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A 2-year multisite study of viticultural and environmental factors affecting rotundone concentration in Duras red wine

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ABSTRACT

Aim: A study was carried out in 2013 and 2014 to determine the key environmental and viticultural variables affecting the concentration of rotundone, the black pepper aroma compound, in *Vitis vinifera* L. cv. Duras red wines at 10 different vineyard blocks.
Methods and results: For each block, data for fruit quality attributes, as well as climatic and agronomical variables, were collected. Rotundone was quantified in wines prepared by microvinification techniques (in a 1-L Erlenmeyer flask). Rotundone concentration varied across blocks from 63 ng/L to 239 ng/L in 2013 and from 25 ng/L to 115 ng/L in 2014. Three separate partial least squares regression models were constructed to predict rotundone concentration in wines in 2013, in 2014, and in both vintages. Gluconic acid, a secondary metabolite of *Botrytis cinerea*, had a substantial contribution to the 2013 and multivintage models, with a negative regression coefficient with rotundone concentration. Other predictors were associated with abiotic factors such as cumulative rainfall, thermal index, hours of sunshine and mean daily irradiation.
Conclusions: Our results indicate that mesoscale climatic variables are the key factors determining rotundone concentration, and also suggest that *Botrytis cinerea* may be involved in rotundone degradation.
Significance and impact of the study: Our findings may assist grape growers producing Duras red wines to select specific vineyard blocks with the aim of producing wines with a desired rotundone concentration. They also open up new fields of investigation into mechanisms involved in possible rotundone degradation by *Botrytis cinerea*.

KEYWORDS

abiotic factors, *Botrytis cinerea*, mesoscale climate, partial least squares regression model, rotundone

Additional tables can be downloaded from https://oeno-one.eu/article/view/2341

INTRODUCTION

Wine is a complex matrix that contains more than 800 aroma compounds (Robinson *et al.*, 2014). Most of the contributors to the varietal aroma of white wines, such as the monoterpenols responsible for floral notes in Muscat varieties (Williams *et al.*, 1981) and the varietal thiols imparting grapefruit and tropical fruit notes in Sauvignon blanc wines (Tominaga *et al.*, 1998), have been widely studied. Knowledge of odorants accounting for the varietal character of red wines, especially free aroma compounds directly extracted from grapes without being released from a precursor, had been limited to green and undesirable aroma methoxypyrazine compounds (Sala *et al.*, 2000) until the discovery of the sesquiterpene rotundone, which is responsible for the black pepper aroma (Wood *et al.*, 2008). Specific anosmia to this potent odorant, which has a detection threshold of 16 ng/L in red wine, has been reported (Wood *et al.*, 2008). In most cases, rotundone is perceived by consumers either positively or in a neutral manner (Geffroy *et al.*, 2018a), and those who appreciate peppery wines are generally wine connoisseurs who pay more than the average consumer for a bottle of wine (Geffroy *et al.*, 2016a).

Aside from Syrah/Shiraz wines, rotundone has so far been identified at very high concentrations (above 100 ng/L) in red wines made from Schioppettino (Caputi *et al.*, 2011), Duras, Pineau d'Aunis (Geffroy *et al.*, 2014), Gamay (Geffroy *et al.*, 2016a) and Maturana tinta (Cullere *et al.*, 2016). Differences in rotundone concentration have been identified between certified clones of Duras, and the grapevine defense response to powdery mildew might result in enhanced rotundone production (Geffroy *et al.*, 2015a). Zhang *et al.* (2016a) have shown that rotundone might not only be produced as a response to herbivore attacks and proposed the existence of other biosynthetic pathways. Rotundone does not appear to be translocated from vegetative tissues (Geffroy *et al.*, 2016b; Zhang *et al.*, 2016a), and in accordance with the hypothesis of local synthesis in grape berries, crop load reduction through grape thinning has no effect on rotundone concentration in wine (Geffroy *et al.*, 2014). The compound is produced by simple aerial or enzymatic oxidation of α-guaiene (Huang *et al.*, 2014; Takase *et al.*, 2016). Although rotundone has been identified at early stages of development in vegetative tissues (Zhang *et al.*, 2016a), it is known to accumulate late in the season in berries, with the concentration increasing from veraison to harvest (Caputi *et al.*, 2011; Geffroy *et al.*, 2014).

Cool and wet vintages promote the production of peppery red wines (Caputi *et al.*, 2011). Soil characteristics (Scarlett *et al.*, 2014), bunch zone air and bunch surface temperatures (Zhang *et al.*, 2015a), vineyard water balance (Zhang *et al.*, 2015b), and vine water status over the veraison-to-harvest period (Geffroy *et al.*, 2014; Geffroy *et al.*, 2015b) have been identified as key variables that explain differences in rotundone concentration between vintages and within a single vineyard block. Thus, the concentration of rotundone has been reported to be enhanced by the water supply through irrigation (Geffroy *et al.*, 2014; Geffroy *et al.*, 2016b). Conflicting results have been reported regarding the effect of bunch exposure, because leaf removal can reduce (Geffroy *et al.*, 2014), increase, or have no effect on rotundone accumulation (Homich *et al.*, 2017; Geffroy *et al.*, 2018b).

The hierarchy between viticultural and environmental factors driving rotundone production, especially temperature and water status, remains unclear. Although several studies have been conducted on rotundone at an intra-block scale (Scarlett *et al.*, 2014; Geffroy *et al.*, 2015b; Zhang *et al.*, 2015a; Bramley *et al.*, 2017), no data are presently available for studies carried out at a mesoscale. To study these aspects and to identify possible correlations between rotundone and vine and climatic variables, a trial was carried out in 2013 and 2014 on 10 *Vitis vinifera* L. cv. Duras vineyard blocks over the Gaillac protected designation of origin in the south-west of France. For each plot, data for the variables were collected or calculated mostly at veraison or between veraison and harvest, due to the late accumulation of rotundone in berries.

MATERIALS AND METHODS

1. Vineyard blocks and vine panels

The characteristics of the 10 commercial dryland vineyard blocks used for this study are shown in Table 1. The plots were selected to capture a wide range of soil and climatic conditions, with the aim of maximizing the range of rotundone concentrations found in the blocks. The 10 blocks were located within the Gaillac

TABLE 1. Characteristics of the 10 vineyard blocks used in the study

Block no.	Clone no.	Rootstock	Plant density (vines/ha)	Training system	Year of plantation	Row orientation	Elevation (m a.s.l.)	Slope (%)
1	554	Gravesac	4545	Guyot	1999	NW to SE	159	1.6
2	554	101-14 MGt	4132	Guyot	2002	NE to SW	118	0.5
3	555	3309C	4545	Guyot	2005	NW to SE	195	4.1
4	555	140R	4545	Guyot	2004	N to S	226	4.0
5	554	3309C	6172	Cordon	2003	N to S	201	4.9
6	554	3309C	4545	Cordon	2005	NE to SW	280	5.5
7	MS	SO4	4761	Goblet	1999	NW to SE	146	2.7
8	554	3309C	4545	Cordon	2001	NW to SE	129	1.2
9	654	SO4	4000	Cordon	1992	NW to SE	174	1.7
10	555	Fercal	4545	Guyot	1996	N to S	271	2.8

E, east; m a.s.l., meters above sea level; MGt, Millardet et de Grasset; MS, massal selection; N, north; S, south; W, west.

protected designation of origin, which covers a surface area of approximately 1000 km^2.

At each block, one 30-vine panel (comprising 10 consecutive vines from three adjacent rows) was selected for data collection and sampling. Vine management was performed by growers, except for fruit-zone leaf removal, which is a practice that is likely to affect rotundone concentration (Geffroy *et al.*, 2014; Homich *et al.*, 2017; Geffroy *et al.*, 2018b). To avoid bias due to differences in the timing and extent of mechanical leaf removal between blocks when the practice had to be performed by growers, all the leaves were removed manually from the basal node to the first node above the top bunch on the morning-sun side of the canopy at mid-veraison, when rotundone starts to accumulate in berries (Caputi *et al.*, 2011). Row orientation was determined using Google Earth Pro software version 7.3.0.3832 (Google Inc., Mountain View, CA, USA). Elevation and slope data were extracted from the RGE ALTI 5 m device, provided by the French National Institute of Geographic and Forest Information.

2. Phenology and vine measurements

For each block, the percentage of veraison was determined by randomly collecting 100 berries from both sides of the row and also several parts of the bunch, and by counting the number of colored berries every third day from the end of July to the end of August. Linear regression tests were performed using Excel software (Microsoft Corp., Redmond, WA, USA) to calculate the onset and end of veraison, the mid-veraison date, and the duration of veraison.

Exposed leaf area (m^2/m^2 soil), which is the leaf area that can be exposed to direct solar radiation, was estimated at mid-veraison using the protocol proposed by Murisier (1996). Measurements were made for six consecutive vines on each of the three rows constituting the 30-vine panel. The ratio between canopy height and row spacing, which is an indicator of the shade of one vine row provided by an adjacent row (Smart, 1985), was also calculated.

For each sampled vine, the number of bunches per vine was counted and the yield at harvest (kg/vine) was monitored by weighing individual crop loads, using a Precia Molen C20 K balance (Precia SA, Privas, France). Mean bunch weights were also calculated for each block.

Leaf area to crop weight ratio, which is an indicator of vine balance and ability to produce high-quality fruit and wines (Kliewer and Dokoozlian, 2005) was calculated as follows:

leaf area to crop weight ratio = [exposed leaf area (m^2/m^2 soil) ´ 10 000] / [yield at harvest (kg/vine) ´ plant density (vines/ha)]

In 2014 only, powdery mildew (*Erysiphe necator*) infections were detected on bunches from vines of one of the blocks. For this vintage, the severity of the infection was assessed at harvest by visually estimating the percentage of the bunch area colonized by the pathogen on 100 grape bunches (50 on each side of the vine). In 2013 and 2014, the severity of bunch rot (*Botrytis cinerea*) was assessed by determination of the gluconic acid content of the grapes (see the section *Berry sampling and analyses*), which

correlates with the visually estimated percentage of rotten grapes (Bocquet *et al.*, 1995).

Pruning wood weight (kg/vine) was estimated by weighing 1-year-old shoots of 10 out of 30 vines in January 2014 and 2015, using a Precia Molen C20 K balance.

3. Weather measurements and water-balance model

Daily high-resolution data provided by Météo France (Toulouse, France), the French weather service, were obtained using ANTILOPE (Champeaux *et al.*, 2011) for rainfall and AROME (Brousseau *et al.*, 2011) for air temperature and evapotranspiration. Resolutions were 1 km^2 and 4 km^2 for ANTILOPE and AROME, respectively. These data were used to calculate the average minimum, maximum and mean daily air temperature for each block between veraison and harvest; to calculate the Huglin index over the period from 1 April to 30 September (Tonietto and Carbonneau, 2002); to determine cumulative rainfall over different periods; and to run a water-balance model.

The percentage of soil covered by grass differed greatly between the blocks, and the water balance for intercropped systems (WaLIS) model was used to simulate the stem water potential (ψ_{stem}) of the vine (Celette *et al.*, 2010; Dufourcq *et al.*, 2013) at different time points between 15 days before mid-veraison and harvest for each block. The minimal and maximal values of ψ_{stem} over the mid-veraison to harvest period were also identified for each block. Calibration of the model was carried out in 2013 and 2014 based on three measurements of ψ_{stem} that were obtained between the beginning of July and the end of August for each block. Each measurement was taken from 3 of the 30 vines and from two mature exposed leaves per vine, according to the protocol proposed by Choné *et al.* (2001). These three vines were marked, and ψ_{stem} was always measured on these selected plants. On cloudless days, leaf blades were initially enclosed in plastic bags between 11:00 and 12:00 hours, and measurements were made between 14:00 and 15:00 hours.

The hours of sunshine, solar irradiation, and hygrometry data over the mid-veraison to harvest period, with a 9-km^2 spatial resolution, were obtained for each block from HelioClim-3, a satellite-based surface solar irradiation database (Blanc *et al.*, 2011; Eissa *et al.*, 2015).

In April 2015, just before budburst, trunk circumference, which is an index of vine vigor that correlates well with the rotundone concentration in vines within the same block (Geffroy *et al.*, 2015b), was determined for every vine within each block. We used the average of measurements obtained at three different heights: just above the graft, 10 cm under the trellis wire, and halfway between the two first points of measurement.

4. Berry sampling and analyses

Previous modeling of the kinetics of rotundone accumulation in berries has shown that the concentration increases from veraison and reaches a plateau after a certain time (Zhang *et al.*, 2015b). According to Zhang *et al.*, this plateau is higher and is reached earlier during cool and wet seasons. In 2011 and 2012, two hot and dry vintages that did not promote high rotundone concentration in the grapes of vines grown in the south-west of France, this plateau was achieved for Duras wines 44 days after mid-veraison (Geffroy *et al.*, 2014).

To collect grapes with the maximum rotundone concentration, each block was harvested in 2013 and 2014 exactly 44 days after mid-veraison. For each block and for each year, two samples, consisting of 200 berries from both sides of the row and several parts of the bunch (four berries per vine on each side of the row), were collected.

Before the start of the analysis, the mass of the 200 berries in each sample was measured. The berries were then crushed gently, and the musts obtained were centrifuged (14,000 *g* for 6 min) to enable conventional laboratory and $\delta^{13}C$ analyses. Sugar concentration (°Brix) was determined with a PAL digital handheld pocket refractometer (Atago, Tokyo, Japan). Titratable acidity was measured according to the methods of the Organisation Internationale de la Vigne et du Vin (2009), and pH was measured with an HI 3221 pH meter (Hanna Instruments, Woonsocket, RI, USA).

A Konelab Arena 20 sequential analyzer (Thermo Fisher Scientific, Waltham, MA, USA), in conjunction with enzyme kits provided by several suppliers, was used to determine the concentration of amino acids, ammonium, gluconic acid (Megazyme, Bray, Ireland) and malic acid (Thermo Fisher Scientific). Potassium concentration was determined by flame photometry (Bio Arrow, France) according to the

TABLE 2. Variables for which data were collected

Block	Phenology	Vine	
Variable	Variable	Variable	Period of measurement
Age of the plot	15 days before veraison	Pruning wood weight	Winter
Slope	Onset of veraison	Trunk circumference	Winter
Clone	Mid-veraison	Exposed leaf area	Veraison
Rootstock	End of veraison	Height-to-row spacing ratio	Veraison
Training system	Duration of veraison	Leaf area to crop weight ratio	Veraison/harvest
Row orientation		Stem water potential (ψ_{stem})	15 days before veraison
Plant density		Stem water potential (ψ_{stem})	Onset of veraison
Elevation		Stem water potential (ψ_{stem})	Mid-veraison
		Stem water potential (ψ_{stem})	End of veraison
		Stem water potential (ψ_{stem})	Harvest
		Stem water potential (ψ_{stem})	Minimum veraison – harvest
		Stem water potential (ψ_{stem})	Maximum veraison – harvest
Crop		Climate	
Variable	Period of measurement	Variable	Period of measurement
Yield per vine	Harvest	Mean air temperature	Veraison – harvest
No. of bunches per vine	Harvest	Minimum air temperature	Veraison – harvest
Bunch weight	Harvest	Maximum air temperature	Veraison – harvest
Sugar concentration	Veraison,[a] harvest	Huglin index	1 April – 30 September
Mass of 200 berries	Harvest	Mean air hygrometry	Veraison – harvest
Titratable acidity	Veraison,[a] harvest	Mean daily irradiation	Veraison – harvest
pH	Veraison,[a] harvest	Hours of sunshine	Veraison – harvest
Malic acid concentration	Veraison,[a] harvest	Cumulative rainfall	1 January – 30 December
Tartaric acid concentration	Harvest	Cumulative rainfall	1 April – 30 September
Amino acids concentration	Veraison,[a] harvest	Cumulative rainfall	15 days before veraison – veraison
Ammonium concentration	Veraison,[a] harvest	Cumulative rainfall	Veraison – harvest
Total phenolic index	Harvest	Evapotranspiration	Veraison – harvest
Anthocyanins content	Harvest		
$\delta^{13}C$	Veraison,[a] harvest		
Gluconic acid concentration	Harvest		
Severity of powdery mildew	Harvest[a]		
Skin-to-juice ratio	Harvest		

[a]Determined only in 2014 and excluded from the multivintage model. Data included in the partial least squares regression models to predict rotundone concentration in wine

methods of the Organisation Internationale de la Vigne et du Vin (2009), and the concentration of tartaric acid was determined by colorimetric titration (Hill and Caputi, 1970). $\delta^{13}C$, a proxy for vine water status (van Leeuwen *et al.*, 2009) was determined at harvest according to the protocol described by Geffroy *et al.* (2014). All the analytical determinations were carried out twice.

The berry skins that had not been used for the previous analyses were crushed for 2 min at low speed (600 rpm) using a Faciclic food blender (Moulinex, Ecully, France), in preparation for phenolic analyses. The concentration of anthocyanins and the total phenolic index of the grapes were quantified according to the method described by Cayla *et al.* (2002). In 2014 only, samples of 240 berries were also collected from the 10 blocks at mid-veraison to determine the weight of 200 berries, $\delta^{13}C$, sugar concentration, titratable acidity, pH, concentration of malic acid and amino acids, and ammonium content, according to previously described methods.

5. Microvinification and rotundone analysis

Rotundone content was analyzed indirectly in wines fermented under microvinification conditions. For each block and for each year, two grape samples, comprising 800 g of berries from both sides of the row and several parts of the bunch, were collected at harvest 44 days after mid-veraison. Microscale fermentation was carried out in a 1-L Erlenmeyer flask, according to the protocol described by Geffroy *et al.* (2014). After 8 days of fermentation, the amount of wine and of skins was measured to determine the skin-to-juice ratio at pressing (%). The wines were centrifuged (14,000 *g* for 6 min), and sulfite (80 mg/L) was added before bottling and storage at 4°C or less until the rotundone analysis.

The rotundone concentration of the wines was determined by solid-phase microextraction-multidimensional gas chromatography–mass spectrometry, according to the protocol described by Geffroy *et al.* (2014). The analyses were performed in two different years but during the same period of each year, to reduce potential variation associated with different post-bottling times.

6. Data treatment

Details of the variables for which data were collected are shown in Table 2. Rotundone concentration and data for the other variables, collected for the 10 blocks over the 2 years of the study, were subjected to analysis of variance, with the vintage as a factor and blocks as repetitions, using Xlstat software version 19.5 47062 (Addinsoft, Paris, France). Fisher's least significant difference test was used as a post-hoc comparison of means at P £ 0.05.

Based on previous work conducted with the aim of modeling 3-isobutyl-2-methoxypyrazine concentration in Cabernet Franc grapes (Scheiner *et al.*, 2011), a multivariate analysis using partial least squares regression (PLSR) was carried out to identify key factors affecting rotundone concentration in wine. The data were averaged over the replicates before PLSR was performed. For the multi-vintage model, variables that had been determined only in 2014 were discarded. A first set of PLSR for which the number of latent variables was determined by the lowest predicted residual sum of squares was conducted, using Xlstat software to model rotundone concentration in 2013, in 2014, and in both vintages (Wold *et al.*, 1984). Variables with a variable importance in projection score below 1 were removed manually and the model was regenerated. Once the number of variables remaining in the model was lower than the number of observations (10 for 2013 and 2014, and 20 for the multi-vintage data set), PLSR was conducted with Unscrambler X software version 10.4 (Camo, Oslo, Norway).

The results were validated by a full cross-validation (leave-one-out) procedure. Variables that did not contribute sufficiently to the model (absolute values of the loading weights below 0.1) were removed manually and the model was regenerated. For the multivintage model, one outlier (block no. 6 in 2013) detected using principal component analysis was removed.

RESULTS AND DISCUSSION

The data collected for each vineyard block in 2013 and 2014 are presented as supplementary material (Supplementary tables 1 and 2).

1. Main differences between the vintages

The 2013 vintage was characterized by a cold spring and regular and heavy rainfall events over the vine vegetative cycle that resulted in severe bunch rot (*Botrytis cinerea*) damage, ripening difficulties and delay of harvest. The mean harvest date for the 10 blocks was 6 October ± 4 days. In 2014, the winter was rainy and warm and summer was extremely rainy between mid-July and the end of August, whereas conditions during ripening were dry and hot. The mean harvest date was 22 September ± 3 days. Compared with 2014, the 2013 vintage was significantly wetter as well as cooler over the entire winegrowing and ripening periods, as reflected by the cumulative rainfall between 1 April and 30 September (423 ± 22 mm in 2013, 358 ± 19 mm in 2014) and between veraison and harvest (115 ± 18 mm in 2013, 63 ± 13 mm in 2014), the Huglin index (1862 ± 51 in 2013, 1969 ± 48 in 2014), and the mean air temperature over the veraison-to-harvest period (18.50 ± 0.56°C in 2013, 19.48 ± 0.42°C in 2014). The values of ψ_{stem} were significantly lower at mid-veraison during the first year of the study (–0.87 ± 0.20 MPa in 2013, –0.60 ± 0.12 MPa in 2014). Owing to the larger quantities of rainfall received in 2013 over the veraison-to-harvest period, the level of water deficit was higher at harvest in 2014

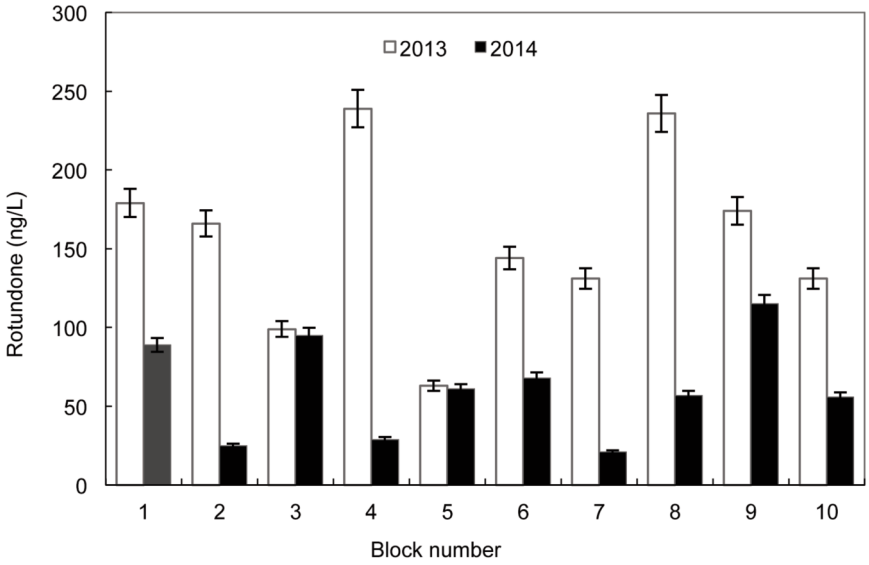

FIGURE 1. Rotundone concentration in wines made from grapes from the 10 vineyard blocks of Duras N in 2013 and 2014.

(–0.51 ± 0.17 MPa in 2013, –1.06 ± 0.14 MPa in 2014).

Vintage had a weak effect on crop and grape analytical characteristics, because no significant differences in yield (1.75 ± 1.01 kg/vine in 2013, 2.65 ± 1.22 kg/vine in 2014), sugar concentration (21.8 ± 0.9°Brix in 2013, 21.8 ± 0.6°Brix in 2014), pH (3.06 ± 0.12 in 2013, 3.05 ± 0.07 in 2014), titratable acidity (9.20 ± 1.13 g/L as tartaric acid in 2013, 9.10 ± 1.22 g/L as tartaric acid in 2014), amino acid concentration (120 ± 33 mg/L in 2013, 136 ± 48 mg/L in 2014) or total phenolic index (79.6 ± 10.8 in 2013, 88.5 ± 15.2 in 2014) were observed between 2013 and 2014. Concentrations of anthocyanins (872 ± 143 mg/L in 2013, 1048 ± 194 mg/L in 2014) and ammonium (40.9 ± 20.3 mg/L in 2013, 63.8 ± 20.4 mg/L in 2014) were the only fruit compound variables that differed significantly between the two vintages.

Rotundone concentration was significantly higher ($P < 0.001$) in the cooler and wetter 2013 growing season (Figure 1). This result is consistent with those of previous studies (Caputi *et al.*, 2011). Surprisingly, some blocks that had high rotundone concentration in 2013 (i.e. blocks no. 2 and no. 8), with values among the highest ever reported for Duras, had low to moderate concentrations in 2014. In the same way, some blocks that had high concentrations in 2014 (i.e. blocks no. 3 and no. 5) had low to moderate rotundone concentrations in 2013. This observation runs counter to the belief that some vineyards within a given wine region may always be prone to yielding wines with substantial amounts of rotundone. This led us to the notion that qualitative or quantitative fixed variables such as year of plantation, elevation, training system, clone or rootstock do not make a substantial contribution to the rotundone model.

2. Partial least squares regression rotundone models

Basic statistics of the models, regression coefficients, means and standard deviations of the variables included in the best models, are shown in Table 3. Regression plots of predicted rotundone concentration versus observed rotundone concentration in 2013, in 2014, and in both vintages are presented in Figure 2. The performance of the 2013 vintage model, reflected by its values of R^2 and root mean square error, was better than that of the other models. However, all the models appear to be appropriate for predicting rotundone concentration using a limited number of input variables for blocks with low or high rotundone concentrations.

The loadings of the latent variables used in the regression models are shown in Table 4. The first latent variable in the 2013 model was mainly characterized by gluconic acid concentration. For 2013, this variable was

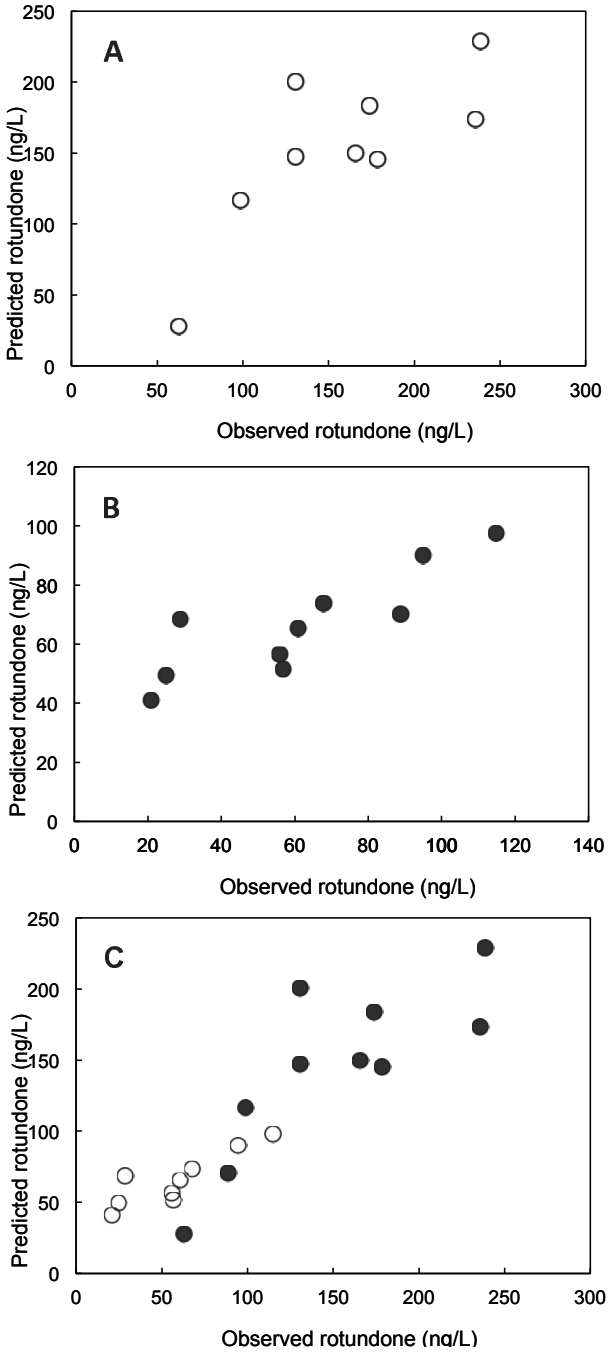

FIGURE 2. Predicted versus observed rotundone concentration.
Predicted versus observed rotundone concentration, using data from the models described in Table 3: A, 2013 (R^2 = 0.95); B, 2014 (R^2 = 0.78); and C, multi-vintage (R^2 = 0.81).

significantly negatively correlated with rotundone concentration ($P < 0.01$, $R^2 = 0.69$) (Figure 3). It also contributed to the multi-vintage model. This observation was somewhat unexpected, because laccase, the polyphenol oxidase produced by *Botrytis cinerea*, has the ability to convert α-guaiene to rotundone via a non-specific oxidation reaction in the presence of chemical mediators (Schilling *et al.*, 2013). However, it has also been documented that *Botrytis cinerea* can withstand the toxic effects of plant compounds such as sesquiterpenes by laccase-mediated oxidation and detoxification (Mayer and Staples, 2002). Further studies will be necessary to confirm this hypothesis and to better understand the mechanisms involved in rotundone degradation by the fungus.

Other variables contributing to the latent variables of the models were associated with abiotic factors, which indicates that mesoscale climate is the key factor driving rotundone production. Notably, the Huglin index, a thermal index based on a degree-day approach from 1 April to 30 September, was a strong contributor to the 2014 model. This is consistent with the finding that cool vintages promote rotundone accumulation in grapevine berries (Caputi *et al.*, 2011). A contribution to the models from mean air temperature between veraison and harvest would have been expected, because post-veraison berry surface temperature has been identified as the main determinant of rotundone concentration in grape berries (Zhang *et al.*, 2015a).

Bunch temperature does not fully reflect air temperature, because it is also determined by absorbed radiation and convective heat loss (Dry, 2009). The extent to which bunch temperature exceeds air temperature depends on degree of exposure, radiation load, wind velocity, berry or bunch size, berry color and bunch compactness. With the exception of wind velocity, which was not assessed, most of the data for these variables were collected directly or depend on data collected for other variables, such as bunch weight, mass of 200 berries, training system, rootstock, row orientation and exposed leaf area. If bunch temperature were the key variable driving rotundone concentration, the best rotundone model would include air temperature and other variables affecting bunch exposure and penetration of solar radiation through the canopy. It has recently been reported that the percentage of degree hours above 25°C (Dh_{25}) in the fruit zone from veraison to harvest correlates with rotundone concentration in berries (Zhang *et al.*, 2015a). Our study was designed and conducted 2 years before publication of these findings. Dh_{25} could not be calculated *a posteriori*, because collection of the data for this variable would

TABLE 3. Basic statistics of the partial least squares regression models

Variable	Model			Mean and standard deviation of model	
	2013	2014	2013–2014	2013	2014
Cumulative rainfall, veraison – harvest (mm)	–0.90[a]	NI	NI	115 ± 18*	63 ± 13
Hours of sunshine	0.72[a]	NI	0.61[a]	273 ± 17	362 ± 5*
Gluconic acid concentration (mg/L)	–0.83[a]	NI	–0.001[a]	72.4 ± 69.1*	14.3 ± 21.2
Cumulative rainfall, 1 April – 30 September (mm)	NI	0.85[a]	0.77[a]	423 ± 22*	358 ± 19
Huglin index	NI	–0.63[a]	NI	1862 ± 51	1969 ± 48*
Cumulative rainfall, 1 January – 31 December (mm)	NI	NI	0.66[a]	19.1 ± 12.6	50.1 ± 7.9*
Mean daily irradiation, veraison – harvest (W/m^2)	NI	NI	0.59[a]	1505 ± 81	1863 ± 50*
No. of latent variables	2	2	2	NA	NA
Root mean square error	11.4	14.1	28.5	NA	NA
R^2 (calibration)	0.95	0.78	0.81	NA	NA
Root mean square error of cross-validation	14.9	20.8	41.3	NA	NA
R^2 (validation)	0.92	0.53	0.59	NA	NA

NA, not applicable; NI, variable not included in the model. *Significantly higher of the means for 2013 and 2014 (least significant difference, P £ 0.05). [a]Regression coefficient between the variable and rotundone concentration
Regression coefficients, means and standard deviations of variables for the best rotundone models

TABLE 4. Loadings of the variables contributing to the best rotundone models

Variable	2013		2014		2013–2014	
	LV 1	LV 2	LV 1	LV 2	LV 1	LV 2
Gluconic acid concentration	–0.99	0.32	NI	NI	0.17	–0.84
Hours of sunshine, veraison – harvest	0.11	0.78	NI	NI	–0.21	0.05
Cumulative rainfall, veraison – harvest	–0.18	–0.55	NI	NI	NI	NI
Cumulative rainfall, 1 April – 30 September	NI	NI	0.26	0.88	0.14	0.20
Huglin index	NI	NI	–1.00	0.48	NI	NI
Cumulative rainfall, 1 January – 31 December	NI	NI	NI	NI	0.43	0.42
Mean daily irradiation, veraison – harvest	NI	NI	NI	NI	–0.88	0.29
Explained variance (%)	76.1	19.1	51.9	25.7	40.8	39.9

LV, latent variable; NI, variable not included in the model.

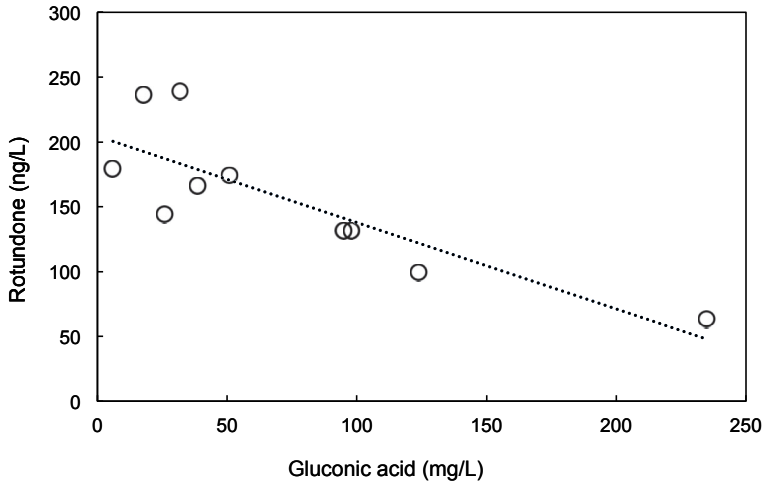

FIGURE 3. Relation between gluconic acid concentration and rotundone concentration.
Relation between gluconic acid content and rotundone concentration in wines in 2013 (n = 10). Linear regression model: y = –0.6667x + 204.47, P < 0.01, R^2 = 0.69.

have required the use of temperature loggers located within the canopy. We cannot completely exclude the possibility that Dh_{25} would have been included in the models.

Mean daily irradiation between veraison and harvest had a substantial contribution to the multi-vintage model. Hours of sunshine was also included in the best rotundone models for 2013 and 2013–2014. These two variables, reflecting the quantity of ultraviolet radiation received by the blocks, were positively correlated with rotundone concentration. Several studies have investigated the light dependency of sesquiterpene emission, with evidence that emission of some sesquiterpenes is solely temperature-controlled whereas emission of others is also affected by light (Duhl *et al.*, 2008). Although additional studies would be needed to confirm this hypothesis, our data suggest that ultraviolet rays may stimulate the production of rotundone. This conclusion is consistent with recent research conducted in cool-climate vineyards on Noiret and Fer cultivars, indicating that grape exposure by leaf removal could promote rotundone accumulation (Homich *et al.*, 2017; Geffroy *et al.*, 2018b).

The amount of rainfall between 1 January and 31 December and during the period of vine vegetative growth (between 1 April and 30 September) had a positive contribution to the 2014 and multi-vintage models, whereas rainfall during the late stage of development (i.e. between veraison and harvest) was negatively correlated with rotundone concentration in 2013. These results are somewhat unexpected, because irrigation around veraison, when rotundone starts to accumulate in berries, stimulates its production (Geffroy *et al.*, 2014; Geffroy *et al.*, 2016b). For the 2013 rainy vintage, even if skin-to-juice ratio and mass of 200 berries were not included in the model, we cannot exclude the possibility that the large excess of water may have resulted in a dilution effect through an increase in berry size.

The contribution to the models from cumulative rainfall between 1 April and 30 September and between 1 January and 31 December deserves further comment. Rotundone is found at elevated concentrations in the early berry stages, notably in flower caps (Zhang *et al.*, 2016b). Rotundone concentration then decreases to reach a minimal concentration at veraison, before increasing again from veraison to harvest (Zhang *et al.*, 2016b). It could be assumed that the precipitation regimen over the entire berry development period, not only during the last stage of maturation, may have affected the final concentration of rotundone through degradation or emission before veraison. Another hypothesis is that increased water availability over the period of vegetative growth and between 1 January and 31 December would have led to higher-vigor vines, resulting in greater bunch shading and lower bunch-zone air temperature. However, this hypothesis is unlikely, because the level of water deficit experienced by the vines at mid-veraison, when shoot growth stops, was minor (ψ_{stem} = –0.87 ± 0.20 MPa in 2013, ψ_{stem} = –0.60 ± 0.12 MPa in 2014) and insufficient to affect vine vegetative expression (van Leeuwen *et al.*, 2009). Additionally, no significant correlation at $P < 0.05$ could be established for the whole data set between cumulative rainfall and either pruning wood weight or exposed leaf area.

Consistent with the fact that the hierarchy of blocks for rotundone concentration can differ greatly from one vintage to the other, none of the rotundone models included any fixed variables. This is somewhat unexpected for the clone, because higher rotundone concentrations have been reported in Duras wines from clones 554 and 654 than from clone 555 (Geffroy *et al.*, 2015a). In the same way, the rootstock could have affected the concentration of rotundone in wine. The rootstock is likely to affect some key variables driving rotundone production, such as scion water status and bunch surface, through its effect on vine vigor, leaf area and penetration of solar radiation (Koundouras *et al.*, 2008). However, it may be difficult to draw firm conclusions, due to the small number of observations for each level of these two factors and the fact that not all clone–rootstock combinations were available.

It has been suggested that the grapevine defense response to powdery mildew could enhance rotundone production in berries (Geffroy *et al.*, 2015a). This hypothesis could not be confirmed under in the present study, because severity of powdery mildew infection was not included in the 2014 model. Period of infection by *Erysiphe necator* was not assessed in that previous study nor in the present study. On the one hand, as rotundone accumulates around veraison, differences in timing of infection, which usually occurs when bunches are receptive (from

blooming to veraison), may play a role in defense mechanisms leading to production of rotundone. On the other hand, powdery mildew damage was noticed only in 2014 at just one block, and it remains difficult to draw firm conclusions regarding the contribution of this biotic factor.

Consistent with the findings that rotundone is not translocated from vegetative tissues (Geffroy *et al.*, 2016b; Zhang *et al.*, 2016a) and that its accumulation does not depend on source–sink relations (Geffroy *et al.*, 2014), it should be pointed out that the leaf area to crop weight ratio was not included in any of the rotundone models.

The absence of contribution to the models from sugar concentration at harvest is somewhat unexpected. Although recent studies have shown that an increased alcohol content of wine improves rotundone extraction rate from grapes into wine (Zhang *et al.*, 2017), our data suggest that ethanol concentration in the resulting wines had no effect on rotundone concentration. This may be explained by the relatively small amplitude of difference between the lowest and highest sugar concentrations (20.1°Brix and 22.8°Brix, respectively) recorded between blocks for the 2 years of the study.

We cannot rule out the possibility that some blocks were harvested in 2013 and 2014 before rotundone concentration reached its maximum. This would mean that the proposed models would be valid for prediction of rotundone concentration only in wines from grapes harvested exactly 44 days after mid-veraison and may not be applicable to grapes harvested beyond that time. However, this hypothesis is unlikely, because 2013 and 2014 were characterized by substantial concentrations of rotundone in Duras wines (Geffroy *et al.*, 2016b). Therefore, considering previous findings (Zhang *et al.*, 2016a), the rotundone plateau and the maximum rotundone concentration were expected to have been reached earlier than in 2011 and 2012, and before 44 days after mid-veraison.

With only 2 years of data, we cannot completely disregard the possibility that our model may not be generalizable. This is particularly true because the two vintages of the study were relatively cool and wet for the area, as illustrated by the data for Huglin index and cumulative rainfall between 1 April and 30 September. The 2015 vintage, which was hotter and dryer, was characterized by a different climatic pattern than that of 2013 and 2014. The equation of the multi-vintage model was used to predict rotundone concentration in wine fermented under the same microvinification conditions from grapes collected in 2015 on a Duras plot 44 days after mid-veraison. Based on measurements made on this block for mean daily irradiation between veraison and harvest (1930 W/m^2), hours of sunshine between veraison and harvest (419), cumulative rainfall between 1 April and 30 September (348 mm), cumulative rainfall between 1 January and 31 December (516 mm) and gluconic acid concentration (12 mg/L), the predicted rotundone concentration was 30.8 ng/L; the observed value was 26.0 ng/L. In light of this prediction, we believe that the model may be sufficiently robust to also allow estimation of rotundone concentration during hot vintages.

CONCLUSION

Our study allowed us to model and assess the effect of environmental and viticultural factors on rotundone concentration in wine. Gluconic acid, a secondary metabolite of *Botrytis cinerea* that is negatively correlated with rotundone concentration, was included in the best rotundone model for the 2013 vintage and multi-vintage (2013–2014) models. This observation suggests that the fungus may be involved in rotundone degradation mechanisms, probably through its laccase activity. Other variables included in the PLSR models were abiotic factors. This indicates that mesoscale climate is the key factor driving rotundone production. In most cases, cumulative rainfall and thermal index for the entire vine vegetative period seem to be better predictors than variables calculated during the maturation period only. The positive contribution of mean daily irradiation between veraison and harvest and hours of sunshine suggest that rotundone production may be stimulated by ultraviolet exposure. Our findings may assist grape growers producing *Vitis vinifera* L. cv. Duras red wines from vineyards scattered across the studied viticultural area, to select specific blocks with the aim of producing wines with a specific rotundone concentration. They also open new fields of investigation into the mechanisms involved in possible rotundone degradation by *Botrytis cinerea*.

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