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 an alcohol concentration that is too high and titratable acidity that is low, along with a high pH level without the desired level of phenolic maturity. Final wine quality notably depends on the phenolic composition of the 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 and 2013) in a cool-climate wine region (Eger, Hungary). The spray acted as an elicitor, stimulating the synthesis of several secondary metabolites. The 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 were evaluated for grapes and wines, as well as for resveratrol. Grapes from the treated vines had thicker skins than the controls at all sampling dates in both vintages. The phenolic potential (especially anthocyanin concentration and extractability) of the foliar spray-treated grapes was greatly improved. Our experiment showed that phenolic ripening can be enhanced using the foliar spray, and that its application was useful in different vintages.

INTRODUCTION

Nowadays, wine consumers prefer well-structured wines with deep colour, fruit scents, soft tannins and a pleasant mouthfeel (Bruwer *et al*., 2011). These kinds of wines can be made from well-ripened grapes with an optimal level of phenolic and technological (sugar) maturity, but not from over-ripened 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 wines that are too alcoholic. 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, the ripening is slowed in a rainy, cool vintage, and late ripening varieties (such as Cabernet Sauvignon, Cabernet Franc and 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; 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

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et al., 2007; Prajitna *et al.*, 2007). There are also some papers dealing with the 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 was examined for its effects. In addition, Syrah is a new cultivar to the Eger wine region, where there is only limited cultivation experience with it.

The aim of this study was to 1) describe the effects of the application of this new foliar spray on grape phenolic maturity and 2) 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 at Nagy-Eged hill, a commercial vineyard (lat. 47°55'31.84" N; long. 20°24'42.32" W, elevation 430 m above sea level, asl) in the Eger wine region (northeast Hungary). 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 Nagy-Eged hill, leading to alcoholic, unbalanced wines. Besides, the desired level of phenolic maturity cannot be achieved in most vintages. The trial 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 a south-north row orientation were investigated. The 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 six rows was selected for each treatment (three control (unsprayed, C) and three treated (sprayed, LM) rows). Each row was divided into three blocks. One block contained 25 to 29 vines. At the same harvest time, three blocks per treatment were harvested, resulting in three replicates per 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 the process of authorisation in others. Two applications of 1 kg/ha were done. The first one was at the beginning of véraison, the second one 12 days later. The powder was diluted in water without using an adjuvant. The whole canopy was sprayed using a motorised backpack sprayer.

There were three harvest dates (2012-09-06., 2012-09-13., 2012-09-27, and 2013-09-12, 2013-09-19 and 2013-10-03) in each vintage for both the control and treated vines. Establishing as reference the commercial harvest date of the Gróf Buttler Winery, which effectively was the second harvest in our trial, the first harvest took place 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. Véraison 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 from the trial site.

Berry sampling
Three sets of 20 kg of grapes, with each set from 25 to 29 vines, were carefully harvested by hand for both treatments at each harvest date, and transported immediately to the experimental winery. Three 1 kg samples from 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 analysed within two hours after the harvest.

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

A total of 150 berries were selected separately for phenolic measurements (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, and 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).

Assessment of grape phenolic maturity
The phenolic potential of the 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 four hours with buffer solutions at two pH values (1.0 and 3.4). The original method proposed a buffer of pH 3.2, 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 and 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:
$$EA\,(\%) = [(A1 - A3.4) / A1] \times 100$$
$$SM\,(\%) = [(A280 - ((A3.4 / 1000) \times 40)) / A280] \times 100$$

Measurements of berry physical properties
A TA.XTplus Texture Analyzer (Stable Micro System, Surrey, UK) with a HDP/90 platform and 30 kg load cell was used to follow the grape physical properties. Exponent 6.1.4.0 software was used for the 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 analyser. 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 pedicels were removed from the bunch, laid on the plate of the analyser and then 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 Exponent 6.1.4.0 software. Berry skin thickness (Sp_{sk}) was measured using a P/2 probe with 2 mm diameter. For this measurement, approximately 0.25 cm^2 of 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, after which the test was conducted as described by other authors previously (Letaief *et al.*, 2008a; 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 with 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 using Exponent 6.1.4.0.

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

The total phenolics of the wines were analysed by the Folin-Ciocalteu method (Singleton & Rossi, 1965) and the results expressed as gallic acid equivalents (GAE mg/L). The quantity of leuco-anthocyanins (flavan-3,4-diols) was determined as described by Flanzy *et al.* (1969). The bisulphite bleaching method was used to determine the anthocyanin content of the grape extracts and wines (Ribéreau-Gayon & Stonestreet, 1965), while the total catechins (flavan-3-ols) were measured using the vanillin assay according to Amerine and Ough (1980). The colour 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 gelatine and HCl indices (Ribéreau-Gayon *et al.*, 2006) were also calculated. All the measurements were performed in triplicate.

Qualitative and quantitative determination of resveratrol components in wines by HPLC
The analysis of the resveratrol compounds was carried out according to Kállay and 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 were as follows: a HP Series 1050 HPLC-apparatus with a normal phase LiChrospher® 100 CN

(250 x 4 mm, 5 μm) column (Merck, Germany) was used for the measurements. The detector was a HP Series 1050. The flow was set at 2 mL/min at 30°C with the detection wavelength at 306 nm. The methanol and acetonitrile used for the experiment were of HPLC grade, while 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 were 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 of grapes were crushed, destemmed and sulphited (1 mL of 5% aqueous SO$_2$ solution for every 1 L of mashed grapes) in the experimental winery at each harvest date. Macerations were conducted in 30 L plastic containers, and all grape repetitions were fermented separately. Three experimental wine replicates were made at each harvest time for each respective treatment. After the grapes had been processed 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 also were 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 for several days until the moment of the analysis, and no sulphur was added prior to analysis.

Sensory analysis
All the wines were tasted by a group of 17 expert oenologists. 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 vintages. The wines were evaluated sensorially using the 100-point OIV (1994) method. In all the cases, the objective was for the tasters to name which wines they preferred and for what reason.

Statistical analysis
Statistical analysis was conducted using 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-subject effect tests. The homogeneity of variances was checked by Levene's test. In the case of significant effect of the harvest dates, Tukey's test or the Games-Howell post hoc test was used for mean separation, according to whether or not the homogeneity of the variances held.

RESULTS

Climatic characteristics for 2012 and 2013
Fig. 1 shows the climatic characteristics of the two vintages. The weather in 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, and 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 that of 2013 by 15 to 25%. There also were notable differences in the case of titratable acidity, with the values in 2013 being significantly higher. The lowest concentration was 8.6 g/L. The weight loss of the berries during ripening was due to 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 of 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% varied 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 ripening. The significant increase observable in 2012 was due to the rainfall during
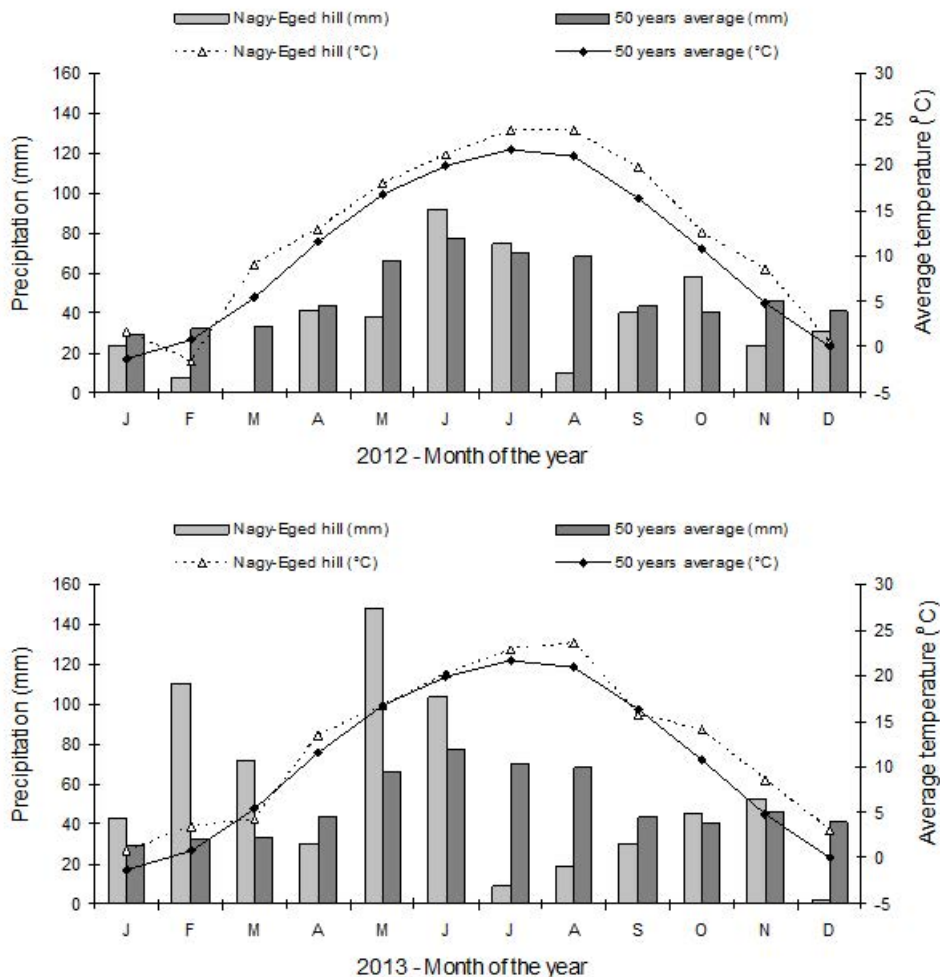

FIGURE 1
Average air temperature (lines) and monthly sum of precipitation (bars) for 2012 and 2013 at the experimental site (data from automatic weather stations).

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 three 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 ± 34aα	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 test and the Games-Howell post hoc test were used at p = 0.05. Each value represents the average ± standard error of three replicates. C = control, LM = foliar sprayed

TABLE 3
Berry physical properties.

Parameter	Vintage	Harvest date					
		Treatment					
		2012-09-06 / 2013-09-12		2012-09-13 / 2013-09-19		2012-09-27 / 2013-10-03	
		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 ± 0.042aαβ	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 test and the Games-Howell post hoc test were used at p = 0.05. Each value represents the average ± standard error of 50 replicates. C = control, LM = foliar sprayed

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 the 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 summarises the main wine parameters. The wines had a wide range of alcohol concentration (between 11.28% v/v and 15.55% v/v). However, the foliar spray did not influence this parameter. We found significant differences between the titratable acidity and pH in the first phase of 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 leuco-anthocyanin and anthocyanin concentrations were found to be significantly higher in the treated wines in three instances: at the second and the third harvest dates in 2012, and at the second harvest date in 2013 (although only for anthocyanins). The weather conditions in 2012 favoured anthocyanin synthesis up to 796 mg/L. By contrast, the unfavourable vintage in 2013 resulted in a significantly lower anthocyanin concentration (Table 4). The impact of the foliar spray and harvest date on catechin levels is unclear. The colour intensity ($A_{420} + A_{520} + A_{620}$) correlated well with the increasing concentration of anthocyanins. The values of colour hue (A_{420}/A_{520}) represent a bluish tone, but this is typical for young red wines (Boulton, 2001).

The gelatine index increased significantly between the first and the third harvest dates in the foliar spray-treated grapes in 2012. In 2013 the differences between harvest dates were smaller, and the values also were much lower than in 2012 and less than the optimal value due to the unfavourable weather conditions (Ribéreau-Gayon *et al*., 2006). During tastings the wines were characterised by green, unripe tannins. HCl indices showed 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 the treated wines at the second harvest date in 2013. 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 the treated wines. Wines made from foliar spray-treated grapes were preferred and received higher scores than the 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 the 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, favouring 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 also is necessary to take into account the changes in grape skin cell wall composition and structure, because this can modify the extractability (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 for one instance in 2012. At the third harvest date the treated grapes had a lower A1 value. Vintage had a significant influence on all the Glories parameters except for 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 the treated grapes 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}),

TABLE 4
Wine composition parameters.

Parameter	Vintage	Harvest date					
		Treatment					
		2012-09-06 / 2013-09-12		2012-09-13 / 2013-09-19		2012-09-27 / 2013-10-03	
		C	LM	C	LM	C	LM
Alcohol (%v/v)	2012	$14.58 \pm 0.09a\alpha$	$14.43 \pm 0.20a\alpha$	$15.08 \pm 0.26a\alpha\beta$	$15.15 \pm 0.21a\beta$	$15.35 \pm 0.33a\beta$	$15.55 \pm 0.31a\beta$
	2013	$11.28 \pm 0.18a\alpha$	$12.11 \pm 0.62a\alpha\beta$	$11.87 \pm 0.06a\beta$	$11.62 \pm 0.23a\alpha$	$13.80 \pm 0.50a\gamma$	$13.12 \pm 0.26a\beta$
		*	*	*	*	*	*
Titratable acidity (g/L)	2012	$7.03 \pm 0.06a\alpha$	$6.00 \pm 0.20b\alpha$	$5.03 \pm 0.06a\beta$	$5.47 \pm 0.31a\alpha$	$5.87 \pm 0.21a\gamma$	$5.63 \pm 0.06a\alpha$
	2013	$8.33 \pm 0.15a\alpha$	$7.60 \pm 0.10b\alpha$	$7.63 \pm 0.12a\beta$	$7.00 \pm 0.17b\beta$	$6.67 \pm 0.15a\gamma$	$6.97 \pm 0.21a\beta$
		*	*	*	*	*	*
pH	2012	$3.33 \pm 0.01a\alpha$	$3.65 \pm 0.05b\alpha$	$3.72 \pm 0.04a\alpha\beta$	$3.81 \pm 0.07a\beta$	$3.86 \pm 0.04a\gamma$	$3.69 \pm 0.02b\alpha$
	2013	$3.02 \pm 0.03a\alpha$	$3.16 \pm 0.01b\alpha$	$3.15 \pm 0.02a\beta$	$3.07 \pm 0.01b\beta$	$3.11 \pm 0.01a\beta$	$3.12 \pm 0.02a\gamma$
		*	*	*	*	*	*
Total polyphenols (mg/L)	2012	$2562 \pm 64a\alpha$	$2708 \pm 83a\alpha$	$2944 \pm 59a\beta$	$2928 \pm 68a\beta$	$2782 \pm 50a\gamma$	$2850 \pm 69b\alpha\beta$
	2013	$1045 \pm 47a\alpha$	$1035 \pm 78a\alpha$	$1025 \pm 91a\alpha$	$1117 \pm 61a\alpha\beta$	$1304 \pm 165a\alpha$	$1260 \pm 113a\beta$
		*	*	*	*	*	*
Leuco-anthocyanins (mg/L)	2012	$1641 \pm 42a\alpha$	$1582 \pm 105a\alpha$	$1543 \pm 39a\alpha\beta$	$1767 \pm 111b\alpha$	$1449 \pm 43a\beta$	$1770 \pm 50b\alpha$
	2013	$1137 \pm 103a\alpha$	$1248 \pm 89a\alpha$	$1152 \pm 41a\alpha$	$1386 \pm 168a\alpha\beta$	$1526 \pm 102a\beta$	$1626 \pm 141a\beta$
		*	*	*	*	*	*
Catechins (mg/L)	2012	$1517 \pm 73a\alpha$	$1184 \pm 37b\alpha$	$1747 \pm 65a\beta$	$1538 \pm 109b\beta$	$1371 \pm 48a\alpha$	$1421 \pm 52a\beta$
	2013	$962 \pm 85a\alpha$	$916 \pm 64a\alpha$	$820 \pm 33a\alpha$	$997 \pm 62b\alpha$	$1048 \pm 156a\alpha$	$1072 \pm 87a\alpha$
		*	*	*	*	*	*
Anthocyanins (mg/L)	2012	$740 \pm 19a\alpha$	$793 \pm 31a\alpha$	$736 \pm 23a\alpha$	$796 \pm 13b\alpha$	$688 \pm 47a\alpha$	$762 \pm 43a\alpha$
	2013	$340 \pm 56a\alpha$	$406 \pm 10a\alpha$	$408 \pm 9a\alpha$	$463 \pm 21b\alpha\beta$	$526 \pm 39a\beta$	$576 \pm 51a\beta$
		*	*	*	*	*	*
Color intensity ($A_{420} + A_{520} + A_{620}$)	2012	$23.43 \pm 0.86a\alpha$	$23.61 \pm 0.64a\alpha\beta$	$22.18 \pm 0.48a\alpha$	$24.04 \pm 0.07b\alpha$	$22.82 \pm 0.14a\alpha$	$24.47 \pm 0.07b\beta$
	2013	$14.68 \pm 2.33a\alpha$	$20.49 \pm 0.92b\alpha$	$17.70 \pm 0.18a\alpha$	$20.16 \pm 1.67a\alpha$	$23.34 \pm 0.88a\beta$	$25.56 \pm 1.75a\beta$
		*	*	*	*	*	*
Color hue (A_{420}/A_{520})	2012	$0.60 \pm 0.02a\alpha$	$0.64 \pm 0.02a\alpha$	$0.63 \pm 0.02a\alpha\beta$	$0.64 \pm 0.01a\alpha$	$0.65 \pm 0.01a\beta$	$0.63 \pm 0.00a\alpha$
	2013	$0.39 \pm 0.01a\alpha$	$0.37 \pm 0.01b\alpha$	$0.35 \pm 0.00a\beta$	$0.34 \pm 0.00b\alpha$	$0.34 \pm 0.00a\beta$	$0.34 \pm 0.00a\beta$
		*	*	*	*	*	*
HCl index	2012	$4.83 \pm 0.15a\alpha$	$5.06 \pm 3.16a\alpha$	$6.53 \pm 0.35a\beta$	$12.99 \pm 0.03b\beta$	$9.50 \pm 0.36a\gamma$	$11.16 \pm 1.24a\alpha$
	2013	$5.01 \pm 0.53a\alpha$	$6.14 \pm 0.54a\alpha$	$4.97 \pm 0.73a\alpha$	$4.34 \pm 0.61a\beta$	$4.43 \pm 0.68a\alpha$	$6.27 \pm 0.14b\alpha$
		*	*	*	*	*	*
Gelatine index	2012	$46.91 \pm 1.19a\alpha$	$51.58 \pm 0.51b\alpha$	$52.32 \pm 1.65a\beta$	$52.50 \pm 0.21a\alpha$	$52.59 \pm 0.91a\beta$	$56.58 \pm 0.36b\beta$
	2013	$26.40 \pm 2.52a\alpha\beta$	$23.17 \pm 1.85a\alpha\beta$	$23.13 \pm 0.93a\alpha$	$23.23 \pm 0.35a\alpha$	$18.20 \pm 0.30a\beta$	$18.90 \pm 0.30b\beta$
		*	*	*	*	*	*

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 test and the Games-Howell post hoc test were used at p = 0.05. Each value represents the average ± standard error of three replicates. C = control, LM = foliar sprayed

TABLE 5
Resveratrol analysis of wines.

Parameter	Vintage	Harvest date					
		Treatment					
		2012-09-06 / 2013-09-12		2012-09-13 / 2013-09-19		2012-09-27 / 2013-10-03	
		C	LM	C	LM	C	LM
Trans-resveratrol (mg/L)	2012	n.d.	$0.10 \pm 0.01b\alpha$	$0.83 \pm 0.25a\alpha\beta$	$0.41 \pm 0.01b\beta$	$0.30 \pm 0.10a\alpha$	$0.23 \pm 0.08a\alpha\beta$
	2013	n.d.	$0.16 \pm 0.14\alpha\beta$	$0.10 \pm 0.12\alpha$	n.d.	$0.63 \pm 0.10a\beta$	$0.50 \pm 0.11a\beta$
				*	*	*	*
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	$1.07 \pm 0.06a\alpha$	$1.39 \pm 0.04b\alpha$	$0.57 \pm 0.06a\beta$	$1.45 \pm 0.05b\alpha$	$0.50 \pm 0.05a\beta$	$0.55 \pm 0.05a\beta$
	2013	$0.37 \pm 0.28a\alpha$	$0.46 \pm 0.16a\alpha$	$0.41 \pm 0.07a\alpha$	$0.12 \pm 0.11b\beta$	$0.47 \pm 0.32a\alpha$	$0.74 \pm 0.05a\alpha$
		*	*	*	*		*
Cis-piceid (mg/L)	2012	n.d.	$0.93 \pm 0.15\alpha\beta$	$1.20 \pm 0.20a\alpha$	$0.90 \pm 0.00a\alpha$	$0.87 \pm 0.06a\alpha$	$0.61 \pm 0.07b\beta$
	2013	$0.41 \pm 0.09a\alpha$	$0.60 \pm 0.34a\alpha$	$0.25 \pm 0.02a\alpha$	$0.87 \pm 0.19b\alpha\beta$	$1.05 \pm 0.31a\beta$	$1.63 \pm 0.30a\beta$
		*			*		*
Σ (mg/L)	2012	$1.07 \pm 0.06a\alpha$	$2.42 \pm 0.18b\alpha$	$2.60 \pm 0.00a\beta$	$2.76 \pm 0.06a\alpha$	$1.67 \pm 0.20a\gamma$	$1.39 \pm 0.17a\beta$
	2013	$0.78 \pm 0.32a\alpha$	$1.23 \pm 0.26b\alpha$	$0.73 \pm 0.11a\alpha$	$0.99 \pm 0.10a\alpha$	$2.14 \pm 0.69a\beta$	$2.87 \pm 0.23b\beta$
			*	*	*	*	*

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 within the same year and same treatment. * means significant differences between the years within the same treatments and harvest dates. For separation, Tukey's test and the Games-Howell post hoc test were used at p = 0.05. Each value represents the average ± standard error of three replicates. n.d. = not detectable, C = control, LM = foliar sprayed

berry hardness (BH) and seed texture properties were the mostly affected parameters, as can be seen in Table 3. It seems that cooler weather results in harder skin and softer seeds. 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. Furthermore, the harvest date had no effect on these parameters.

Torchio *et al*. (2010) reported a decreasing Young's modulus of the berry skin (E_{sk}) as ripening progressed. 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 berries. 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 gelatine indices for the wines from foliar spray-treated grapes in 2012, and to some extent in 2013, indicate a more polymerised and balanced tannin structure compared to the control wines. The sensory analysis supported these facts. All the tasters were able to differentiate between the control and treated wines. The wines made from foliar spray-treated grapes had more intense flavour, 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 polymerised phenolic compound concentration. The HCl indices of the wines were between 4 and 12. A wine suitable for aging has a value of 10 to 25 (Ribéreau-Gayon *et al*., 2006). Only two wines met this criterion. Both wines were made from foliar spray-treated grapes at the second and third harvest dates in 2012.

Resveratrol synthesis was also positively affected by the foliar spray, especially in the first phase of ripening. However, the differences disappeared by the second harvest in both vintages. A 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 the 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 (Fig. 1) delays stilbene synthesis. The cooler vintage in 2103 also reduced the impact of the foliar spray, resulting in a 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 by vine-pathogen interaction. Vines recognise the yeasts in the foliar spray, which activates some defence 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 was 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 the application of yeast derivatives (LalVigne® MATURE, Lallemand Inc.) on Syrah grape phenolic maturity as well as on wine phenolic composition and concentration. The results from two vintages indicate that its application led 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, and showing more flavours and complexity than the ones made from unsprayed vines. Preliminary evidence was also obtained to suggest that LalVigne® MATURE may 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 the early phases could play an important role in plant protection.

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