AN OVERVIEW OF THE IMPACT OF CLONE, ENVIRONMENTAL FACTORS AND VITICULTURAL TECHNIQUES ON ROTUNDONE CONCENTRATION IN RED WINES

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Abstract:

Context and purpose of the study – Rotundone is the main aroma compound responsible for peppery notes in red wine. This positive and very potent molecule has an odor threshold of 8 ng/L in water and 16 ng/L in red wine. It has been detected in several grape varieties with some of the highest concentrations recorded in Syrah, Duras, Tardif and Noiret, an interspecific hybrid grown in the North-East of the USA. If several winemaking practices have been identified to lower rotundone in wine, up to date, no enological solution has proved its efficiency to maximize it. This means that efforts to produce high rotundone wines must be undertaken in vineyards. This work provides practical ways that can be used by winegrowers to modulate rotundone levels in their wines.

Material and methods – Several field trials have been conducted for more than ten years in the southwest of France on Duras to investigate the impact of environmental factors and viticultural practices on rotundone concentration. This grape variety only grown within the protected designation of origin Gaillac was selected as it is known to exhibit substantial and regular levels of rotundone. These experiments were carried out using, in most cases, randomised complete block design with three replications per treatment. This research includes the study of the effect of clone, disease, ripeness, irrigation, defoliation, grape thinning, and the key factors driving the variability in rotundone concentrations between sites. Rotundone was quantified indirectly in wines fermented under microvinification conditions (1 L Erlenmeyer flask).

Results – Our results highlighted that certified clones had an impact on rotundone concentration in wine. The production of rotundone by the plant could be a response to biotic stress as a significant and positive correlation was established between rotundone concentrations and the severity of powdery mildew (*Erysiphe necator*) on bunches. On the opposite, *Botrytis cinerea* had a negative impact on rotundone in wine as a likely consequence of the activity of its polyphenol oxidase. Rotundone accumulation was also affected by abiotic factors, rising in concentration with an increase in water supply and radiation, while temperature elevation had a depreciative impact. The molecule was not impacted by grape thinning, suggesting the absence of translocation and a likely *in situ* biosynthesis. Harvesting date, irrigation, defoliation were identified as leverages to manipulate rotundone levels in wines.

Keywords: rotundone, *Vitis vinifera* L. Duras, biotic factors, abiotic factors, viticultural practices.

1. Introduction

Rotundone is a grape sesquiterpene responsible for peppery notes in red wine (Wood et al., 2008). This potent aroma compound with an odor threshold of 8 ng/L in water and 16 ng/L in wine is generally positively perceived by wine consumers (Wood et al. 2008, Geffroy et al. 2018). This ubiquitous molecule has been detected in several genotypes with particularly high concentrations levels in Syrah, Duras, Tardif, and Noiret, an interspecific hybrid grown in the North-East of the USA (Geffroy et al. 2020, Homich et al. 2017). If several winemaking practices and enological variables have been identified to lower rotundone in wine such as the use of *Saccharomyces uvarum*, carbonic maceration or a post-fermentative maceration of 6 days (Geffroy et al. 2017), up to date, no enological solution has proved its efficiency to maximize it. This means that efforts to produce high rotundone wines must be undertaken in vineyards. Based on research works conducted over the last decade in the southwest of France, this article provides winegrowers with practical ways to manipulate rotundone levels in wines using viticultural techniques, or to select vineyards with expected high rotundone concentrations.

2. Material and methods

2.1 Exploratory trial on viticultural and environmental factors

A field trial was conducted in 2013 and 2014 to investigate the key environmental and viticultural factors impacting rotundone in Duras red wines. This grape variety only grown within the protected designation of origin (PDO) Gaillac was selected as it is known to exhibit substantial and regular levels of rotundone. For 10 Duras vineyards, grape composition including sugar concentration, titratable acidity, pH, tartaric and malic acids, potassium, amino acids, ammonium, anthocyanins, TPI and gluconic acid were determined 44 days after mid-veraison using previously published methods (Geffroy et al. 2019). For each site, phenological data, exposed leaf area, cluster weights, number of clusters, yields and leaf area / crop load ratio were determined at harvest. Pruning wood mass was measured individually on 1-year wood during winter dormancy. Daily high-resolution data for rainfall, air temperature (minima, maxima, and mean values), hours of sunshine, solar irradiation and hygrometry were provided by MétéoFrance and HelioClim-3. The WAter baLance for Intercropped Systems (WALIS) model was run to estimate stem water potential of the vine at different time points (Dufourcq, et al. 2013). Three separate partial least squares regression models were built using Xlstats (Addinsoft, Paris) to predict rotundone concentration in wines in 2013, in 2014, and in both seasons determined as described in §2.4, and to identity the key variables driving rotundone concentration.

2.2 Clone trial

In 2013 and 2014, the impact of the four certified clones of Duras on rotundone concentration in microvinified wines (§2.5) was studied. These clones designated under the numbers 554, 555, 627 and 654, were virus-tested, certified and the homogeneity of the experimental vineyard was verified through trunk circumference and $\delta^{13}C$ measurements, and NDVI mapping as described in Geffroy et al. (2015). The experimental site consisted of four consecutive rows, each row planted with one of the four studied clones. For each clone, grape sampling for rotundone analysis was replicated on three experimental units per row consisting of twelve continuous vines. Data were analysed using Xlstat software (Addinsoft, France) through a three-way analysis of variance (ANOVA) treatment (vintage × clone × block) with first-order interaction .

2.3 Maturity and viticultural techniques trial

In 2011 and in 2012, two separate experiments, the first one studying the effect of maturity on rotundone and the second one the effect of viticultural techniques, were established in two distinct parts of the same Duras vineyard. These two experiments were conducted using a randomised complete block design with three replicates per treatment. The experimental set up was spread over five adjacent rows, with each experimental unit made of twelve continuous vines. For the maturity trial, wines were produced from grapes sampled at five times, after 30, 37, 44, 51 and 58 days from mid-veraison. For the other trial, four viticultural techniques were compared to a control treatment: 100% fruit zone leaf removal on both sides of the row at mid-veraison (Leaf removal), bunch thinning with an intensity of 40% at mid-veraison (Grape thinning), four Irrigations each equivalent to 10 mm of rain starting 10 days before the expected date of veraison (Irrigation), and an application of exogenous jasmonic acid – an elicitor that might stimulate rotundone biosynthesis through the

mevalonate pathway - 20 days after mid-veraison, using a 1 mmol/L solution sprayed at 200 L/ha (Elicitor). Rotundone was determined as described in §2.4. Data were analysed using Xlstat software (Addinsoft, France) through a three-way ANOVA (vintage × maturity or viticultural treatment × block) with first-order interaction.

2.4 Rotundone quantification

At harvest, one sample consisting of 800 berries was collected for each site or experimental unit, and vinified through microvinification techniques, in a 1-L Erlenmeyer flask, following the procedure proposed by Geffroy et al. (2014). Then, rotundone in wine was determined by solid phase extraction - solid phase microextraction -multidimensional gas chromatography-mass spectrometry according to a previously published protocol (Geffroy et al. 2014).

3. Results and discussion

3.1 Key environmental factors driving rotundone in wine

Rotundone concentration varied across sites from 63 ng/L to 239 ng/L in 2013, and from 25 ng/L to 115 ng/L in 2014. Some sites that exhibited high rotundone concentration in 2013 had low to moderate contents in 2014. In the same way, some vineyards showing high concentrations in 2014 had low to moderate rotundone contents during the previous year. This means that fixed variables such as training system, clone or rootstock, elevation, do not substantially contribute to the rotundone prediction model. Regression coefficients of the variables included in the best PLSR models and coefficients of determination of the models (R^2 calibration) are shown in Table 1. These results emphasized that the concentration in gluconic acid, a secondary metabolite of *Botrytis cinerea*, had a negative substantial contribution to two out of the three models, as a likely consequence of its polyphenol oxidase (laccase). Other predictors were related with abiotic factors such as cumulative rainfall, thermal index, hours of sunshine and mean daily irradiation highlighting that mesoscale climatic variables are the key factors driving rotundone accumulation.

3.2 Differences in rotundone between Duras clones

Rotundone concentrations were significantly higher in wines made from clones number 654 and 554 in both seasons (Figure 1) which indicates that Duras producers should plant these clones if they wish to produce wines rich in rotundone. The rotundone concentrations measured in 2014 are among the highest ever reported in the literature (Geffroy et al. 2020). On the experimental site, a positive logarithmic correlation (R^2 = 0.58) was observed between the intensity of powdery mildew estimated on grapes and rotundone concentrations in experimental suggesting that grapevine defence response could enhance rotundone biosynthesis.

3.3 Effect of maturity and viticultural techniques on rotundone

Our results demonstrated that the time of harvest had a strong impact on the rotundone concentration and that the molecule accumulated late during maturation (Figure 2). Slightly different accumulation kinetics although non-significant from a statistical standpoint were observed during the two years of study. In 2011, rotundone increased rapidly and then reached a plateau 44 days after mid-veraison while in 2012, rotundone accumulation was more steadily.

As for the impact of viticultural techniques, bunch thinning had no impact on the rotundone content in wine which suggests that the accumulation of rotundone is independent of source-sink type relationships and that the molecule is likely to be produced *in situ,* without being conveyed from vegetative organs through the phloem. These findings are in agreement with other results highlighting that the interruption of the sap flow by cutting the fruit branch does not disturb the accumulation of rotundone (Geffroy et al. 2016).

Leaf removal carried out on both sides of the row at veraison strongly reduced rotundone as the likely consequence of the increase in bunch surface temperature, a parameter known to penalize the biosynthesis of this compound (Zhang et al., 2015). The elicitor treatment had no significant effect on rotundone. However, as the efficacy of the application strongly depends on the conditions of spraying (concentration of the solution, volume, timing, and foliage penetration), it remains difficult to draw firm conclusions on the role of jasmonic acid. In our experimental conditions, irrigation induced a significant increase in rotundone which is consistent with our previous observations highlighting the determining role of cumulative rainfall (§3.1).

4. Conclusion

Our work showed that rotundone production could be affected by abiotic (amount of water, irradiation, thermal index) and biotic (infection by *Erysiphe necator* and *Botrytis cinerea*) factors. They also made it possible to identify viticultural leverages to modulate the concentration of rotundone in wines. Thus the date of harvest, the clone, irrigation were identified as practices that can be used by winegrowers to promote rotundone and the peppery expression of their wines.

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6. Literature cited

Dufourcq t., Barraud g., Delpuech x., Gaudin r., 2013. Use of the water balance model walis to predict stem water potential on Colombard variety in Gascony vineyard. Ciência e técnica vitivinícola, 28 75-79.

Geffroy, O., Kleiber, D., & Jacques, A., 2020. May peppery wines be the spice of life? A review of research on the 'pepper'aroma and the sesquiterpenoid rotundone. OENO One, 54(2), 245-262.

Geffroy, O., Descôtes, J., Levasseur-Garcia, C., Debord, C., Denux, J. P., & Dufourcq, T. (2019). A 2-year multisite study of viticultural and environmental factors affecting rotundone concentration in Duras red wine. OENO One, 53(3).

Geffroy, O., Descôtes, J., Serrano, E., Li Calzi, M., Dagan, L., & Schneider, R., 2018. Can a certain concentration of rotundone be undesirable in Duras red wine? A study to estimate a consumer rejection threshold for the pepper aroma compound. Australian Journal of Grape and Wine Research, 24(1), 88-95.

Geffroy, O., Siebert, T., Silvano, A., & Herderich, M., 2017. Impact of winemaking techniques on classical enological parameters and rotundone in red wine at the laboratory scale. American Journal of Enology and Viticulture, 68(1), 141-146.

Geffroy, O., Siebert, T., Herderich, M., Mille, B. and Serrano, E., 2016. On-vine grape drying combined with irrigation allows to produce red wines with enhanced phenolic and rotundone concentrations. Scientia Horticulturae, 207, 208–17.

Geffroy, O., Yobrégat, O., Dufourcq, T., Siebert, T., & Serrano, E., 2015. Certified clone and powdery mildew impact rotundone in red wine from *Vitis vinifera* L. cv. Duras N. Journal International des Sciences de la Vigne et du Vin, 49(4), 231-240.

Geffroy, O., Dufourcq, T., Carcenac, D., Siebert, T., Herderich, M. and Serrano, E., 2014. Effect ripeness and viticultural techniques on the rotundone concentration in red wine made from *Vitis vinifera* L. cv. Duras. Australian Journal of Grape and Wine Research, 20(3), 401–8.

Homich, L. J., Elias, R. J., Heuvel, J. E. V., & Centinari, M., 2017. Impact of fruit-zone leaf removal on rotundone concentration in Noiret. American Journal of Enology and Viticulture, 68(4), 447-457.

Wood, C., T.E. Siebert, M. Parker, D.L. Capone, G.M. Elsey, A.P. Pollnitz, M. Eggers, M. Meier, T. Vossing, S. Widder, G. Krammer, M.A. Sefton, & M.J. Herderich, 2008. From wine to pepper: rotundone, an obscure sesquiterpene, is a potent spicy aroma compound. Journal of Agricultural and Food Chemistry 56, 3738–3744.

Zhang, P., Barlow, S., Krstic, M., Herderich, M., Fuentes, S., & Howell, K. (2015). Within-vineyard, within-vine, and within-bunch variability of the rotundone concentration in berries of *Vitis vinifera* L. cv. Shiraz. Journal of Agricultural and Food Chemistry, 63(17), 4276-4283.

Table 1: Coefficient of determination (calibration) of the best PLSR models to predict rotundone concentrations in 2013, 2014 and 2013-2014 and regression coefficients of the variables included. – variable not included in the best model.

Variable	PLSR model		
	2013	2014	2013-2014
Cumulative rainfall veraison – harvest	-0.90	-	-
Hours of sunshine	0.72	-	0.61
Gluconic acid concentration	-0.83	-	-0.001
Cumulative rainfall 1 April – 30 September	-	0.85	0.77
Huglin index	-	-0.63	-
Cumulative rainfall 1 January – 31 December	-	-	0.66
Mean daily irradiation veraison – harvest	-	-	0.59
R^2 (calibration)	0.92	0.53	0.59

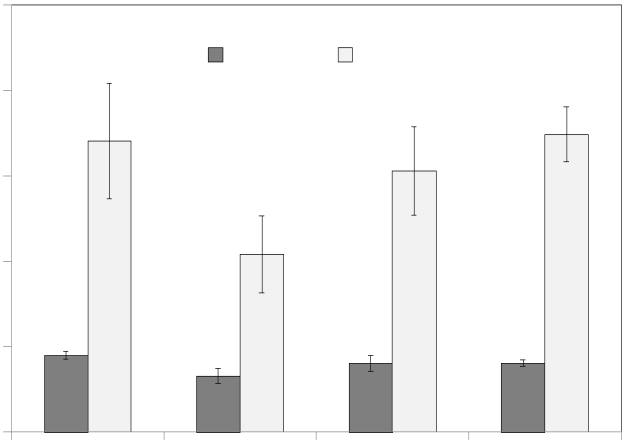

Figure 1: Effect of the four certified Duras clones on rotundone concentration. Different letters referring to both seasons indicate means significantly different at $P< 0.05$ by Fisher test.

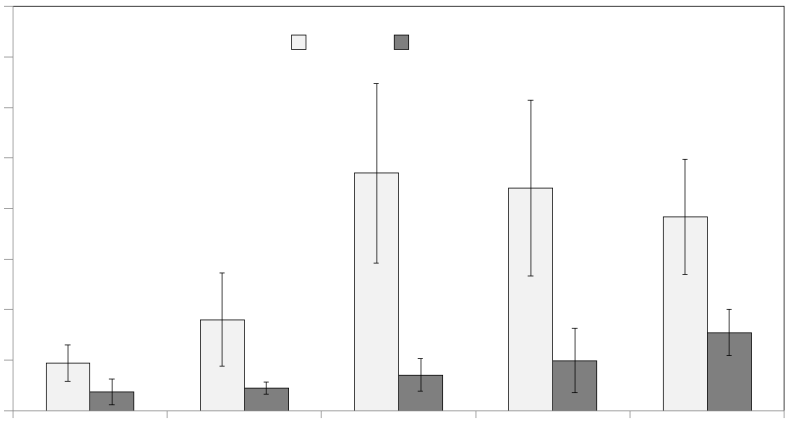

Figure 2: Effect of the maturity on rotundone concentration. Different letters referring to both seasons indicate means significantly different at $P< 0.05$ by Fisher test.

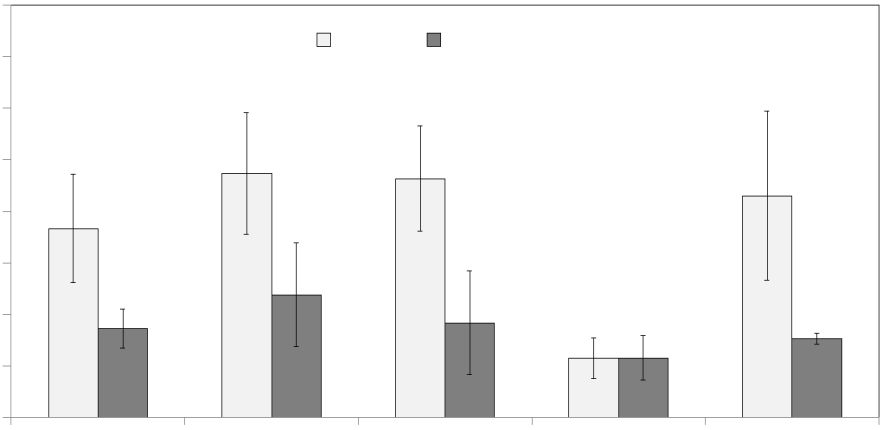

Figure 3: Effect of some viticultural techniques on rotundone concentration. Different letters referring to both seasons indicate means significantly different at $P < 0.05$ by Fisher test.