CERTIFIED CLONE AND POWDERY MILDEW IMPACT ROTUNDONE IN RED WINE FROM *VITIS VINIFERA* L. CV. DURAS N

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Abstract

Aim: Few recent studies have been investigating the effect of clone on aroma compounds. The aim of this research work was to study the impact of certified clones from *Vitis vinifera* L. cv. Duras N on grape quality and rotundone, a sesquiterpene responsible for peppery aroma which has been reported recently in red wines made from this cultivar.

Methods and results: The experimental site consisted of four consecutive rows, each row planted with one of the four certified clones of Duras N (554, 555, 627 and 654). For each clone, measurements were replicated on three experimental units per row. Each experimental unit consisted of twelve continuous vines. Rotundone concentration was measured in wines prepared by microvinification techniques (1-L Erlenmeyer flasks). For both vintages of study, rotundone concentrations were significantly higher in wines from clones 554 and 654 in comparison with clone 555, while clone 627 showed an intermediate level. In 2014, differences in powdery mildew (PM) severity on clusters were identified between the four clones and a positive logarithmic correlation ($r^2 = 0.58$) was reported on the experimental site between rotundone in wines and PM severity on grapes.

Conclusion: Our results found differences in rotundone concentrations in wines made from the four Duras N certified clones. The results also suggested that grapevine defence response to PM could enhance rotundone production in berries. Clonal differences in susceptibility to biotic stress, such as PM, might explain the differences observed in rotundone concentrations between the four studied clones.

Significance and impact of the study: Our results may assist grape growers to produce high quality wines with a desired aroma attribute made from Duras N. One should consider planting clone 554 in order to promote high levels of rotundone in wines made from this cultivar.

Key words: rotundone, Duras N, certified clone, powdery mildew, natural defence

Résumé

Objectif : Peu d'études récentes ont étudié l'effet du clone sur les composés aromatiques. L'objectif de ce travail de recherche était d'évaluer l'impact des clones certifiés de *Vitis vinifera* L. cv. Duras N sur la qualité des raisins et la rotundone, un sesquiterpène responsable des arômes poivrés identifié récemment dans les vins élaborés à partir de ce cépage.

Méthodes et résultats : Le site expérimental se compose de quatre rangs consécutifs, chacun des rangs étant planté avec l'un des quatre clones certifiés de Duras N (554, 555, 627 et 654). Pour chacun des clones, les mesures ont été répétées sur trois placettes expérimentales par rang, chacune d'entre elle se composant de douze pieds consécutifs. La concentration en rotundone a été mesurée dans des vins élaborés en condition de microvinification (Erlenmeyer d'1 L). Pour les deux millésimes d'étude, les concentrations en rotundone ont été significativement supérieures dans les vins des clones 554 et 654 en comparaison avec le clone 555 alors que le clone 627 a présenté un niveau intermédiaire. En 2014, des différences d'intensité de dégâts d'oïdium ont été mises en évidence sur grappes entre les quatre clones et une corrélation logarithmique positive ($r^2 = 0.58$) a pu être établie sur le site expérimental entre la rotundone dans les vins et l'intensité de ces dégâts.

Conclusion : Nos résultats ont permis de mettre en évidence des différences de concentrations en rotundone dans les vins élaborés à partir des quatre clones certifiés de Duras N. Les résultats suggèrent également que les réactions de défense de la vigne vis-à-vis de l'oïdium peuvent induire la production de rotundone dans la baie. Des différences clonales de sensibilité à un stress biotique, comme l'oïdium, pourrait permettre d'expliquer les différences de concentrations en rotundone observées entre les quatre clones étudiés.

Signification et impact de l'étude : Nos résultats sont susceptibles d'aider les producteurs à élaborer des vins de Duras N de qualité possédant des notes aromatiques recherchées. Ces derniers ont intérêt à planter le clone 554 s'ils souhaitent favoriser des concentrations en rotundone élevées dans les vins de ce cépage.

Mots clés : rotundone, Duras N, clone certifié, oïdium, défense naturelle

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INTRODUCTION

According to the definition of the Office International de la Vigne et du Vin (OIV), "one clone is the certified vegetative descent of one vine chosen for its identity, its phenotypic characteristics and its sanitary condition". One clone may have been selected deliberately from a grapevine that has demonstrated desirable traits. That is why differences in phenotypes are often found within certified clones of a given grape variety. Clones can differ in morphological characteristics (leaf, cluster and berry shapes) which can lead to different levels of susceptibility to grape disease such as *Botrytis cinerea*, agronomical behaviour (earliness of ripening, vigour, fertility, sensitivity to flower abortion, number of berries per cluster, cluster weight, grape yield), and berry traits (sugar, acidity, phenolic and aroma contents at harvest). Despite large progress in the chemistry of wine aroma during the last two decades, few recent studies have been investigating the impact of clone on aroma compounds or using aroma analysis as a tool for selecting new clones.

Most of the studies on this topic were conducted on white aromatic cultivars with a focus on free and bound monoterpene contents of the investigated clones. Differences were identified within clones of Gewürztraminer and Weisser Riesling (Marais and Rapp, 1991), Chardonnay (McCarthy, 1988a; Versini *et al.*, 1988) and Muscat à petits grains (McCarthy, 1988b). Comparing Merlot noir clone wines, Kotseridis *et al.* (1998) reported higher levels of 3-isobutyl-2-methoxypyrazine in the wine of French certified clone 182. Another study investigated the aroma of wines from seven clones of Monastrell grapes that allowed some grouping of the clones (Gómez-Plaza *et al.*, 1999).

More recently in Shiraz grapes, Siebert and Solomon (2011) found a higher level of rotundone in the 2626 clone in comparison with the 1127. This key aroma compound, identified for the first time in 2008 in Shiraz wines (Wood *et al.*, 2008), was also reported recently by Geffroy *et al.* (2014) in Duras N wines, a grape variety grown in the South West of France.

Duras N is one of the main red grape cultivars in the Protected Designation of Origin (PDO) Gaillac. Its name is derived from "dur" (meaning "hard"), which refers to the hardness of its shoots. It was first mentioned in Gaillac in a document from 1484, and seems to have been continually cultivated since then in the area. Several hypotheses were advanced to explain the deep origin of this cultivar, considering that Gaillac – one of the oldest vineyards in France – was founded in the second century BC (Viala and Vermorel, 1902; Lavignac, 2001). The presence in forests and riversides around the Gaillac area of many relict populations of *Vitis vinifera* ssp. *sylvestris*, locally called "lambrusques", made some authors think that Duras could be an offspring of this wild vine. Other writers supposed Duras to be the Duracina variety cited by the Latin authors Cato the Elder, Pliny the Elder and Columella (Lavignac, 2001). But no evidence or clue could be provided to confirm these suppositions as no genetic relationships were found between local varieties and wild vines in the area (Olivier Yobrégat, unpubl. data, 2014), and a simple similarity between two cultivar names does not constitute an evidence to pretend they are identical, especially with more than 1 500 years of difference. Recently, Lacombe *et al.* (2013) discovered that Duras N was an offspring of two other ancient cultivars from the North East of France, Savagnin B and Tressot N.

Four different clones of this cultivar, almost exclusively represented in the PDO Gaillac, are presently available. The clones are virus-tested, certified and designated under the numbers 554, 555, 627 and 654. Clones 554 and 555 were the first to be certified in 1978 (MAP, 2007), were the first to be planted for multiplication and are currently the most profuse. In one study (Daniel Goulard, unpubl. data, 1993), the four clones were described as homogenous for pruning wood weight, grape yield and analytical characteristics of the grapes at harvest. However, the intensity of rotundone could vary between them. Therefore, a two-year study, commenced in 2013, was conducted in order to evaluate the rotundone concentration in Duras N wines made from the four certified clones.

MATERIALS AND METHODS

1. Experimental site and design

The experimental vineyard, located in the Gaillac vineyard (lat. 43° 50' 32'' N; long. 01° 51' 7'' E) consisted of four consecutive rows, each row planted with one of the four certified clones of Duras N. Orientation of the vine rows was south-west to north-east with the following sequence: 554, 555, 627 and 654. All measurements were replicated three times on experimental units of 28.5 m^2 consisting of twelve continuous vines. The plot was flat and typical of the area with 2.20 m x 1 m row spacing. It was planted in 1999 with Duras N grapevines grafted onto 140 Ruggeri rootstock (clone 265). Plant materials (rootstocks and scions) came directly from the initial repository of selected clones (IFV Domaine de

l'Espiguette, France) where permanent sanitary controls are performed and traceability information is maintained. Vines were trained with vertical shoot positioning (VSP) associated with Cordon de Royat pruning system. The soil was managed by chemical weed control under the vines, and by grass cover inter-row.

2. Description of the clones and sampling

The official description of the agronomical and technological behaviours of the four certified clones of Duras N is presented in Table I. For the four clones, budburst took place on 10 April in 2013 and 4 April in 2014. Mid-veraison was determined by randomly collecting 100 berries on the experimental site and by counting the number of coloured berries. No differences in mid-veraison date (27 August in 2013 and 12 August in 2014) were observed between the four clones. Due to a delay of 3 weeks in phenology and high risks of *Botrytis cinerea* development in 2013, grape samples were collected on 26 September, which was early in the maturation process and corresponded to 30 days after mid-veraison. In 2014, sample collection took place 40 days after mid-veraison on 19 September. In both vintages, picking dates coincided with commercial harvest of the plot. Each year, twelve grape samples were collected at harvest (three per clone). Each sample consisted of 216 berries from both sides of the row and several parts of the bunch (nine berries per vine on each side of the row). Prior to analysis, the weight of 200 berries was measured. Berries were crushed and 100 mL of must were centrifuged (14 000 × *g* for 6 min) to enable classical laboratory and $\delta^{13}C$ analysis. The remaining amount of must and skin was crushed for 2 min using a blender and was used for phenolics determination. Also, from each experimental unit, 800 g of berries were collected to perform microscale fermentations in a 1-L Erlenmeyer flask according to the protocol proposed by Geffroy *et al.* (2014). After alcoholic fermentation, wines were centrifuged (14 000 × *g* for 6 min), supplemented with sulphite (80 mg/L), bottled and stored at 4°C or below until rotundone analysis. In order to obtain a sufficient amount of juice and wine to carry out the analyses, the samples were only composed of healthy berries.

3. Classical laboratory

Sugar concentration (°Brix) was determined with a digital hand-held Pocket refractometer PAL (Atago, Japan). Titratable acidity was measured according to OIV method (OIV, 2009) and pH measured with a HI 3221 pH meter (Hanna Instruments, France). A Konelab Arena 20 sequential analyser (Thermo Electron Corporation, USA) associated with enzyme kits provided by several suppliers was used to determine amino acids, ammonium (Megazyme, Ireland) and malic acid (Thermo Fisher Scientific, USA). Potassium determination was by flame photometry (Bio Arrow, France) according to OIV method (OIV, 2009) and tartaric acid determination by colorimetric titration (Hill and Caputi, 1970). Anthocyanins and Total Phenolic Index (TPI) were quantified in grapes according to Cayla *et al.* (2002) using an Evolution 100 spectrophotometer (Thermo Electron Corporation, USA). This analytical method for phenolics determination in grapes is based on a1-hour micromaceration in a hydroalcoholic and acid medium. All determinations were carried out in duplicate. The remaining amount of centrifuged must was used for $\delta^{13}C$ determination.

4. Rotundone analysis

Because of quarantine requirements and logistical issues with shipping frozen grapes from France to Australia, rotundone was analysed indirectly in wines fermented under microvinification conditions. For the rotundone, reproducibility tests showed an average coefficient of variation of 5% across the microfermentors. Rotundone in wine was determined by solid phase microextraction-multidimensional gas chromatography-mass spectrometry (Geffroy *et al.*, 2014). The analyses were performed in two different

Table1 - Description of the four certified clones of Duras N in France (MAP, 2007).

Clone number	Breeder	Year of certification	Surface available for multiplication (ha)	Fertility	Cluster weight	Grape yield	Sugar concentration at harvest
554	ENTAV	1978	1.54	Average	Average	Average	Superior
555	ENTAV	1978	0.44	Average	Inferior to average	Average	Superior
627	ENTAV	1979	0.44	Average to superior	Average to superior	Average to superior	Average to superior
654	ENTAV	1980	<0.01	Average to superior	Average to superior	Inferior to average	Inferior to average

years but during the same period of each year to reduce potential variations associated with different post-bottling times.

5. Measurements on vines and evaluation of powdery mildew severity on grapes

At harvest, number of clusters per vine and yield (kg/vine) were monitored for 12 out of 12 vines for each experimental unit by counting the number of clusters and weighing crop loads individually with a Precia Molen C20 K balance (Precia SA, France). Severe powdery mildew (*Erysiphe necator*) infections were recorded on grapes in 2014. Powdery mildew (PM) severity was assessed at harvest by visually estimating the percentage of the cluster area colonized by the pathogen on 100 grape clusters (50 per each side of the vine) within each experimental unit.

6. Weather measurements

In 2013 and 2014, rainfall and air temperature (minima, maxima and mean values) were monitored by a Cim AGRO weather station (Cimel Electronique, France) placed within 200 m of the experimental site. In order to characterize the two vintages, several indices proposed by Tonietto and Carbonneau (2002) were calculated: the Huglin index or heliothermal index over the period of 1 April to 30 September, the cool night index (FNv-r), the mean air temperature (Tv-r), the maximal air temperature (Txv-r), and the thermal amplitude (Av-r) indices over the veraison-harvest period. Cumulative rainfall over the whole calendar year, the budburst-veraison and veraison-harvest periods was also calculated.

7. Homogeneity control of the plot

Since its plantation, the plot has been used for pre-multiplication, has always been virus-tested and judged homogenous especially regarding pruning wood weight and vigour. All ELISA tests carried out in November 2014 were negative for ArMV, GLFV, GLRaV 1, 2 and 3. Recent studies have highlighted large spatial variations in berry and wine rotundone concentrations within a single vineyard (Geffroy *et al.*, 2014; Scarlett *et al.*, 2014). This variability was associated with variation in the land underlying the vineyard and was linked especially with vine water status. Despite its apparent homogeneity, extra controls were carried out on the plot in order to detect any source of variability, especially between the rows, using:

- Trunk circumference (TC): several studies (Tisseyre *et al.*, 2007; Bramley *et al.*, 2011) showed that this index of vine vigour was well correlated to apparent electrical conductivity of the soil (EC_a) and therefore could be used to monitor soil heterogeneity. More recently, studies by Geffroy *et al.* (2015) showed that a link existed between plant architecture (TC) and rotundone, and that TC could be used to approach rotundone spatial distribution. In April 2014 just before budburst, TC was determined for every vine within each experimental site as the average value measured at three different heights: just above the graft;10 cm under the trellis wire; and half way between the two first points of measurement.

- $\delta^{13}C$ measurement: $\delta^{13}C$ measured on grapes at harvest is an integrative indicator of water deficit experienced by the vine during grape ripening (Van Leeuwen *et al.*, 2009). Geffroy *et al.* (2014) identified good correlations ($r^2 = 0.76$ in 2011 and $r^2 = 0.74$ in 2012) between rotundone concentration in wine and $\delta^{13}C$ within the same vineyard. Tin capsules (TIN 6 × 4 mm) were introduced into a 96-well (8 mm) microplate (model 83.1835; Sarstedt, Germany). Five mL of centrifuged grape juice collected at harvest for each experimental unit were introduced into each of the tin capsules using a micropipette. The microplate was then placed in a non-ventilated oven at 60°C for 24 h, sealed and sent to the stable isotope laboratory of the James Hutton Institute (Dundee, Scotland). Samples were analysed for their bulk 13C isotopic composition by standard operation procedures compliant with the International Union of Pure and Applied Chemistry guidelines and recommendations for stable isotope ratio measurements and reporting results thereof (Coplen, 2011). The precision of the $\delta^{13}C$ values obtained was determined by repeat analysis of reference materials. Quality control samples were better than ±0.15%.

- NDVI mapping: in 2014, remotely sensed imagery (airborne multispectral video imagery with a ground resolution of 50 cm) of the plot was obtained (Lamb *et al.*, 2004) at veraison (4 August). This widely used indicator of plant vigour or relative biomass was calculated from the extracted single-pixel or multiple-pixel values using the relationship proposed by Rouse *et al.* (1973).

To our knowledge, for a given cultivar no clonal differences in drought tolerance, vigour or vine architecture (TC) – variables which are impacted by the rootstock – have been previously described in the literature. Therefore, if some differences were to be observed between the clones for these variables, then they are more likely to reflect variations in soil composition within the experimental site.

8. Statistical analysis

Statistical analyses were conducted with Xlstat software (Addinsoft, France). All the analytical data measured both in 2013 and 2014, and rotundone content of the wines were first subjected to a three-way analysis of variance (ANOVA) treatment (vintage × clone × block) with first-order interaction (n = 24; residual degrees of freedom = 6). Because the block effect was never significant and vintage × clone interactions were almost always significant, data excluding rotundone were next subjected for each year to a one-way (clone) ANOVA (n = 12; residual degrees of freedom = 8). Fisher's least significant difference test was used as a post-hoc comparison of means at $P< 0.05$. Other variables which were just determined with repeated measurements during one year of study (i.e. trunk circumference, severity of powdery mildew) were first treated by a two-way ANOVA (clone × block) with first-order interaction. As the block factor was not significant, data were next analysed by a one-way ANOVA. Regression was undertaken with the PM severity data averaged for each experimental unit.

RESULTS AND DISCUSSION

1. Quantitative and qualitative performances of the clones

Results of the three-way ANOVA treatments (Table 2) show that the vintage factor had the strongest overall impact on quantitative and qualitative performances of the clones. As harvest took place respectively 30 days and 40 days after mid-veraison in 2013 and 2014, it is difficult to compare the two vintages from an analytical point of view. No significant block effect or interactions involving that factor were observed on the plot and NDVI spatial pattern (results not shown), trunk circumference and $\delta^{13}C$ measurements did not allow discrimination of the four studied clones. Despite the imperfection of the experimental design, these observations reflect a good homogeneity of the plot and confirm that differences observed are more likely to be due to a real clone effect than to variation in the land underlying the vineyard, especially between the four rows of study.

Vintage × clone interactions were almost always significant. While differences were observed in 2013 between the four clones for most of the measured variables, few analytical data allowed discrimination of the clones in 2014. In 2014, the severity of powdery infections might have brought some perturbation and variability in the data set. In 2013, clone 554 showed better qualitative performances (i.e. higher sugar concentration, TPI and anthocyanins) in comparison with the other clones and especially clone 555. This is not a trivial matter as the two clones that showed the largest amplitude of difference in analytical variables (554 and 555) were the furthest apart in the vineyard. Also, our observations, in terms of grape yield and sugar concentration at harvest, were not particularly in accordance with the information provided by MAP (2007) and shown in Table 1.

Table 2 - Results of the analysis of variance performed on the data measured during the trial.

Parameter	*P*-value					
	Vintage (V)	Clone (C)	Block (B)	V × C	V × B	C × B
200 berry weight	<0.0001	0.152	0.543	0.035	0.593	0.736
Sugar concentration	0.076	0.002	0.764	0.004	0.296	0.115
Total acidity	0.462	0.033	0.203	0.034	0.386	0.556
pH	<0.0001	0.224	0.150	0.025	0.714	0.718
Tartaric acid	<0.0001	0.011	0.806	0.006	0.290	0.160
Malic acid	<0.0001	0.005	0.223	0.001	0.092	0.103
Amino acids	0.005	0.106	0.777	0.046	0.851	0.148
Ammonium	0.559	0.301	0.502	0.323	0.524	0.506
Potassium	<0.0001	0.481	0.069	0.106	0.982	0.218
Total Phenolic Index (TPI)	0.007	0.009	0.397	0.014	0.917	0.534
Anthocyanins	0.006	0.020	0.353	0.011	0.256	0.400
Number of clusters per vine	<0.0001	0.951	0.126	0.218	0.179	0.390
Yield at harvest	<0.0001	0.125	0.217	0.198	0.397	0.148
$\delta^{13}C$	0.980	0.701	0.961	0.969	0.415	0.521
Severity of powdery mildew	-	<0.0001	0.123	-	-	0.075
Trunk circumference	-	0.478	0.652	-	-	0.603
Rotundone concentration	<0.0001	0.049	0.842	0.127	0.597	0.579

Neither the number of clusters per vine nor yields (kg per vine) at harvest were impacted in 2013 and 2014 by clone type. As significant differences in severity of PM on grapes at harvest were observed between the clones in 2014 and PM infection leads to lighter bunch weight, differences in yields could have been expected. The PM infections could have impacted the analytical variables measured on grapes at harvest as previously reported on Chardonnay grapes and wines, which showed an increase in titratable acidity and total phenolics corresponding with increasing severity of PM infection (Stummer *et al.*, 2005). However, this phenomenon was not observed during our study because only healthy berries were collected for laboratory analyses, minimizing the effects of PM. Differences in susceptibility of grapevine cultivars to PM (Doster and Schnathorst, 1985) and in clonal susceptibility to downy mildew (*Plasmopara viticola*) on leaves (Boso *et al.*, 2004) have been previously described. To our knowledge, this is the first time that clonal differences in powdery mildew susceptibility have been observed on grapes. However, as the experimental site consisted of four rows – each one planted with one different clone – and PM infection was only observed in 2014, it is difficult to draw definitive conclusions. Indeed, the differences observed could reflect variations in quality of the phytosanitary treatment targeted against PM in relation with the spraying equipment. However, there was no direct relationship between the sequence of planting of the four clones (554, 555, 627 and 654) and the severity of powdery mildew recorded on grapes in 2014 (555 < 554 < 627 < 654). Differences in canopy density could have affected PM severity between the four clones; for example, canopy management designed to optimize sunlight exposure of grape clusters was shown to have a beneficial impact on powdery mildew (Austin *et al.*, 2011). Nevertheless, NDVI measurements conducted in August 2014 did not show any significant differences between the four rows and consequently between the clones for this vegetation index. As summarized by Schnee (2008) in his doctoral thesis, the difference of susceptibility to PM could have been induced by i) constitutive factors in relation with physical properties or barriers that limits the penetration of the pathogen (i.e. cuticle) or ii) induced factors in relation with defence responses (i.e. phytoalexin). A large variability in severity of powdery mildew was observed within the clones. This can be attributed to the fact that this variable was determined with repeated measurements for each block and that standard deviations refer both to variability within one block and to inter-block variability.

2. Rotundone in wines

Unlike the other variables measured on grapes and discussed previously, no vintage × clone interaction was observed for rotundone. Concentrations of rotundone were significantly higher in wines from clones 554 and 654 in comparison with clone 555, while clone 627 showed an intermediate level (Figure 1). As rotundone was measured indirectly in wines prepared by microvinification techniques (1-L Erlenmeyer flask), differences observed in sugar content of the grapes in 2013, and thus leading to distinct ethanol concentrations in the resulting wines, might have influenced the extraction of the hydrophobic rotundone from the berry skin. However, under the winemaking conditions of this study, the effect of the ethanol differences would be minimal, which is consistent with other research where the impact of ethanol on the extraction of hydrophobic compounds, such as proanthocyanidins, from the berry skin did not exceed a few percent on mature or almost mature grapes (Canals *et al.*, 2005).

Rotundone concentrations observed in 2014 (more than 300 ng/L on average for three of the studied clones) (Figure 1), are among the highest ever reported and the strong vintage effect observed on the experimental site deserves further discussion. Caputi *et al.* (2011) suggested that higher concentrations were most likely to accumulate in grapes in cooler vintages while Geffroy *et al.* (2014) proposed that summer water stress was a more important variable than air temperature in explaining differences in rotundone between seasons. More recently, Zhang *et al.* (2015) showed that berry temperature exceeding 25°C between veraison and harvest negatively affects the rotundone concentration in Shiraz. The 2013 vintage was characterized by a cold spring and regular, large rainfall events over the vine vegetative cycle which induced a delay of 3 weeks at harvest. In 2014, winter was rainy and warm; summer was extremely rainy with 160 mm of rain recorded between mid-July and the end of August; and conditions during maturation were dry and hot. Climatic indices and cumulative rainfall calculated for the two vintages of study allow the two vintages to be compared in more detail (Table 4). The 2013 vintage was cooler, especially during the veraison-harvest period, and less rainy than 2014 whose rainfall events mostly occurred as summer storms, and the two vintages were characterized by a similar water deficit as reflected by $\delta^{13}C$ measurements (Table 3). Consequently, as bunch temperature appeared to be the main determinant to explain the differences in rotundone between the two vintages, larger concentrations were expected in 2013. As

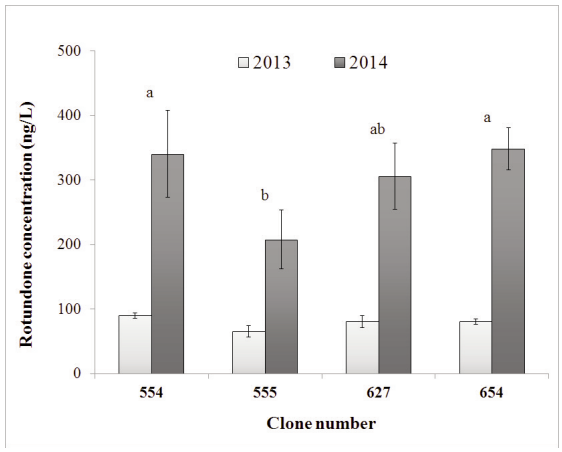

Figure 1 - Rotundone concentration in experimental wines made from the four certified clones of Duras N.
Different letters which refer to both years indicate means significantly different at P< 0.05 by Fisher test.

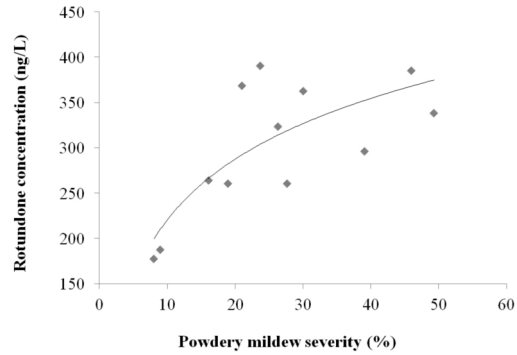

Figure 2. Relationship between rotundone concentration in wines and powdery mildew severity on grapes at harvest in 2014 (n = 12).
Linear model gave y = 96.72 ln(x) – 2.270;
r^2 = 0.58; RMSE = 49.2.

grapes were harvested 10 days earlier in 2013 for the clonal study, this difference in lapse of time after mid-veraison must have impacted rotundone concentrations in the resultant wines. Geffroy *et al.* (2014) showed, in the same viticultural context, that rotundone concentration greatly increased between mid-veraison +30 days and mid-veraison +44 days in red wines from Duras N. As a result, differences in rotundone concentrations expected between the vintages for the clonal study should remain small and in no case as large as determined in our study. This suggests that another mechanism(s) is involved.

Host responses to PM have been characterized in various resistant species such as *Vitis labrusca*, *Vitis rupestris* (Doster and Schnathorst, 1985), *Vitis aestivalis* (Fung *et al.*, 2008) and *Vitis pseudoreticulata* (Weng *et al.*, 2014), a wild Chinese species. This last study carried out on leaves showed that infection by PM induced differential expression of 6541 genes with several of them belonging to the defence, salicylic acid (SA) and jasmonic acid categories. One of the few studies which have been investigating the transcriptional response to PM in susceptible *Vitis vinifera* (Fung *et al.*, 2008) showed that endogenous SA levels were lower in *Vitis vinifera* than in *Vitis aestivalis* in the absence of fungus and that SA concentrations increased with PM in *Vitis vinifera*. This suggests that PM infection can also stimulate natural defence mechanism in *Vitis vinifera*. The biosynthesis and biological function of rotundone has not been determined, but as with several other sesquiterpenes, it could be involved in the natural defence mechanisms of the vine, especially through the mevalonate/jasmonic acid pathway (D'Onofrio *et al.*, 2009; Dicke and Baldwin, 2010). In the latter study, methyl jasmonate and jasmonic acid induced a wide range of sesquiterpenes in berry cell suspension cultures of Cabernet Sauvignon and Riesling. On the experimental site, a correlation (r^2 = 0.58) was observed between the rotundone concentration in experimental wine and PM severity (Figure 2). As experimental wines were made from healthy berries, these results suggest that infected berries could induce plant defence mechanisms leading to synthesis of rotundone in the neighboring healthy berries. As the correlation observed is positive – with clones 555 showing the lowest PM severity and rotundone in wines – mechanisms involved in the clonal susceptibility discussed previously are more likely to be related to constitutive than to induced factors. Even though in 2013 no pathogen infection (i.e. *Botrytis cinerea*) could be visually detected or identified through determination of gluconic acid (results not shown), our results, which did not show any vintage × clone interaction for rotundone, suggest that clonal differences in susceptibility to biotic stress such as PM through constitutive mechanisms might help explain the differences observed in rotundone between the clones.

In 1998, a Duras N repository containing 150 clones was established in Gaillac. After initial investigations, seven clones were selected to be compared with clone 554 and a new plot dedicated to clonal selection was planted in 2007. Rotundone determinations in wines will be carried out and will bring additional information to most commonly agronomical and enological data for clone selection. If higher concentrations in rotundone were to become a criterion for a new clone selection, then it is important to confirm whether this ability to produce high levels of peppery aroma is related to susceptibility to PM or to any other disease.

Table 3 - Effect of clone on TC, $\delta^{13}C$, classical laboratory analysis of the grape at harvest and production aptitude.

Parameter	Clone number				*Significance*
	554	555	627	654	*P*
Trunk circumference (cm)	19.2 ± 1.2 a	19.6 ± 1.4 a	19.2 ± 1.2 a	19.6 ± 1.8 a	0.574
2013					
200 berry weight (g)	387 ± 9 a	398 ± 8 a	385 ± 5 a	390 ± 8 a	0.260
Sugar concentration (°Brix)	21.1 ± 0.2 a	19.9 ± 0.2 c	20.1 ± 0.3 b	21.0 ± 0.2 a	<0.0001
Total acidity (mEq/L)	163 ± 2 b	175 ± 4 a	157 ± 2 c	158 ± 0 c	0.001
pH	3.02 ± 0.03 a	2.99 ± 0.01 a	3.01 ± 0.02 a	3.03 ± 0.01 a	0.104
Tartaric acid (g/L)	4.21 ± 0.06 a	3.69 ± 0.18 c	3.84 ± 0.06 bc	3.97 ± 0.23 b	0.005
Malic acid (g/L)	7.49 ± 0.16 b	8.42 ± 0.11 a	7.32 ± 0.10 b	7.41 ± 0.11 b	<0.0001
Amino acids (mg/L)	77.2 ± 2.1 a	78.5 ± 0.4 a	75.5 ± 3.7 a	76.1 ± 3.2 a	0.548
$NH4^+$ (mg/L)	38.9 ± 3.9 a	38.2 ± 3.2 a	31.9 ± 3.1 a	39.8 ± 5.6 a	0.150
K^+ (g/L)	1.15 ± 0.08 a	1.16 ± 0.05 a	1.16 ± 0.02 a	1.22 ± 0.06 a	0.438
Total Phenolic Index (TPI)	89.2 ± 2.5 a	73.1 ± 0.4 c	77.7 ± 1.8 b	77.9 ± 1.8 b	<0.0001
Anthocyanin (mg/kg)	1128 ± 48 a	908 ± 58 b	939 ± 24 b	952 ± 40 b	0.001
Number of clusters per vine	16.4 ± 4.1 a	15.6 ± 4.1 a	16.2 ± 5.7 a	16.3 ± 4.7 a	0.939
Yield (kg/vine)	2.29 ± 0.66 a	1.98 ± 0.64 a	2.47 ± 1.01 a	2.20 ± 0.81 a	0.196
$\delta^{13}C$	-28.6 ± 0.0 a	-28.4 ± 0.1 a	-28.5 ± 0.1 a	-28.5 ± 0.1 a	0.287
2014					
200 berry weight (g)	469 ± 22 b	471 ± 17 b	496 ± 20 a	465 ± 2 b	0.002
Sugar concentration (°Brix)	20.7 ± 0.2 a	20.6 ± 0.1 a	20.6 ± 0.2 a	20.6 ± 0.2 a	0.887
Total acidity (mEq/L)	165 ± 6 a	159 ± 5 a	162 ± 3 a	157 ± 3 a	0.193
pH	2.82 ± 0.04 b	2.88 ± 0.04 a	2.83 ± 0.03 b	2.83 ± 0.01 b	0.025
Tartaric acid (g/L)	3.19 ± 0.06 ab	3.34 ± 0.09 a	2.96 ± 0.01 b	3.29 ± 0.23 ab	0.029
Malic acid (g/L)	8.66 ± 0.38 a	8.40 ± 0.34 a	8.63 ± 0.26 a	8.36 ± 0.21 a	0.543
Amino acids (mg/L)	79.4 ± 11.9 a	81.9 ± 8.8 a	95.1 ± 7.7 a	99.2 ± 14.3 a	0.086
$NH4^+$ (mg/L)	35.0 ± 5.8 a	41.5 ± 11.2 a	44.8 ± 9.8 a	42.9 ± 7.9 a	0.383
K^+ (g/L)	1.35 ± 0.05 a	1.30 ± 0.05 a	1.30 ± 0.03 a	1.29 ± 0.01 a	0.235
Total Phenolic Index (TPI)	75.1 ± 6.2 a	74.0 ± 1.4 a	72.4 ± 0.9 a	77.6 ± 1.5 a	0.341
Anthocyanin (mg/kg)	897 ± 62 a	920 ± 69 a	882 ± 5 a	932 ± 8 a	0.365
Number of clusters per vine	11.6 ± 6.1 a	9.1 ± 4.7 a	10.3 ± 4.2 a	11.7 ± 5.1 a	0.186
Yield (kg/vine)	1.46 ± 0.97 a	1.00 ± 0.58 a	1.20 ± 0.64 a	1.13 ± 0.50 a	0.260
$\delta^{13}C$	-28.6 ± 0.2 a	-28.5 ± 0.2 a	-28.4 ± 0.2 a	-28.5 ± 0.0 a	0.929
Severity of powdery mildew (%)	20.0 ± 22.2 c	12.1 ± 15.8 d	33.4 ± 30.7 b	41.5 ± 28.7 a	<0.0001

Means followed by the same letter within a row are not significantly different (Fisher's least significant difference, P≤ 0.05).

CONCLUSION

Our study allowed characterizing the impact of the four certified Duras N clones on grape quality and rotundone in wines. While vintage × clone interactions were observed for most of the classical analytical variables, rotundone concentrations were higher in 2013 and 2014 in clones 554 and 654 in comparison to clone 555, with clone 627 showing intermediate level. Currently, clones 554 and 555 are the most available clones in the nurseries. Thus, winegrowers who are willing to promote rotundone concentration in their red wines from Duras N should consider planting clone 554. In 2014, differences in PM severity were reported between the four clones and PM infection seems to be a key variable to explain the extremely high rotundone concentrations found in wines from the 2014 vintage. In 2014, a positive correlation ($r^2 = 0.58$) was reported on the experimental site between rotundone in wines and PM severity on grapes, which suggests that grapevine defence response to PM could enhance rotundone production in berries. If the differences in Duras N clone susceptibility to PM were to be confirmed, then our results suggest that the mechanisms involved are more likely to be related to constitutive factors rather than induced factors. Our findings also suggest that clonal differences in susceptibility to biotic stress such as PM might help explain the differences observed in rotundone between the clones.

Table 4 - Characterization of the 2013 and 2014 vintages thanks to several climatic indexes and cumulative rainfall calculated over the whole calendar year, the budburst-veraison and veraison-harvest periods.

	Huglin index (IH)	Cool night index (FNv-r)	Mean air temperature (Tv-r)	Maximal air temperature (Txv-r)	Thermal amplitude (Av-r)	Cumulative rainfall (mm)		
						01/01 - 31/12	Budburst - veraison	Veraison - harvest
2013	1901	12.3	17.8	24.7	12.4	700	313	43
2014	2019	13.3	19.2	26.1	12.8	782	347	48

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