeled bag and placed on a stove at 70°C with forced ventilation until a constant weight was reached. Once dry, each sample was weighed to obtain the dry weight and percentage of water.

The humidity level of the stems and bunches was applied to all stems and bunches in the repetition.

Physiology

To determine the effect of the experimental products on the physiological activity of the plant, foliar water potential (ψf) and the exchange of gasses in the atmosphere were measured on individual leaves.

The measurements took place at two moments of the day, at optimal environmental conditions for photosynthetic activity and at solar noon. Optimal conditions for photosynthesis were determined according to Sánchez-de-Miguel (2007).

These measurements were taken twice during the cycle. At pre-veraison five leaves of each treatment were sampled and at full maturity five leaves of each treatment were sampled.

The foliar water potential was evaluated using a Scholander pressure chamber (Model 3000 Soil Moisture Equipment Corp., Santa Barbara, CA, USA) with a reading resolution of 0.01 MPa.

Via an open system of infrared gas exchange measurement (IRGA) (LI-6400 portable photosynthesis system from LI-COR®, Lincoln, Nebraska, USA), the instantaneous rate of CO_2 (An) assimilation in µmol CO_2/m^2 leaf and stomatal conductance (gs) in mol H_2O/m^2 leaf was obtained. This measurement was taken only on the Shiraz cultivar.

Yield Components (Three Harvests)

Harvest Weight

The harvest weight was determined at three different moments, defined as the average °Brix of the different repetitions from the same cultivar. The °Brix intervals that were set to decide the times of harvest were equivalent to an early harvest, an industrial harvest and an overripe harvest.

Harvest weight was measured in six plants by experimental plot and time of harvest. Shoots and bunches were counted for each vine, and the bunches from the six plants were weighed on a 30 kg maximum 5 g precision electronic scale (Calitrol Control Gram model PM-30, Barcelona, Spain). Using these data and the average weight of the berries, the following yield components were determined: berry weight, kg/vine, number of bunches per shoot and bunch weight.

Evolution of the Berry Composition During the Ripening Period

Technological Maturity

Weekly samplings were performed of 100 berries per elemental plot from pre-veraison to time of harvest. Each

sample was weighed on a scale with a sensitivity of 0.01 g (OHAUS brand Adventurer Pro Model AP AV412) to determine average berry weight. The must from these 100 berries was extracted with a strainer and, after centrifugation, the supernatant material was collected for analysis of total soluble solids (°Brix), pH and total titratable acidity.

The total soluble solids were measured using a portable digital refractometer (PALETTE WM-7, ATAGO Inc., Kirkland, Washington, USA), with results shown in °Brix; total titratable acidity was measured using an automatic titrator (736 GP Titrino, METROHM AG, Herisau, Switzerland), with the values shown in g tartaric acid/L; and pH was determined using a pH meter (micropH 2001, CRISON, Barcelona, Spain) that had been previously calibrated.

a. Phenolic Maturity

Samples were taken of 150 berries during each of the three harvests from every one of the experimental Shiraz plots and then triturated in a blender (Royal Blender Turbo 10-speed, Princess).

The extraction method proposed by Glories and Augustin (1993) was used to calculate the total polyphenol index (TPI) and the concentration of total and extractable anthocyanins. Two standards were applied: one at pH 1 (HCl 0.1 N) for extracting all anthocyanins and another at pH 3.2 (5 g/L tartaric acid solution) for extracting the extractable anthocyanins. Absorbency measurements were taken at 280 nm and at 520 nm with a spectrophotometer *(J.P. Selecta*, SPECTROPHOTOMETER UV-2005). Tannins were extracted from the berry using methodology from the Standard Methods of the Australian Wine Research Institute (AWRI) (Iland et al. 2004).

Quantification of the tannins was performed using the method of Methyl Cellulose Precipitation (MCP) (Sarneckis et al. 2006, and Mercurio and Smith 2008). This reading was obtained by using a spectrophotometer (J.P. Selecta, SPECTROPHOTOMETER UV-2005).

1.1.1 Fermentations

Separate fermentations were conducted for the 16 repetitions during the three specified harvests, resulting in a total of 48 micro-fermentations. Fermentations were achieved by following the micro-fermentation method described by Sampaio et al. (2007). The fermentation micro-deposit consisted of a glass jug (1 gallon) with a cap for alimentary use perforated in the centre, through which passed the fermentation value, which was thermosealed with food-grade silicone.

1.1.2 Sensory Analysis

The wines from the two experiments were subjected to sensory evaluation. These were performed by a group of 11 professional tasters made up of professional oenologists and researchers from the viticulture research group at the Universidad Politécnica de Madrid.

The method of analysis involved triangle taste tests that showed whether or not the tasters were able to perceive a difference in treatments. This procedure allows information to be collected on sensory differences and similarities for a wide variety of products (Blancher et al. 2007 and Cartier et al. 2006).

The results of the triangle taste tests were calculated statistically according to the tables of Roessler et al. (1948) for levels of significance of 5%, 1% and 0.1%.

After the triangular tests, treatment wines that were significantly different according to the aforementioned underwent a descriptive test to determine which wine the tasters preferred.

1.1.3 Analysis of Results

Due to its experimental design, statistical analysis of a large part of the results was performed through variance analysis. The significance of the variance analysis was determined for probability levels $p<0.05$ (*), $p<0.01$ (**) y $p<0.001$ (***). Whenever this showed significant effects of treatment, the averages were compared using Duncan's multiple range test for a probability level of $p<0.05$. The program SPSS, version 18.0 (SPSS Inc. Chicago, Illinois) was used for all statistical analyses.

2. RESULTS AND DISCUSSION

All media data obtained in the field and laboratory during the campaign was reported and compared with data obtained by other authors, with particular attention paid to differences possibly attributable to different yeast derivative treatments. See table 1.

2.1 Vegetative Growth

The most reliable estimator of plant vigour is pruning weight (Huglin 1986). It gives the best estimation of plant yield, growth and development, and thus the productive potential of the vine for given conditions (Yuste 1995).

The limits of shoot weight cited in the literature are 20-40 g/shoot. Sauvignon data showed low shoot vigour due to high crop load. Shiraz shoot weight was within normal values mentioned in the literature.

None of the treatments with yeast derivatives affected the vegetative growth of Shiraz or Sauvignon Blanc – neither

Table 1. RD-LM treatment effect on Shiraz and RD-LA treatment effect on Sauvignon Blanc re pruning weight (kg/m²), shoot weight (g) and % of shoot humidity. Statistical significance: *, **, ***, ns: significant differences for p≤0.05, 0.01, 0.001, or not significant, respectively.

Variety	Treatment	PW (kg/m2)	Shoot weight (g)	% Hum shoot
Shiraz	Control	0.23	30.18	55.9
	RD-LM	0.25	30.43	55.8
	Sig.	ns	ns	ns
Variety	**Treatment**	**PW (kg/m2)**	**Shoot weight (g)**	**% Hum shoot**
Sauvignon Blanc	Control	0.17	16.80	60.0
	RD-LA	0.15	15.28	61.9
	Sig.	ns	ns	ns
Variety	**Treatment**	**PW (kg/m2)**	**Shoot weight (g)**	**% Hum shoot**
Shiraz	Control	0.23	30.18	55.9
	RD-LM	0.25	30.43	55.8
	Sig.	ns	ns	ns
Variety	**Treatment**	**PW (kg/m2)**	**Shoot weight (g)**	**% Hum shoot**
Sauvignon Blanc	Control	0.17	16.80	60.0
	RD-LA	0.15	15.28	61.9
	Sig.	ns	ns	ns
Sauvignon Blanc	Control	0.17	16.80	60.0
	RD-LT	0.14	14.40	63.6
	Sig.	ns	ns	ns

the vigour estimated by the shoot weight nor the vine's vegetative expression as evidenced by the pruning weight. This was to be expected, since the yeast derivatives were applied after vegetative growth was well underway and in some aspects (such as on the stems) almost complete. (The conditions of the shoots, like humidity content, were not affected during the ripening period.)

2.2 Vegetative/Productive Balance

Dry Weight

Knowing that dry weight production indicates a vineyard's capacity or potential (Carbonneau and Casteran 1986) and that dry weight accumulated on renewable parts provides a good approximation of the overall productivity of the vine by assuming 88–93% of the dry weight produced annually (Williams 1996), we can conclude from treatments that there was no effect on vineyard capacity or potential.

Like with vegetative growth, the experimental treatments had no effect on the humidity conditions of leaves and bunches, which change during the ripening period.

Dry Weight Distribution

According to the dry weight distribution data obtained by various authors (Fregoni 1980, Fernandez et al. 1997, Williams and Grimes 1987, and Williams and Biscay 1991), our trial showed a lower % of dry weight accumulated in stems and a higher % in leaves. The % in bunches was similar to that obtained by these authors. This indicates the low vigour of the shoots, especially in Sauvignon Blanc, due to the high number of shoots per plant and the water stress present.

Dry weight distribution between leaves, stems and bunches did not have a significant effect, and where grape harvests varied the values were within normal ranges (between 54% and 64% of dry weight corresponding to renewable parts).

Table 2. RD-LM treatment effect on Shiraz and RD-LA treatment effect on Sauvignon Blanc re dry weight and % of humidity in leaves (LDW and %LH) and dry weight and % of humidity in the bunches (BDW and %BH). Statistical significance: *, **, ***, ns: significant differences for p≤0.05, 0.01, 0.001, or not significant, respectively.

		LEAVES		BUNCHES				LEAVES		BUNCHES	
Variety	**Treatm.**	**LDW**	**%LH**	**BDW**	**%BH**	**Variety**	**Treatm.**	**LDW**	**%LH**	**BDW**	**%BH**
Shiraz	Control	55.22	63.6	80.20	70.1	Sauvignon Blanc	Control	36.04	61.0	47.94	72.1
	RD-LM	51.14	62.2	68.39	71.2		RD-LA	33.03	61.2	58.71	72.2
	Sig.	ns	ns	ns	ns		Sig.	ns	ns	ns	ns

Table 3. RD-LM treatment effect on dry weight distribution in the renewable parts of Shiraz vines, and RD-LA treatment effect on dry weight distribution in the renewable parts of Sauvignon Blanc vines. Statistical significance: *, **, ***, ns: significant differences for p≤0.05, 0.01, 0.001, or not significant, respectively.

Variety	Treatm.	% stems	% leaves	% bunches	Variety	Treatm.	% stems	% leaves	% bunches
Shiraz	Control	12.14	32.03	55.84	Sauvignon Blanc	Control	11.44	31.31	57.25
	RD-LM	14.02	31.08	54.90		RD-LA	9.53	26.67	63.80
	Sig.	ns	ns	ns		Sig.	ns	ns	ns

Physiology

Leaf Water Potential

The leaf water potential measured at noon (ψ mid) decreased as the cycle progressed, even under conditions of sufficient moisture (Williams and Matthews, 1990), remaining relatively stable after veraison (Williams and Grimes, 1987). In Table 4 we see that in maturation, the ψ mid decreased with respect to pre-veraison values.

Furthermore, according to Williams and Mathews (1990), leaf water potential measured at noon must be lower than -1.3 MPa for premature leaf abscission to occur, and Kriedman and Smart (1971) observed that leaf water potential measured at 12 p.m. began to limit photosynthesis from -1.2 MPa. According to the values obtained by these authors, Shiraz and Sauvignon Blanc vines in our trial experienced since pre-veraison limiting values for photosynthesis, and explain the senescence of basal leaves already present on this period.OK

According to Vallone et al. (1997), leaf water potential at noon in the ripening period has an effect on yield components.

The leaf water potential although in some cases showed significant differences only in the LA treatment on Sauvignon Blanc is worthy of consideration. For LA treatment, the leaf water potential were significantly reduced compared to the control during the ripening period.

Gas Exchange

According to the schema by Medrano et al. (2002) on vine photosynthetic response to drought with stomatal conductance used as a reference parameter, we see that the values at the time of maximum photosynthetic activity suggest moderate drought. The stomatal effects are predominant, and photosynthesis is restored once the leaf is rehydrated (Flexas et al., 1999), and no stomatal effects are detectable (Naor et al., 1994, Flexas et al., 2002, and Maroco et al ., 2002). For measurements performed at noon, the values show severe water stress with predominance of non-stomatal effects and no recovery of photosynthesis after the leaf is rehydrated (Dühring 1988).

Unlike leaf water potential, physiological measurements at the leaf level in Shiraz were not significantly affected either at maximum photosynthesis or at noon by the application of LM. We consider the significant reduction in the net assimilation rate at midday with respect to maximum photosynthesis noteworthy, as values at maturation are much lower than pre-veraison values, which shows that the effects of water status are more limiting at noon and when the ripening period has advanced.

2.4 Yield Components

Yield components are already determined at the time when the yeast derivatives are applied, except berry weight, which tends to double during ripening. As might

Table 4. RD-LM treatment effect on Shiraz and RD-LA treatment effect on Sauvignon Blanc leaf water potential (MPa) at maximum photosynthesis (ψ max) and at midday (ψ mid). Statistical significance: *, **, ***, ns: significant differences for p≤0.05, 0.01, 0.001, or not significant, respectively.

Variety	Date	Treatment	Ψmax	Ψmid	Variety	Date	Treatment	Ψmax	Ψmid
Shiraz	Pre-veraison	Control	-1.50	-1.44	Sauvignon Blanc	Pre-veraison	Control	-1.26	-1.35
		RD-LM	-1.33	-1.38			RD-LA	-1.22	-1.39
		Sig.	**	ns			Sig.	ns	ns
	Maturation	Control	-1.60	-1.67		Maturation	Control	-1.22	-1.58
		RD-LM	-1.46	-1.62			RD-LA	-1.64	-1.72
		Sig.	*	ns			Sig.	**	*

Table 5. RD-LM treatment effect on Shiraz re net photosynthesis rate (µmol CO2/m2 leaves) and stomata conductance (mol H_2O/m2 leaves). Statistical significance: *, **, ***, ns: significant differences for p≤0.05, 0.01, 0.001, or not significant, respectively.

Variety	Date	s.h.	Treatm.	Photos.	Cond.	Date	s.h.	Treatm.	Photos.	Cond.
Shiraz	Pre-veraison	maximum	Control	7.48	0.0876	Maturation	maximum	Control	7.70	0.1948
			RD-LM	8.39	0.1002			RD- LM	6.71	0.1638
			Sig.	ns	ns			Sig.	ns	ns
		midday	Control	3.11	0.0454		midday	Control	1.57	0.0248
			RD-LM	3.37	0.0311			RD-LM	1.43	0.0206
			Sig.	ns	ns			Sig.	ns	ns
		s.h. *applic.	Sig.	ns	ns		s.h. *applic.	Sig.	ns	ns

be expected, yield components were not significantly affected in any of the treatments or varieties. Overall values for fertility (number of clusters/shoots), bunch weight and berry weight were quantitatively small, probably due to the high number of shoots present in the vineyard and the very limited water availability.

2.5 Evolution of Berry Composition during Ripening

a. Berry Weight

Berry weight is a basic estimator of growth and the quality/quantity of the harvest.

Significant differences were found only in the sampling from August 29, which coincided with the first harvest, showing greater berry weight vs. the control in the case of RD-LA treatment, as we saw earlier in the yield components.

b. Total Soluble Solids (°Brix)

During ripening, grapes are a major sink for carbohydrates from the active leaves and reserve structures (Roubelakis-Angelakis 2009). We can see in figure 2 (next page) that treatments did not have any impact on carbohydrate accumulation during the ripening period.

c. Total Acidity

Evolution of the total titratable acidity (expressed as g/L of tartaric acid) was characterized by an increase in the herbaceous phase followed by a decrease throughout maturation, coinciding with the increase in sugar content and increased berry weight (Esteban et al. 1999 and Hrazdina et al. 1984). In figure 3 (next page) we see that treatments did not affect the degradation of acids during ripening.

d. pH

The pH of the must depends on the balance between concentrations of free organic acids and their salts, with potassium as the primary cation (Smart and Coombe, 1983). In figure 4 we see that treatments did not affect this balance.

During the ripening period, the technological maturity of the pulp did not undergo significant changes in sugar con-

Table 6. RD-LM treatment effect on Shiraz and RD-LA treatment effect on Sauvignon Blanc yield components. Statistical significance: *, **, ***, ns: significant differences for p≤0.05, 0.01, 0.001, or not significant, respectively.

Variety	Date	Treatm.	Bunch Weight (g)	Kg /vine	Berry Weight (g)	Num. Bunches / Shoot	Variety	Date	Treatm.	Bunch Weight (g)	Kg /vine	Berry Weight (g)	Num. Bunches / Shoot
Shiraz	1st Harvest 09/03/2013	Control	94.21	3.30	0.96	1.39	Sauvignon Blanc	1st Harvest 08/29/2013	Control	81.63	3.23	0.78	1.38
		RD-LM	96.37	3.43	0.98	1.38			RD-LA	83.13	3.92	0.86	1.61
	2nd Harvest 09/18/2013	Control	100.50	3.54	0.98	1.49		2nd Harvest 09/10/2013	Control	75.39	3.52	0.78	1.41
		RD-LM	94.45	3.37	0.94	1.40			RD-LA	83.62	3.82	0.82	1.39
	3rd Harvest 09/24/2013	Control	96.30	3.18	0.93	1.35		3rd Harvest 09/18/2013	Control	67.67	2.91	0.78	1.41
		RD-LM	80.65	2.85	0.85	1.37			RD-LA	72.37	3.21	0.86	1.40
	Harvest	Sig.	ns	ns	ns	ns		Harvest	Sig.	ns	ns	ns	ns
	Application	Sig.	ns	ns	ns	ns		Application	Sig	ns	ns	ns	ns
	Harv. * Applic.	Sig.	ns	ns	ns	ns		Harv. * Applic.	Sig.	ns	ns	ns	ns

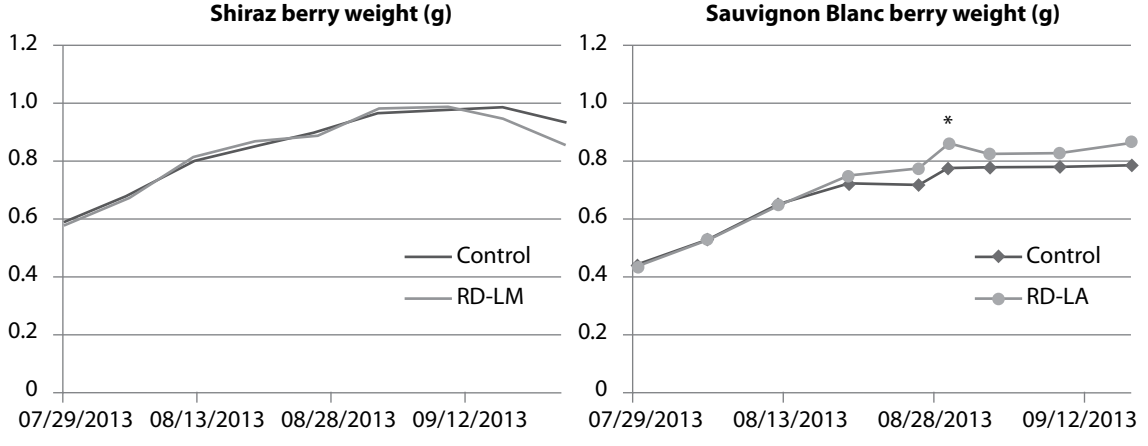


Figure 1. RD-LM treatment effect on Shiraz and RD-LA treatment effect on Sauvignon Blanc berry weight (g) from pre-veraison to the third harvest

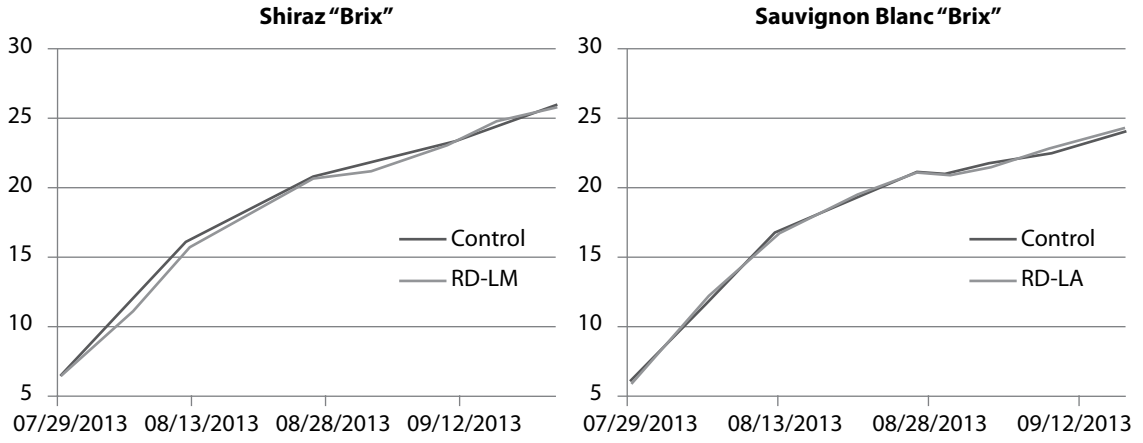


Figure 2. RD-LM treatment effect on Shiraz and RD-LA treatment effect on Sauvignon Blanc re °Brix from pre-veraison to the third harvest

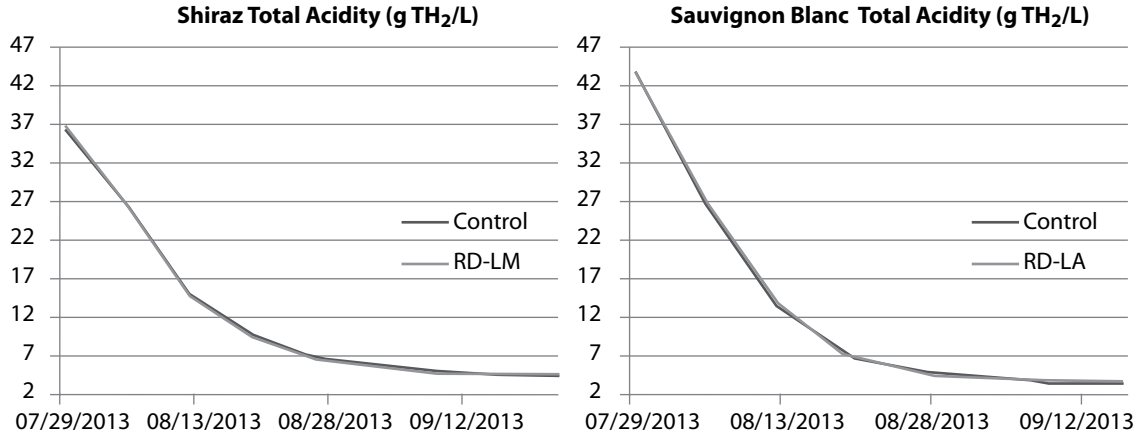


Figure 3. RD-LM treatment effect on Shiraz, and RD-LA and RD-LT treatment effect on Sauvignon Blanc re total ccidity (g TH_2/L) from pre-veraison to the third harvest

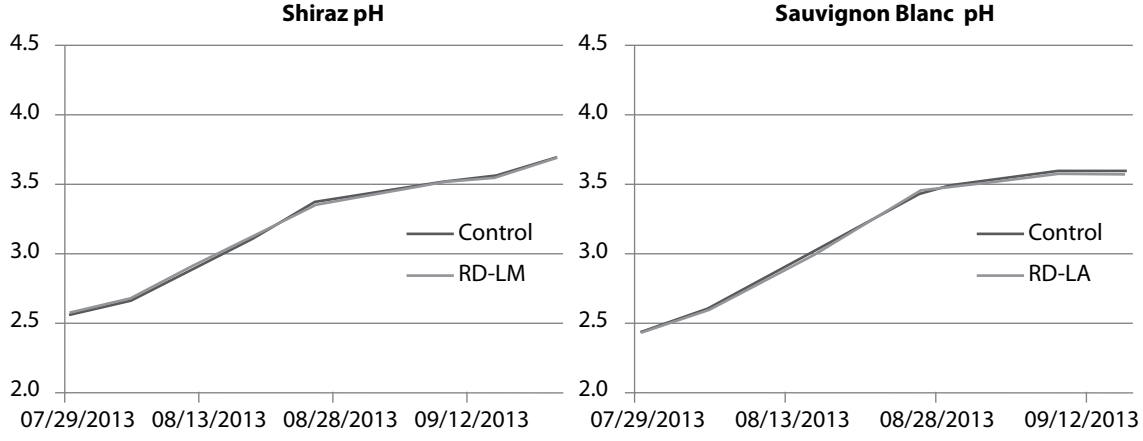


Figure 4. RD-LM treatment effect on Shiraz and RD-LA treatment effect on Sauvignon blanc re pH from pre-veraison to the third harvest

tent, expressed as °Brix, total acidity and pH. The control and yeast derivative treatments had similar values.

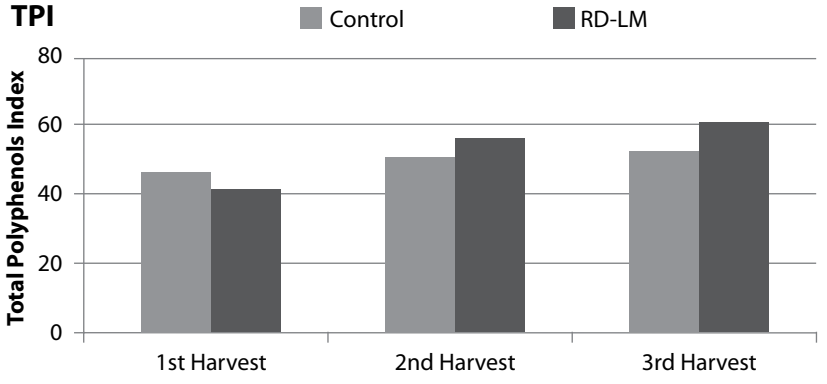
e. Total Polyphenol Index

The Index of Total Polyphenols theoretically represents the sum of the contribution of phenolic anthocyanins from the skins, the tannins from the skins and the tannins from the seeds. (See figure 5)

f. Total Anthocyanins and Extractable Anthocyanins

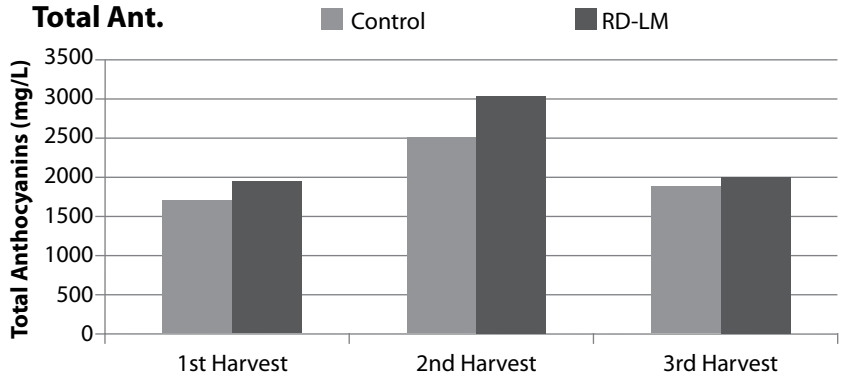
In figure 6, as expected, the anthocyanin content increases during ripening to a maximum at second harvest, then decreases due to "overripening" linked to a phenomenon of degradation and usually an aging phenomenon in the berry. As a consequence of this cellular aging, a substantial decrease in cohesion between the cells occurs, with degradation of cell walls and membranes. This increases the extractability of anthocyanins.

g. Tannins

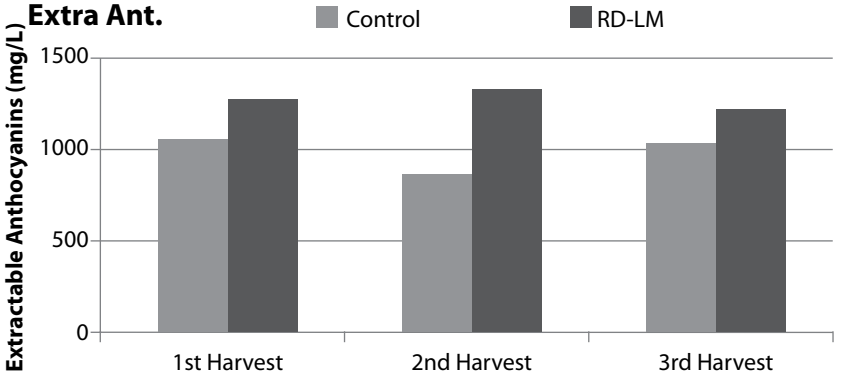


SHIRAZ	IPT
Harvest x Application	***
Harvest	*
Application	ns

Figure 5. RD-LM treatment effect on Shiraz re Total Polyphenol Index (TPI) from pre-veraison to the third harvest. Statistical significance: *, **, ***, ns: significant differences for p≤0.05, 0.01, 0.001, or not significant, respectively.

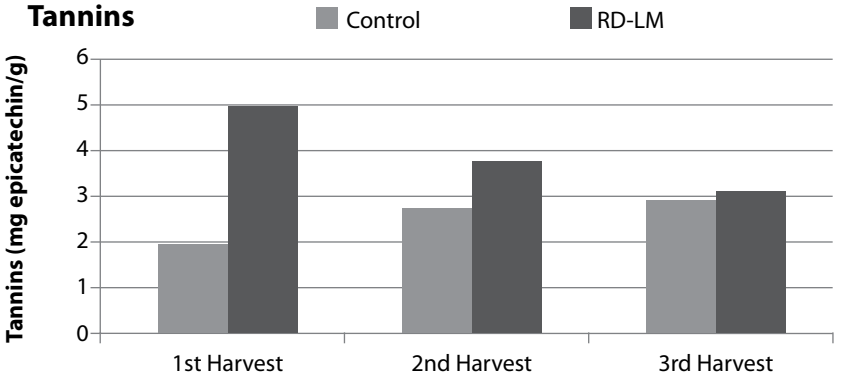


SHIRAZ	Ant TOTAL
Harvest x Application	***
Harvest	*
Application	ns



SHIRAZ	Ant EXTRA
Harvest x Application	***
Harvest	***
Application	*

Figure 6. RD-LM treatment effect on Shiraz re total anthocyanins and extractable anthocyanin concentration (mg malvidin/L) at the three harvests. Statistical significance: *, **, ***, ns: significant differences for p≤0.05, 0.01, 0.001, or not significant, respectively.



SHIRAZ	Tannins
Harvest x Application	***
Harvest	ns
Application	***

Figure 7. RD-LM treatment effect on Shiraz re tannin concentration (mg epicatechin/g) at the three harvests. Statistical significance: *, **, ***, ns: significant differences for $p \leq 0.05, 0.01, 0.001$, or not significant, respectively.

Tannins are synthesized during the first stage of berry growth or "herbaceous growth," and synthesis ends shortly after veraison.

When skin maturity was studied in Shiraz to ascertain the influence of yeast derivatives (LM) on phenolic maturity, it was found that the effect depended on harvest time and generally on interaction between harvest time and treatment with yeast derivatives. This was the case both for the total polyphenol index and for total and extractable anthocyanins. In the latter case, the treatment regardless of the harvest date was also shown to have a significant effect. Also noteworthy was that Shiraz tannin content increased significantly at all harvest times when yeast derivatives were applied.

We find this significant effect on Shiraz phenolic maturity when LM yeast derivatives are applied of particular note. Values were always higher on the last two harvest dates compared to the control, only lower on the first harvest date in the case of total polyphenols. The best and maximum values obtained with yeast derivatives products suggest that research needs to continue

2.6 Vinification

All repetitions were fermented at the three harvest times in both cultivars and all treatments studied, for a total of

Table 7. Actions and timing of winemaking process on Shiraz and Sauvignon Blanc at the three harvest times for each treatment. In both cases the yeast used for fermentation was EC-1118 (Lallemand Inc.). The enzyme added in Sauvignon Blanc musts was lalzyme Cmax (Lallemand Inc).

Variety	Harvest	Action						
		Harvest + Vatting + Inoculation	Nutrient Vit™ Addition	Reduless™ Addition	End of Fermentation	End of Maceration Devatting	End of Stabilization Homogenized + Bottled	Tasting
Shiraz	1st Harvest	03 Sep	04 Sep	09 Sep	15 Sep	17 Sep	27 Sep	10 Feb 09 May
	2nd Harvest	18 Sep	19 Sep	25 Sep	03 Oct	03 Oct	14 Oct	10 Feb 09 May
	3rd Harvest	24 Sep	25 Sep	03 Oct	16 Oct	18 Oct	05 Nov	10 Feb 09 May

Variety	Harvest	Action						
		Harvest + Vatting + Enzyme + Refrigeration	Clarification + Inoculation	Nutrient Vit™ Addition	Reduless™ Addition	End of Fermentation Devatting	End of Stabilization Homogenized + Bottled	Tasting
Sauvignon Blanc	1st Harvest	29 Aug	01 Sep	05 Sep	09 Sep	12 Sep	23 Sep	20 Jan 09 May
	2nd Harvest	10 Sep	12 Sep	19 Sep	22 Sep	24 Sep	04 Oct	20 Jan 09 May
	3rd Harvest	18 Sep	20 Sep	25 Sep	03 Oct	08 Oct	18 Oct	20 Jan 09 May

Table 8. RD-LA treatment effect on Sauvignon Blanc and RD-LM treatment effect on Shiraz re triangle taste test. Level of significance 5%, 1% and 0.1% for a panel of 11 tasters (according to Roessler et al. 1948).

Variety/ Product	Harvest	Level of significance	Harvest	Level of significance	Harvest	Level of significance
Sauvignon Blanc RD-LA	1st Harvest	ns	2nd Harvest	5%	3rd Harvest	1%
	1st Harvest	1%	2nd Harvest	5%	3rd Harvest	ns
	1st Harvest	5%	2nd Harvest	5%	3rd Harvest	5%
Shiraz RD-LM	1st Harvest	5%	2nd Harvest	1%	3rd Harvest	1%
	1st Harvest	5%	2nd Harvest	1%	3rd Harvest	1%
	1st Harvest	1%	2nd Harvest	1%	3rd Harvest	1%

48 microvinifications. The table on page 74 shows the actions and dates relative to the winemaking process for the experimental vinifications in both cultivars.

2.7 Sensory Evaluations

Triangle Test

The Triangle Taste Test makes it possible to determine differences between two products with similar qualities. Tasters are offered three samples at random, two of which are from the same wine. Three repetitions of each treatment were tested.

For a panel of 11 tasters, the level of significance according to Roessler et al. (1948) for the triangle taste test is 5% if 7 out of 11 answers are correct, 1% if 8 out of 11 answers are correct and 0.1% if 10 out of 11 answers are correct. Below are the results:

Except in one of the comparisons of the first harvest and in another of the third harvest, the difference was significant between Sauvignon Blanc wines from vines treated with RD-LA versus those from untreated vines.

In the case of Shiraz, the wines from vines treated with RD-LM showed high levels of significance when compared to wines from untreated vines.

It is worth noting that the differences in many cases exceeded 70%, which adds substantial weight to the sensory analysis that revealed a general preference for wines from vines treated with yeast derivatives.

Descriptive Test

A sensory descriptive analysis was performed after it was determined that the wines from vines treated with RD-LM and RD-LA were significantly different from the wines from untreated vines.

Figures 9 and 10 (next page) present the results of the descriptive analysis.

SAUVIGNON BLANC

The particularly interesting sensory analysis of Sauvignon Blanc reveals that depending on the time of harvest, the wines are more aromatic and feature a more enjoyable mouthfeel when treated with yeast derivatives. In the case of Shiraz, significantly favourable mouthfeel and color with LM treatment always depended on the time of harvest.

Conclusions

Applying LA in Sauvignon Blanc and LM in Shiraz improved the aromatic expression of Sauvignon Blanc wines and the phenolic maturity of Shiraz, with higher anthocyanin and tannin content and enhanced sensory impressions in mouthfeel.

Applying yeast derivatives between veraison and the start of ripening (LA and LT in Sauvignon Blanc and LM in Shiraz) did not affect the functionality of the vine, leaf activity, yield components, vegetative growth or technological maturity of the grapes.

These conclusions should be consolidated with results of future trials

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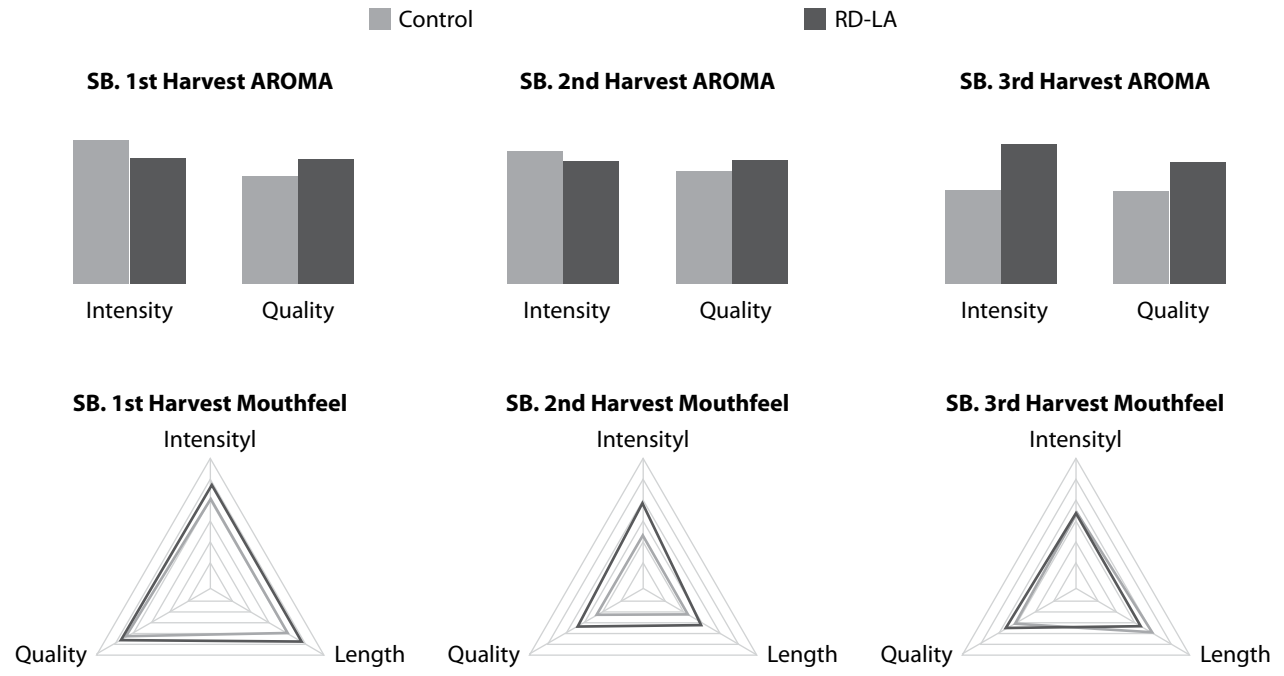

Figure 8. Tasting analysis examining the impact of RD-LA treatment on Sauvignon Blanc wines at the three different harvest times. Statistical significance: *, **, ***, ns: significant differences for p≤0.05, 0.01, 0.001, or not significant, respectively.

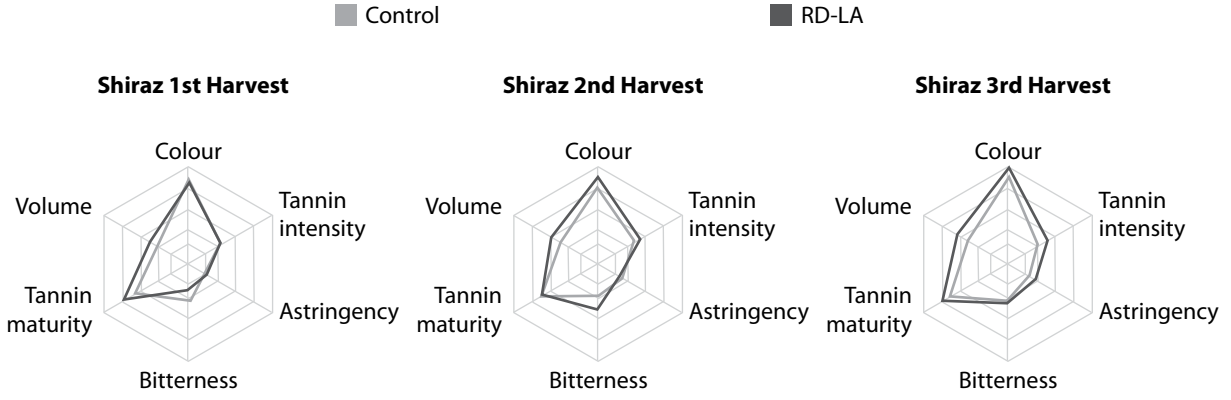

Figure 9. Palette evaluation examining the impact of RD-LM treatment on Shiraz wines at three different harvest times. Statistical significance: *, **, ***, ns: significant differences for p≤0.05, 0.01, 0.001, or not significant, respectively.

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