Enhancing Phenolic Maturity of Syrah with the Application of a New Foliar Spray

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Climate change is inducing earlier grape ripening, especially in warm vintages. This phenomenon is resulting in unbalanced wines with too high an alcohol concentration and low titratable acidity along with a high pH level, without the desired level of phenolic maturity. Final wine quality notably depends on the phenolic composition of grapes and the extractability of these compounds. This research was designed to test a new foliar spray, called LalVigne® MATURE for its capacity to create a balance between sugar development and phenolic maturity. It is a formulation of 100% natural, inactivated wine yeast derivatives. This foliar spray was tested on Syrah vines in two vintages (2012, 2013) in a cool climate wine region (Eger, Hungary). It was acting as an elicitor, stimulating the synthesis of several secondary metabolites. Changes in anthocyanin extractability and texture characteristics of the grape berries were followed during ripening. Experimental wines were made at three separate harvest times in each vintage. Standard analytical parameters for grapes and wines as well as resveratrol were evaluated. Grapes from treated vines had thicker skins than controls at all sampling dates in both vintages. The phenolic potential (especially anthocyanin concentration and its extractability) of the foliar spray treated grapes was greatly improved. Our experiment showed that phenolic ripening can be enhanced using the foliar spray, and its application is useful in different vintages.

INTRODUCTION

Nowadays wine consumers prefer well structured wines with deep color, fruit scents, soft tannins and pleasant mouthfeel (Bruwer *et al.,* 2011). This kind of wines can be made from well-ripened with an optimal level of phenolic and technological (sugar) maturity, but not from overripened grapes. Nevertheless, the changing climate modifies the ripening process notably. In cool climate wine regions such as the Eger wine district in Hungary we can count on more frequent extreme weather events including uneven precipitation, heat waves and droughts (Schultz 2000). In dry and hot vintages the ripening process is faster, and the balance between phenolic and technological (sugar) maturity may not be maintained (Hannah *et al.,* 2013). This results in an increase in the sugar concentration, and in parallel, a rapid decrease in the titratable acidity resulting in unbalanced and too alcoholic wines. At the same time, the lack of optimal phenolic maturity results in wines with green and astringent tannins (Jones *et al.,* 2005). On the other hand, in a rainy, cool vintage the ripening is slowed, and late ripening varieties (such as Cabernet Sauvignon, Cabernet Franc, Syrah) cannot reach optimal maturity (Jackson & Lombard 1993).

Several technological applications can be used in order to reduce these negative effects. Cluster thinning (Guidoni *et al.,* 2002; Prajitna *et al.,* 2007), girdling (Singh Brar *et al.,* 2008; Koshita *et al.,* 2011) and early defoliation (Poni *et al.,* 2006; Poni *et al.,* 2009; Kemp *et al.,* 2011; Gatti *et al.,* 2012; Lee & Skinkis 2013) are reported to have a beneficial effect on phenolic maturity especially on anthocyanin and flavonoid synthesis. The resveratrol content of the grape varies considerably and depends on many viticultural factors including climate, terroir, grape variety, fungal infections and yield (Jeandet *et al.,* 1995; Bavaresco 2003; Bavaresco *et al.,* 2007; Prajitna *et al.,* 2007). There are also some paper which are dealing with increasing resveratrol concentration in grapes using elicitors (Vezzulli *et al.,* 2007; Santamaria *et al.,* 2011).

Beyond the above mentioned techniques a new foliar spray for enhancing phenolic maturity was developed recently, and it was examined for its effects. In addition, Syrah is a new cultivar to the Eger wine region, with only limited cultivation experience with it.

The aim of this study is 1) to describe the effects of the application of this new foliar spray on grape phenolic maturity and 2) to describe some aspects of the responses of a "new" variety (Syrah, *Vitis vinifera* L.) in a cool climate wine region (Eger, Hungary).

MATERIALS AND METHODS

Description of the experimental site and the experimental design

The experiment took place in the Eger wine region (in North-East Hungary) in a commercial vineyard (lat. 47°55'31.84" N; long. 20°24'42.32" W, elevation: 430 m asl). The vineyard's shallow soil is based on limestone. This site met the criteria for an investigation of a new foliar spray designed to enhance phenolic maturity, because in warm vintages the sugar accumulation is very fast at the Nagy-Eged-hill, leading too alcoholic, unbalanced wines. Besides, the desired level of phenolic maturity cannot be achieved in most of the vintages. The trail was performed over two consecutive vintages in 2012 and 2013.

Ten-year-old Syrah (clone ENTAV-INRA® 877) vines grafted onto Teleki 5C at a spacing of 2.4 m x 0.8 m with south-north row orientation were investigated. Vines were trained to a unilateral cordon at a height of 0.6 m, and were pruned to four spurs, each bearing two nodes. A trial site of 6 rows were selected for each treatment (3 control (unsprayed, C) and 3 treated (sprayed, LM) rows). Each row was divided into 3 blocks. One block contained 25-29 vines. At the same harvest time 3 blocks/treatment were harvested resulted in 3 replicates/treatment. The leaf spray, LalVigne® MATURE is a formulation of 100% natural, inactivated wine yeast (*Saccharomyces cerevisiae*) derivatives (specifically designed to be used with the patent foliar application technology WO/2014/024039, Lallemand Inc., Canada). It is non-pathogenic, non-hazardous, food grade and non-GMO. The product is already registered in many countries and in process of authorization in others. Two applications of 1 kg/ha were done. The first one was at the beginning of veraison, the second one 12 days later. The powder was diluted in water without using an adjuvant. The whole canopy was sprayed with a motorized backpack sprayer.

There were three harvest dates (09.06., 09.13., 09.27. in 2012 and 09.12., 09.19., 10.03. in 2013) in each vintage for both the control and treated vines. Establishing as reference the second harvest that was defined by commercial harvest date done by Gróf Buttler winery, the first harvest date was done one week earlier and the third harvest two weeks later than the reference. One vine block represented one wine repetition per treatment at each harvest date. Veraison commenced in the first week of August in 2012, and one week later in 2013.

Climatic data

Climatic data were monitored by an automatic weather station (Boreas Ltd. Érd, Hungary), approximately 300 m far from the trial site.

Berry sampling

Three sets of 20 kg grapes, each set from 25-29 vines were carefully harvested for both treatments at each harvest date by hand, and transported immediately to the experimental winery. Three one kg samples for each treatment were collected at random from several clusters before vinification. The berries were selected randomly from the upper, middle, and lower parts of the bunches. All the berry samples were prepared and analyzed within 2 hours after the harvest.

For the texture analysis, 50 berries were randomly removed from the clusters with pedicels and visually examined before texture analysis. One berry represents one repetition by this measurement. Damaged berries were rejected.

150 berries were separately selected for phenolic measurement (Glories method) and these berries were subdivided into two equal groups for the pH 1 and pH 3.4 solutions. The measurement was done in triplicate. 25 berries were used for each repetition.

Three additional sets of 100 grape samples were selected for weight determination and grape composition analysis.

Grape analysis

The analytical methods recommended by the OIV (2014) were used to determine titratable acidity and the pH of the grapes. The sugar content (expressed as °Brix) of the grape juices was determined at 20 °C using a hand-held refractometer (Atago MASTER-α, Japan).

Assesment of grape phenolic maturity

The phenolic potential of grapes was calculated according to the method described by Saint-Cricq *et al.* (1998). This involved grinding the grapes with a blender and macerating for 4 hours with buffer solutions at two pH values (1.0 and 3.4). The original method proposed a pH 3.2 buffer, but this was adjusted to 3.4, as it is more relevant to the grapes from this region. The indices of phenolic maturity were calculated according to Glories & Augustin (1993): potential anthocyanins (A1), extractable anthocyanins (A3.4), cell maturity index (EA%) and seed maturity index (SM%). All the measurements were done in triplicate.

The following equations were used:

$$EA\ (\%) = [(A1 - A3.4) / A1] \times 100$$

SM (%) = [(A280 − ((A3.4 / 1000) × 40)) / A280] × 100

Measurements of berry physical properties

A TA.XTplus Texture Analyzer (Stable Micro System, Surrey, UK) with HDP/90 platform and 30 kg load cell was used to follow grape physical properties. The Exponent 6.1.4.0 software was used for data evaluation. All operative conditions were applied according to Letaief *et al.* (2008b) and Zsófi *et al.* (2014). Briefly, a P/35 probe was used to determine berry hardness (BH). Berries of approximately the same size, with their pedicel attached, were gently removed from the bunch and laid on the plate of the analyzer. After this, they were compressed to 25% of their diameter. The P/2N needle was applied to conduct a puncture test. A second set of berries with their pedicel were removed from the bunch, they were laid on the plate of the analyzer and then they were punctured in the lateral face (Letaief *et al.,* 2008a). The skin break force (F_{sk}), skin break energy (W_{sk}) and Young's modulus of berry skin (E_{sk}) were calculated from the puncture test data using the software Exponent 6.1.4.0. Berry skin thickness (Sp_{sk}) was measured using a P/2 probe with 2 mm diameter. For this measurement, approximately 0.25 cm^2 skin was removed from the lateral face of the berry. The skin was carefully and gently cleaned of pulp, and then placed on the platform and the test was conducted as described by other authors previously (Letaief *et al.,* 2008a; Letaief *et al.,* 2008b; Río Segade *et al.,* 2008). The skin thickness is given by the distance (travel) between the point corresponding to the probe contact with the berry skin and the platform base during the compression test. For seed hardness tests one seed was removed from the berry and placed on the platform on its lateral side. The seeds were crushed by the P/35 probe. The seed break force (F_s), seed break energy (W_s) and Young's modulus of the seed (E_s) were also calculated by Exponent 6.1.4.0.

Wine analysis

The analytical methods recommended by the OIV (2014) were used to determine ethanol content, titratable acidity and pH of the wines.

Total phenolics of the wines were analyzed by the Folin-Ciocalteu method (Singleton & Rossi 1965) and the results expressed as gallic acid equivalents (GAE mg/L). The quantity of leucoanthocyanins (flavan-3,4-diols) was determined as described by Flanzy *et al.* (1969). The bisulfite bleaching method was used to determine the anthocyanin content of grape extracts and wines (Ribéreau-Gayon & Stonestreet 1965) while the total catechins (flavan-3-ols) were measured using the vanillin assay according to Amerine & Ough (1980). The color intensity ($A_{420}+A_{520}+A_{620}$) and hue (A_{420}/A_{520}) of the wines were determined using the method described by Glories (1984). Phenolic components were measured by spectrophotometer (UVmini-1240 CE UV-VIS, Shimadzu, Japan). The gelatin and HCl indices (Ribéreau-Gayon *et al.,* 2006) were also calculated. All the measurements were preformed in triplicate.

Qualitative and quantitative determination of resveratrol components in wines by HPLC

The analysis of resveratrol compounds was carried out according to Kállay & Török (1997). The wine samples were filtered first on filter paper, then on a membrane of 0.45 µm. The eluent for the isocratic HPLC analysis consisted of a 5 : 5 : 90 mixture (v/v%) of acetonitrile : methanol : redistilled water. All the measurements were done in triplicate, and the wine samples were directly injected after filtration without dilution, in a quantity of 20 µl. Operating conditions and chromatograph settings are as follows: a HP Series 1050 HPLC-apparatus with a normal phase LiChrospher® 100 CN (250x4mm, 5 µm) column (Merck, Germany) was used during the measurements. The detector was a HP Series 1050. The flow was set 2 mL/min at 30 °C with detection wavelength at 306 nm. The methanol and acetonitrile used for the experiment are of

HPLC grade, other chemicals were of analytical purity. *Trans*-resveratrol (99%) standard was purchased from Sigma-Aldrich (Germany). *Trans*-piceid standard was received from the San Michele all'Adige Research and Innovation Centre. *Cis*-isomers are produced by UV irradiation of the *trans*-isomers (Sato *et al.,* 1997). The detection limit was 0.1 mg/L.

Microvinification process

Three sets of 20 kg grapes were crushed, destemmed and sulfited (1 mL of 5% aqueous SO_2 solution for every 1 L of mashed grape) in the experimental winery at each harvest date. Macerations were conducted in 30 L plastic containers, and all grape repetitions were separately fermented. Three experimental wine replicates were made at each harvest time for each treatment respectively. After grape processing the containers were transported immediately to the cellar to ensure constant ambient temperature (13 °C) from the beginning to the end of maceration. After 24 hours of cold maceration selected active dry yeasts (20 g of dry yeast / 100 kg of processed grapes) (Uvaferm VN, Lallemand Inc.) and yeast nutrients (30 g / 100 kg of processed grapes) (Uvavital, Lallemand Inc.) were added. The maceration lasted for 23 days. The cap was punched down twice a day throughout the skin contact period. The wines were also inoculated with 10 mg/L lactic acid bacteria (Uvaferm Alpha, Lallemand Inc.) at the end of alcoholic fermentation. After 23 days the wines were pressed at 1.5 bar in a 30 L membrane press. Free-run and press wines were mixed. After malolactic fermentation had occurred, the wines were racked, and transported to the laboratory for analysis. All the wines were stored at 13 °C until the moment of the analysis for several days, and no sulfur was added prior to analysis.

Sensory analysis

All the wines were tasted by a group of 17 expert enologists. Blind tests were carried out by comparing in pairs (control (C) vs. treated (LM)) the wines obtained from the three different harvest dates in both vintage. The wines were sensory evaluated by the 100-point OIV (1994) method. In all the cases, the objective was to name which they prefer and for what reason.

Statistical analysis

Statistical analysis was conducted by IBM SPSS 20 (IBM Corp., Armonk, NY, USA) software. Values were compared by multivariate ANOVA test with three factors (the effects of vintage: 2012, 2013, treatment: C (control), LM (LalVigne® MATURE) and harvest dates) followed by between-subjects effect test. Homogeneity of variances was checked by Levene's test. In case of significant effect of harvest dates, Tukey's or Games-Howell post hoc test was used for mean separation, according to whether the homogeneity of variances were held or not.

RESULTS

Climatic characteristics for 2012 and 2013

Fig. 1 shows the climatic characteristics of the two vintages. The weather of 2012 can be considered as dry (total rainfall was 439.2 mm compared to the 50-year average of 589.6 mm) and warm (average year temperature was 12.5 °C compared to the 50-year average of 10.7 °C). On the other hand, 2013 can be regarded as a cooler vintage (total rainfall: 663 mm, average year temperature: 12.2 °C), although the weather was somewhat cooler with more rain during the flowering and ripening stage, than in 2012.

Yield, grape juice sugar concentration, acidity, pH, berry weight, cell and seed maturity indices

The average yield per vine was 0.63 kg (control) and 0.65 kg (treated) in 2012, 0.99 kg (control) and 0.92 kg (treated) in 2013. An average of seven bunches were grown per vine in both years.

Table 1 shows the standard grape juice parameters. The grapes reached a greater level of technological maturity in 2012 (maximum sugar concentration: 24.3 °Brix) compared to 2013 (maximum sugar concentration: 21.2 °Brix). Indeed, the berry sugar concentration in 2012 exceeded 2013 by 15-25%. There were also notable differences in the case of titratable acidity with the values in 2013 being significant higher. The lowest concentration was 8.6 g/L. The weight loss of the berries during ripening is due to the dehydration. There was some rain between the second and the third harvest dates in 2012, however, which resulted in heavier berries. Clearly, the vintage had a very strong effect on all the parameters as can be seen in Table 1.

The Glories indices, which provide a prediction on phenolic compounds in the resulting wines (Kontoudakis *et al.,* 2010) are given in Table 2. In general, the lower the EA% and SM% values, the riper the berry. In most cases the regular range for A1, EA% and SM% varies between: 500 to 2,000 mg/L, 70% to 20% and 60% to 0%, respectively (Ribéreau-Gayon *et al.,* 2006). The A1 and A3.4 values indicate a good anthocyanin concentration especially in 2012. Interestingly, the EA% values showed an increase in some cases during ripening, implying that the extractability of the anthocyanins decreased. None of the factors affected the seed maturity index (SM%).

Grape texture properties

Table 3 shows the texture parameters of the berries. The berries became softer (BH) during the ripening. The significant increase observable in 2012 is due to the rainfall during the second and third harvest periods. Changes in skin break force (F_{sk}) showed a very similar pattern to W_{sk} related to the treatments and the harvest time. The impact of the leaf spray caused a significant increase in skin thickness (Sp_{sk}). The values were above 0.2 mm in the case of treated grapes at all harvest dates and in both years. There was no correlation between skin thickness (Sp_{sk}) and skin break force (F_{sk}) values. The seed texture parameters remained unchanged despite the treatment between the harvest dates. However, the vintage had a very strong effect on these parameters.

Wine composition

Table 4 summarizes the main wine parameters. The wines had a wide range of alcohol concentration (between 11.28 %v/v and 15.55 %v/v). The foliar spray did not influence this parameter, however. We found significant differences between the titratable acidity and pH in the first phase of the ripening, but the differences were no longer significant by the second and third harvest dates.

The total polyphenol values were independent of the foliar spray treatment. In 2012 we measured significantly higher (above 2,000 mg/L) values than in 2013 (concentration between 1,025 and 1,304 mg/L). The leucoanthocyanin and anthocyanin concentrations were found to be significantly higher in the treated wines in three instances: in 2012 at the second and the third harvest dates, and in 2013 at the second harvest date (although only for anthocyanins). The weather conditions in 2012 favored anthocyanin synthesis up to 796 mg/L. By contrast, in 2013, the unfavorable vintage resulted in significantly lower anthocyanin concentration (Table 4). The impact of the foliar spray and harvest date on catechin levels is unclear. The color intensity (A_{420}+A_{520}+A_{620}) correlated well with the increasing concentration of anthocyanins. The values of color hue (A_{420}/A_{520}) represent bluish tone, but this is typical for young red wines (Boulton 2001).

The gelatin index increased significantly in 2012 between the first and the third harvest dates in the foliar spray treated grapes. In 2013 the differences between harvest dates were smaller, and the values were also much lower than in 2012 and less than the optimal value due to the unfavorable weather conditions (Ribéreau-Gayon *et al.,* 2006). During tastings the wines were characterized by green, unripe tannins. HCl indices show a marked variation from 4.34 to 12.99. The foliar spray treatment increased this parameter, but the difference was significant only at the second harvest date in 2012, and at the third harvest date in 2013.

Table 5 shows the changes in resveratrol concentration in the wines. The majority of resveratrol was found in the wines as the isomeric forms of piceid (resveratrol glycoside). In 2012 and 2013, *cis*- and *trans*-resveratrol were not detected in the control wines at the first harvest date. *Trans*-resveratrol was also absent in 2013 in the treated wines in the second harvest date. Treated wines contained this compound from the first harvest date. Under the effect of the foliar spray total resveratrol concentration increased especially in the first phase of ripening. The differences in total resveratrol concentration were not significant in three cases: at the second harvest dates in both years, and at the third harvest date in 2012.

Sensory analysis

All the tasters were able to differentiate between the control and treated wines. Wines made from foliar treated grape were preferred and received higher scores than controls (data not shown).

Vintage had a very strong effect on the sensory quality. In 2013 the average points were much lower for all the wines, but the positive impact of the foliar spray remained sensible.

DISCUSSION

The foliar spray treatment had a significant effect on titratable acidity and pH of the grapes with the treated berries containing less acid. This is probably due to the higher berry respiration as an effect of faster ripening (Sweetman *et al.,* 2009). There was a positive effect of the leaf spray treatment on both total (A1) and potential (A3.4) anthocyanins, favoring their accumulation in both years and at nearly all harvest dates. Several phenomena may generally trigger the higher anthocyanin concentration of the wines. These include a beneficial change in the berry skin/flesh ratio (Kennedy *et al.,* 2002; Ojeda *et al.,* 2002), increased extractability (Río Segade *et al.,* 2011) and intensive anthocyanin synthesis (Downey *et al.,* 2004; Yamane *et al.,* 2006; Koshita *et al.,* 2011). In addition, during anthocyanin extraction in winemaking, it is also necessary to take into account the changes in grape skin cell-wall composition and structure, because this can modify the extractability process (Hanlin *et al.,* 2010). The foliar spray treated grapes reached a greater level of phenolic maturity in both years as can be seen in the results for the first and third harvests (values of EA (%) are lower, see Table 3). The absolute (A1) and extractable pigment (A3.4) concentration were also higher due to the foliar spray in both years, except one instance in 2012. At the third harvest date the treated grape had a lower A1 value. Vintage had a significant influence on all the Glories parameters except SM%. As can be seen from the data in Table 2, SM% values did not match the optimal criteria (Ribéreau-Gayon *et al.,* 2006) for ripeness in several cases. Values higher than 60% mean that the seeds were not sufficiently ripe, and thus a long fermentation maceration would not be recommended. Neither the vintage, nor the foliar spray treatment affected the SM% values significantly.

The foliar spray resulted in a significant increase in berry skin thickness (S_{psk}) at all sampling dates. The harvest date and the vintage did not influence the skin thickness significantly. The skin hardness (F_{sk}) values were significant lower for treated gape in three cases (first harvest date in 2012, second and third harvest dates in 2013). Our results show that the concentration of anthocyanins was higher in the thicker skins and also in the case of lower skin hardness (F_{sk}). This is the opposite of other findings, where thinner (Río Segade *et al.,* 2011) and harder skins (Rolle *et al.,* 2008, 2009) contained more anthocyanins. However, thicker and softer skins may also contain more anthocyanins due to the increased flavonoid synthesis and higher berry skin/flesh ratio. The enhanced pigment accumulation due to the foliar spray is also supported by Duo *et al.* (2014) and Lissarrague *et al.* (2014). Berry texture parameters were strongly modified by vintage effect as seen before (Letaief *et al.,* 2008a; Río Segade *et al.,* 2008). Young's modulus of berry skin (E_{sk}), berry hardness (BH) and seed texture properties were the mostly affected parameters as can be seen in Table 3. It seems cooler weather results in harder skin and softer seed. In 2012 the seeds were harder than in 2013. In 2013 the F_s values remained under 36 N and the values of work needed for the break (W_s) were under 6 mJ, indicating softer seeds. There was no difference in seed texture parameters (F_s, E_s, W_s) between the control and treated berries. Further, the harvest date had no effect on these parameters.

Torchio *et al.* (2010) reported decreasing Young's modulus of the berry skin (E_{sk}) as ripening progresses. This was observed only in the 2013 season and can most probably be explained by the combined effects of changes in the cell-wall structure, ripening processes and the water content of the berry. With respect to other berry physical properties, only the BH values, which reflect berry softness, decreased with ripening as expected. The only increase in BH values (Table 3) can be seen between the second and third harvest dates in 2012 due to a rainy period at that time.

The increased values of HCl and gelatin indices for the wines from foliar spray treated grapes in 2012, and to some extent in 2013, indicate a more polymerized and balanced tannin structure compared to control wines. Sensory analysis supported these facts. All the tasters were able to differentiate between the control and treated wines. The wines made from foliar sprayed grapes had more intense flavor, better mouthfeel, higher varietal character and a longer finish. In all cases, the tasters preferred wines made from treated grapes. This capacity to achieve a higher phenolic maturity is a potential benefit of the foliar spray treatment. Interestingly, there was a lower concentration of monomeric catechins in wines from the foliar spray treated grapes in 2012. This observation may be explained by the higher polymerized phenolic compound concentration. HCl indices of the wines were between 4 and 12. A wine suitable for aging has a value of 10-25 (Ribéreau-Gayon *et al.,* 2006). Only two wines met this criterion. Both wines were made from foliar spray treated grapes in 2012 at the second and third harvest dates.

Resveratrol synthesis was also positively affected by the foliar spray especially in the first phase of ripening. The differences disappeared by the second harvest in both vintages, however. Significantly higher concentration was found for the first treated wines in both vintages and for the third treated wine in 2013. The causes may be the same as in the case of higher anthocyanin concentration since resveratrol can also be found in the berry skins. Vintage strongly affected the amount of total resveratrol. It seems the lower average temperature during the ripening phase (Figure 1) is delaying stilbene synthesis. The cooler vintage in 2103 also reduced the impact of the foliar spray resulting in lower resveratrol concentration at the first harvest date. *Trans*-piceid was the most abundant stilbene compound. This is in accordance with other findings (Bavaresco *et al.,* 2007).

The observed changes (the treated berries had higher anthocyanin content along with thicker skins) could be explained with vine-pathogen interaction. Vine recognizes the yeasts in the foliar spray, which is activating some defense mechanisms (Langcake & Pryce 1976; Hahn 1996; Garcia-Brugger *et al.,* 2006; Santamaria *et al.,* 2011). In this way secondary metabolism is enhanced in the berries (Zhao *et al.,* 2005).

Overall, it seems that the impact of the foliar spray is stronger in the earlier phases of the grape ripening process. As the ripening went forward the differences decreased between the treatments, while remaining noticeable until the end of the ripening.

CONCLUSION

We examined the impacts of yeast derivatives applications (LalVigne® MATURE, Lallemand Inc.) on Syrah grape phenolic maturity as well as wine phenolic composition and concentration. The results from two vintages indicate that its application leads to more optimal harvest conditions. In addition, a higher level of phenolic maturity was achieved in both warm (2012) and cool (2013) vintages. The application of this foliar spray results in wines that are more balanced, showing more flavors and complexity than the ones made from unsprayed vines. Preliminary evidence was also obtained to suggest that LalVigne® MATURE may also help in cooler and less optimal vintages by enhancing the ripening process leading to wines with greater oenological potential. Moreover, thicker grape skins and accumulation of resveratrol in early phases could also play an important role in plant protection.

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Figure caption

FIGURE 1 Average air temperature (lines) and monthly sum of precipitation (bars) for 2012 and 2013 at the experimental site (data from automatic weather stations)

TABLE 1 Standard grape composition parameters.

Parameter	Vintage	Harvest date					
		2012.09.06. / 2013.09.12.		2012.09.13. / 2013.09.19.		2012.09.27. / 2013.10.03.	
		Treatment					
		C	LM	C	LM	C	LM
°Brix	2012	22.9 ± 0.3aα	23.6 ± 0.1bα	23.7 ± 0.1aβ	24.0 ± 0.2bβ	24.3 ± 0.1aγ	24.3 ± 0.1aβ
	2013	18.5 ± 0.2aα	18.2 ± 0.1aα	19.0 ± 0.31aα	20.4 ± 0.2bβ	21.2 ± 0.3aβ	21.0 ± 0.2aγ
		*	*	*	*	*	*
Titratable acidity (g/L)	2012	7.6 ± 0.1aα	6.3 ± 0.0bα	5.1 ± 0.1aβ	5.3 ± 0.1bβ	5.5 ± 0.1aγ	5.9 ± 0.0bγ
	2013	10.8 ± 0.1aα	9.4 ± 0.1bα	10.2 ± 0.1aβ	8.9 ± 0.1bβ	8.6 ± 0.1aγ	9.2 ± 0.1bγ
		*	*	*	*	*	*
pH	2012	3.14 ± 0.02aα	3.23 ± 0.00bα	3.32 ± 0.01aβ	3.34 ± 0.01bβ	3.25 ± 0.01aγ	3.34 ± 0.01bβ
	2013	2.90 ± 0.01aα	2.89 ± 0.00aα	2.93 ± 0.01aβ	3.02 ± 0.02bβ	2.94 ± 0.01aβ	2.91 ± 0.01bα
		*	*	*	*	*	*
Weight of 100 berries (g)	2012	127.83 ± 1.39aα	134.68 ± 2.16bα	125.23 ± 3.10aα	121.54 ± 1.24aβ	134.60 ± 2.51aβ	136.92 ± 3.09aα
	2013	173.45 ± 3.43aα	178.98 ± 4.61aα	171.41 ± 6.89aα	175.60 ± 6.06aα	147.11 ± 5.47aβ	147.46 ± 5.79aβ
		*	*	*	*	*	*

Values marked with different Roman letters mean significant differences between the treatments within the same year and same harvest date. Different Greek letters mean significant differences between harvest dates within the same year and same treatment. * means significant differences between the years within the same treatments and harvest dates. For separation, Tukey's and Games-Howell's post hoc test was used at p=0.05. Each value represents the average ± standard error of 3 replicates. C=control, LM=foliar sprayed.

TABLE 2

Measures of phenolic maturity in grapes.

Parameter	Vintage	Harvest date					
		2012.09.06. / 2013.09.12.		2012.09.13. / 2013.09.19.		2012.09.27. / 2013.10.03.	
		Treatment					
		C	LM	C	LM	C	LM
A1 (mg/L)	2012	1754 ± 41aα	1781 ± 82aα	1781 ± 48aα	1888 ± 34bα	1834 ± 124aα	1736 ± 112aα
	2013	1084 ± 61aα	1273 ± 68bα	1038 ± 58aα	1386 ± 49bαβ	1356 ± 57aβ	1433 ± 46aβ
		*	*	*	*	*	*
A3.4 (mg/L)	2012	828 ± 79aα	958 ± 26bα	801 ± 84aα	839 ± 26aβ	725 ± 49aβ	792 ± 16bγ
	2013	559 ± 37aα	702 ± 40bα	593 ± 22aα	734 ± 47bα	602 ± 28aα	761 ± 29bβ
		*	*	*	*	*	
EA (%)	2012	52.9 ± 3.4aα	46.1 ± 3.8bα	54.9 ± 5.8aα	55.5 ± 1.7aβ	60.4 ± 3.2aβ	54.2 ± 2.9aβ
	2013	48.2 ± 6.4aα	44.7 ± 5.6aα	42.6 ± 5.1aα	46.9 ± 4.8aα	55.6 ± 1.5aβ	46.9 ± 1.4bα
					*		*
SM (%)	2012	58.3 ± 2.7aα	55.8 ± 2.5aα	55.8 ± 9.1aα	65.4 ± 1.0aα	66.5 ± 5.8aα	56.2 ± 8.7aα
	2013	69.5 ± 3.5aα	65.5 ± 3.8aα	57.5 ± 10.6aα	67.3 ± 2.0aα	49.0 ± 14.2aα	56.1 ± 14.0aα
		*	*				

Values marked with different Roman letters mean significant differences between the treatments within the same year and same harvest date. Different Greek letters mean significant differences between harvest dates within the same year and same treatment. * means significant differences between the years within the same treatments and harvest dates. For separation, Tukey's and Games-Howell's post hoc test was used at p=0.05. Each value represents the average ± standard error of 3 replicates. C=control, LM=foliar sprayed.

TABLE 3

Berry physical properties.

Parameter	Vintage	Harvest date					
		2012.09.06. / 2013.09.12.		2012.09.13. / 2013.09.19.		2012.09.27. / 2013.10.03.	
		Treatment					
		C	LM	C	LM	C	LM
BH (N)	2012	3.271 ± 0.578aαβ	3.552 ± 0.672bαβ	3.114 ± 0.667aα	3.252 ± 0.684aα	3.450 ± 0.737aβ	3.822 ± 0.947bβ
	2013	3.940 ± 0.899aα	4.011 ± 0.873aα	3.751 ± 0.745aα	3.183 ± 0.617bβ	3.266 ± 0.768aβ	3.134 ± 0.692aβ
		*	*	*		*	*
F_{sk} (N)	2012	0.472 ± 0.066aα	0.433 ± 0.063bα	0.409 ± 0.073aβ	0.422 ± 0.087aα	0.442 ± 0.077aαβ	0.453 ± 0.102aα
	2013	0.450 ± 0.106aα	0.434 ± 0.097aα	0.469 ± 0.098aα	0.414 ± 0.105bα	0.458 ± 0.094aα	0.415 ± 0.089bα
				*			
E_{sk} (N/mm)	2012	0.437 ± 0.111aα	0.451 ± 0.107aα	0.455 ± 0.091aαβ	0.450 ± 0.128aα	0.489 ± 0.076aβ	0.520 ± 0.148aβ
	2013	0.559 ± 0.103aα	0.525 ± 0.085aα	0.476 ± 0.077aαβ	0.499 ± 0.077aα	0.332 ± 0.042aβ	0.371 ± 0.061bβ
		*	*		*	*	*
W_{sk} (mJ)	2012	0.270 ± 0.102aα	0.260 ± 0.075aα	0.232 ± 0.075aβ	0.252 ± 0.104aα	0.244 ± 0.071aβ	0.247 ± 0.096aα
	2013	0.226 ± 0.081aα	0.233 ± 0.088aα	0.283 ± 0.100aβ	0.224 ± 0.101bα	0.342 ± 0.102aγ	0.271 ± 0.082bβ
		*		*		*	
Sp_{sk} (mm)	2012	0.185 ± 0.038aα	0.227 ± 0.042bα	0.197 ± 0.028aα	0.220 ± 0.037bα	0.197 ± 0.038aα	0.228 ± 0.030bα
	2013	0.190 ± 0.033aα	0.210 ± 0.028bα	0.191 ± 0.030aα	0.219 ± 0,030bα	0.190 ± 0.030aα	0.223 ± 0.035bα
F_s (N)	2012	38.50 ± 8.26aα	38.88 ± 9.64aα	38.52 ± 9.17aα	37.61 ± 8.12aα	37.68 ± 8.11aα	39.91 ± 10.51aα
	2013	30.77 ± 7.13aα	33.85 ± 5.78aα	35.60 ± 6.02aβ	34.61 ± 6.42aα	33.35 ± 6.14aαβ	33.14 ± 8.11aα
		*	*		*	*	*
E_s (N/mm)	2012	69.66 ± 14.51aα	73.46 ± 11.82aα	68.31 ± 12.29aα	68.58 ± 14.79aα	73.94 ± 15.33aα	73.12 ± 15.33aα
	2013	77.67 ± 13.75aα	78.64 ± 12.91aα	82.55 ± 15.22aα	87.36 ± 13.18aβ	82.86 ± 14.24aα	80.37 ± 16.54aα

		*	*	*	*	*	*
W_s (mJ)	2012	9.73 ± 2.90aα	9.77 ± 3.42aα	9.92 ± 3.65aα	9.56 ± 3.15aα	9.48 ± 3.13aα	10.25 ± 3.65aα
	2013	5.77 ± 2.24aα	6.85 ± 1.88aα	7.13 ± 2.13aβ	6.59 ± 2.32aα	6.37 ± 1.78aαβ	6.50 ± 2.27aα
		*	*	*	*	*	*

Values marked with different Roman letters mean significant differences between the treatments within the same year and same harvest date. Different Greek letters mean significant differences between harvest dates within the same year and same treatment. * means significant differences between the years within the same treatments and harvest dates. For separation, Tukey's and Games-Howell's post hoc test was used at p=0.05. Each value represents the average ± standard error of 50 replicates. C=control, LM=foliar sprayed.

TABLE 4

Wine composition parameters.

Parameter	Vintage	Harvest date					
		2012.09.06. / 2013.09.12.		2012.09.13. / 2013.09.19.		2012.09.27. / 2013.10.03.	
		Treatment					
		C	LM	C	LM	C	LM
Alcohol (%v/v)	2012	14.58 ± 0.09aα	14.43 ± 0.20aα	15.08 ± 0.26aαβ	15.15 ± 0.21aβ	15.35 ± 0.33aβ	15.55 ± 0.31aβ
	2013	11.28 ± 0.18aα	12.11 ± 0.62aαβ	11.87 ± 0.06aβ	11.62 ± 0.23aα	13.80 ± 0.50aγ	13.12 ± 0.26aβ
		*	*	*	*	*	*
Titratable acidity (g/L)	2012	7.03 ± 0.06aα	6.00 ± 0.20bα	5.03 ± 0.06aβ	5.47 ± 0.31aα	5.87 ± 0.21aγ	5.63 ± 0.06aα
	2013	8.33 ± 0.15aα	7.60 ± 0.10bα	7.63 ± 0.12aβ	7.00 ± 0.17bβ	6.67 ± 0.15aγ	6.97 ± 0.21aβ
		*	*	*	*	*	*
pH	2012	3.33 ± 0.01aα	3.65 ± 0.05bα	3.72 ± 0.04aβ	3.81 ± 0.07aβ	3.86 ± 0.04aγ	3.69 ± 0.02bα
	2013	3.02 ± 0.03aα	3.16 ± 0.01bα	3.15 ± 0.02aβ	3.07 ± 0.01bβ	3.11 ± 0.01aβ	3.12 ± 0.02aγ
		*	*	*	*	*	*
Total polyphenols (mg/L)	2012	2562 ± 64aα	2708 ± 83aα	2944 ± 59aβ	2928 ± 68aβ	2782 ± 50aγ	2850 ± 69bαβ
	2013	1045 ± 47aα	1035 ± 78aα	1025 ± 91aα	1117 ± 61aαβ	1304 ± 165aα	1260 ± 113aβ
		*	*	*	*	*	*
Leucoanthocyanins (mg/L)	2012	1641 ± 42aα	1582 ± 105aα	1543 ± 39aαβ	1767 ± 111bα	1449 ± 43aβ	1770 ± 50bα
	2013	1137 ± 103aα	1248 ± 89aα	1152 ± 41aα	1386 ± 168aαβ	1526 ± 102aβ	1626 ± 141aβ
		*	*	*	*		
Catechins (mg/L)	2012	1517 ± 73aα	1184 ± 37bα	1747 ± 65aβ	1538 ± 109bβ	1371 ± 48aα	1421 ± 52aβ
	2013	962 ± 85aα	916 ± 64aα	820 ± 33aα	997 ± 62bα	1048 ± 156aα	1072 ± 87aα
		*	*	*	*	*	*
Anthocyanins (mg/L)	2012	740 ± 19aα	793 ± 31aα	736 ± 23aα	796 ± 13bα	688 ± 47aα	762 ± 43aα
	2013	340 ± 56aα	406 ± 10aα	408 ± 9aα	463 ± 21bαβ	526 ± 39aβ	576 ± 51aβ

		*	*	*	*	*	*
Color intensity (A_{420}+A_{520}+A_{620})	2012	23.43 ± 0.86aα	23.61 ± 0.64aαβ	22.18 ± 0.48aα	24.04 ± 0.07bα	22.82 ± 0.14aα	24.47 ± 0.07bβ
	2013	14.68 ± 2.33aα	20.49 ± 0.92bα	17.70 ± 0.18aα	20.16 ± 1.67aα	23.34 ± 0.88aβ	25.56 ± 1.75aβ
		*	*	*	*		
Color hue (A_{420}/A_{520})	2012	0.60 ± 0.02aα	0.64 ± 0.02aα	0.63 ± 0.02aαβ	0.64 ± 0.01aα	0.65 ± 0.01aβ	0.63 ± 0.00aα
	2013	0.39 ± 0.01aα	0.37 ± 0.01bα	0.35 ± 0.00aβ	0.34 ± 0.00bβ	0.34 ± 0.00aβ	0.34 ± 0.00aβ
		*	*	*	*	*	*
HCl index	2012	4.83 ± 0.15aα	5.06 ± 3.16aα	6.53 ± 0.35aβ	12.99 ± 0.03bβ	9.50 ± 0.36aγ	11.16 ± 1.24aβ
	2013	5.01 ± 0.53aα	6.14 ± 0.54aα	4.97 ± 0.73aα	4.34 ± 0.61aβ	4.43 ± 0.68aα	6.27 ± 0.14bα
		*	*	*	*	*	*
Gelatin index	2012	46.91 ± 1.19aα	51.58 ± 0.51bα	52.32 ± 1.65aβ	52.50 ± 0.21aα	52.59 ± 0.91aβ	56.58 ± 0.36bβ
	2013	26.40 ± 2.52aαβ	23.17 ± 1.85aαβ	23.13 ± 0.93aα	23.23 ± 0.35aα	18.20 ± 0.30aβ	18.90 ± 0.30bβ
		*	*	*	*	*	*

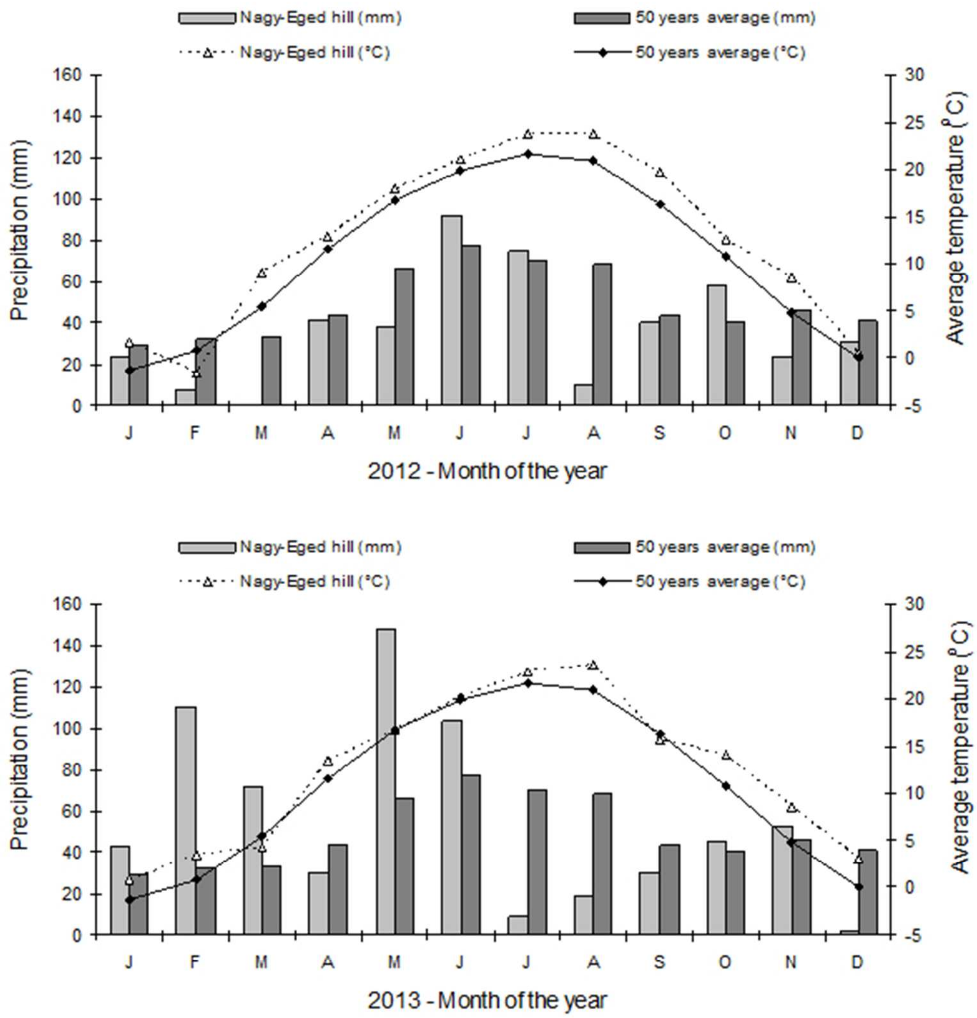
Values marked with different Roman letters mean significant differences between the treatments within the same year and same harvest date. Different Greek letters mean significant differences between harvest dates within the same year and same treatment. * means significant differences between the years within the same treatments and harvest dates. For separation, Tukey's and Games-Howell's post hoc test was used at p=0.05. Each value represents the average ± standard error of 3 replicates. C=control, LM=foliar sprayed.

TABLE 5

Resveratrol analysis of wines.

Parameter	Vintage	Harvest date					
		2012.09.06. / 2013.09.12.		2012.09.13. / 2013.09.19.		2012.09.27. / 2013.10.03.	
		Treatment					
		C	LM	C	LM	C	LM
Trans-resveratrol (mg/L)	2012	n.d.	0.10 ± 0.01α	0.83 ± 0.25aα	0.41 ± 0.01bβ	0.30 ± 0.10aα	0.23 ± 0.08aαβ
	2013	n.d.	0.16 ± 0.14αβ	0.10 ± 0.12α	n.d.	0.63 ± 0.10aβ	0.50 ± 0.11aβ
				*	*	*	*
Cis-resveratrol (mg/L)	2012	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	2013	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trans-piceid (mg/L)	2012	1.07 ± 0.06aα	1.39 ± 0.04bα	0.57 ± 0.06aβ	1.45 ± 0.05bα	0.50 ± 0.05aβ	0.55 ± 0.05aβ
	2013	0.37 ± 0.28aα	0.46 ± 0.16aα	0.41 ± 0.07aα	0.12 ± 0.11bβ	0.47 ± 0.32aα	0.74 ± 0.05aα
		*	*	*	*		*
Cis-piceid (mg/L)	2012	n.d.	0.93 ± 0.15αβ	1.20 ± 0.20aα	0.90 ± 0.00aα	0.87 ± 0.06aα	0.61 ± 0.07bβ
	2013	0.41 ± 0.09aα	0.60 ± 0.34aα	0.25 ± 0.02aα	0.87 ± 0.19bαβ	1.05 ± 0.31aβ	1.63 ± 0.30aβ
		*		*	*		*
Σ (mg/L)	2012	1.07 ± 0.06aα	2.42 ± 0.18bα	2.60 ± 0.00aβ	2.76 ± 0.06aα	1.67 ± 0.20aγ	1.39 ± 0.17aβ
	2013	0.78 ± 0.32aα	1.23 ± 0.26bα	0.73 ± 0.11aα	0.99 ± 0.10aα	2.14 ± 0.69aβ	2.87 ± 0.23bβ
			*	*	*	*	*

Values marked with different Roman letters mean significant differences between the treatments within the same year and same harvest date. Different Greek letters mean significant differences between harvest dates within the same year and same treatment. * means significant differences between the years within the same treatments and harvest dates. For separation, Tukey's and Games-Howell's post hoc test was used at $p=0.05$. Each value represents the average ± standard error of 3 replicates. n.d. = not detectable, C=control, LM=foliar sprayed.



Average air temperature (lines) and monthly sum of precipitation (bars) for 2012 and 2013 at the experimental site (data from automatic weather stations)
159x171mm (96 x 96 DPI)