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May peppery wines be the spice of life? A review of research on the 'pepper' aroma and the sesquiterpenoid rotundone

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ABSTRACT

Rotundone is the main aroma compound responsible for peppery notes in wines. Since its discovery in 2008, this potent and fascinating odorant has been the subject of many research studies worldwide. The aim of this review is to summarize these works. Rotundone is a rather ubiquitous molecule that has been detected in many grape varieties. As with other sesquiterpenes, the proposed biosynthetic scheme for rotundone involves both the methylerythritol phosphate (MEP) and the mevalonate (MVA) pathways. The production of the compound by the plant, which could be a response to biotic stress, is also affected by abiotic factors. In most cases, studies showed that rotundone was neutrally or positively perceived by consumers, which makes the peppery note a desirable character in vine. Practical ways were identified to modulate rotundone levels in wines using viticultural and enological techniques. Climate change, through the expected alteration of precipitation regime and a rise in temperature over the grape maturation period, should have a depreciative impact on rotundone accumulation. Adaptation strategies in this context, together with other perspectives of research, are discussed in concluding this review.

KEYWORDS

peppery aroma, rotundone, occurence, biosynthesis, consumer acceptance, modulation techniques

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INTRODUCTION

The aromatic component of a wine is a key element in its appreciation by consumers, as the olfactory assessment takes place just after the visual examination, which is seldom discriminatory. Aromas are also perceived in the palate through the retronasal olfaction as the oral and nasal cavities are connected to each other. Thus, the persistence of the aromas in the mouth, which is one of the last sensory memories left by the wine after swallowing or spitting it out, also contributes to the overall appreciation. With more than 800 compounds according to Robinson *et al.* (2014), or more than 1000 according to other authors (IFV 2013), wine is one of the food products richest in volatile molecules. All these volatile compounds do not necessarily have an odorant property and odorants are often characterized by a moderate molecular weight, low polarity, good water solubility, high vapor pressure and a lipophilic character (Meierhenrich *et al.*, 2005). The bouquet of wine may involve 80 molecules (Dagan, 2006), originating from grapes for the varietal aroma fraction or synthesized during pre-fermentation operations, alcoholic and malolactic fermentations and aging. Most contributors to the varietal aroma of white wines have been widely studied, for example the monoterpenols responsible for floral notes in Muscat-like varieties (Terrier and Boidron, 1972), or the varietal thiols imparting passion fruit and grapefruit aroma in Sauvignon wines (Tominaga *et al.*, 1998). Until 2008, knowledge of the aromatic compounds that account for the varietal character of red wines, especially free compounds directly extracted from grapes without being released from a precursor, was limited to methoxypyrazines, responsible for undesirable green notes in wines (Lacey *et al.*, 1991). The discovery in an Australian Syrah wine of rotundone, a sesquiterpene responsible for peppery notes (Wood *et al.*, 2008), made a major contribution to this knowledge. Despite its sensory significance, this molecule has remained undetected for a long time in wine and other food products, including pepper. Rotundone has been the subject of much research since its discovery, notwithstanding the lack of availability of commercial standards, a constraint that involves achieving its organic synthesis. This review article addresses the state of the literature on 'pepper' aroma and especially on rotundone. In light of the factors affecting rotundone concentration, adaptation strategies are proposed that will assist in producing wines with consistent rotundone levels in the context of climate change.

1. Generalities on pepper and pepper aroma

Black pepper (*Piper nigrum*), native to the Southeast coast of India, is one of the most widely used spices in the world, with sensory qualities that have been recognized for millennia. It was the main spice imported by the Romans and during the siege of Rome at the end of the Western Roman Empire, the Visigoths claimed 3000 pounds of pepper as a ransom payment (Bury, 1889). In the Middle Ages, pepper was an essential commodity in the European economy, as tolls were paid in pepper in the South of France, Germany, Italy and Poland (Schoell, 1834). This strong demand for pepper led to the trade development toward the East. In search of alternatives to the long and perilous spice routes, Vasco de Gamma crossed the Cape of Good Hope and Christopher Columbus discovered America (Demolins, 1901). Today Vietnam, India, Brazil and Indonesia are the main pepper producers in the world.

Green, black, gray and white pepper are different forms of pepper products on the market (Variyar *et al.*, 1988). Green and black peppercorns are produced using green berries harvested before full ripeness is reached. The green peppercorn berries are usually boiled, treated with sulfur dioxide and frozen or dried to preserve their color. Black pepper, the most noble pepper, is obtained by fermenting these green berries. Red pepper is made of red-orange berries harvested at full maturity. White pepper is traditionally obtained from red pepper that has been immersed in water for several days to eliminate the pericarp. The powdered gray pepper comes from crushing lower-quality black pepper berries.

Other species from the genus *Piper* are also used to produce pepper. The berries of *P. longum* produce long pepper, *P. cubeba* gives cubeb or tailed pepper and *P. borbonense* is used to make Voatsiperifery pepper. By analogy, other spices misuse the vernacular name of 'pepper'. These berries are obtained from species with different botanical characteristics and that do not belong to the genus *Piper*. Sichuan pepper (*Zanthoxylum piperitum*) and pink pepper (*Schinus terebinthifolius*) are among the best-known of these 'fake' peppers. Because of their distinctive peppery flavor some plant species are sometimes also described as 'peppery', such as peppermint (*Mentha piperita*), which results from a spontaneous hybridization between water mint (*Mentha aquatica*) and spearmint (*Mentha spicata*).

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Piperine and its stereoisomer chavicine are the main active compounds of pepper (De Cleyn and Verzele, 1972). These cyclic secondary amines belonging to the piperidine family are the alkaloids responsible for the pungent character of pepper. This sensation relies on the trigeminal nerve and is independent of the chemical receptors located in the buccal cavity responsible for the five basic tastes (sweet, salty, sour, bitter and umami). This nerve provides both a sensory function and the motor function for biting, chewing and swallowing. In addition to the pungent sensation, it is also involved in the sensations of touch, pain and freshness. During the storage of peppercorns, chavicine is slowly transformed into piperine, which leads to a decrease in the pungent character (Kozukue *et al.*, 2007). Pepper also contains many volatile compounds, mostly monoterpenes and sesquiterpenes (SQT) (Table 1). Before the discovery of rotundone (Wood *et al.*, 2008), Jagella and Grosch (1999) concluded that *α*-pinene, *β*-pinene, myrcene, *α*-phyllandrene, limonene, linalol, 2-methylpropanal, 2- and 3-methylbutanal, butyric acid and 3-methylbutyric acid, were the main odorants of *Piper nigrum*. Other research highlighted that the volatile composition of black and white peppers differed slightly (Chen *et al.*, 2011).

The spicy or peppery character has always been associated with the world of wine. Spices were commonly used at the time of the Roman Empire to elaborate the *conditum paradoxum*. The recipe for this spicy wine is given in the book *De Re Coquinaria*, written in the 1st century AD by the gourmet Apicius. A wine, usually of poor quality, was boiled with honey and then supplemented with spices (pepper, mastic, nard, laurel and saffron) and dried fruits (dates). The product obtained was diluted with quality wine and hot coals were added to promote conservation. This tradition also existed in the Middle Ages with *hypocras* which was a beverage made from wine, honey, spices and herbs. Nowadays, the spicy dimension has an important place in the aromatic description of wine. The work led by Noble *et al.* (1987) classified the aroma of wine into 12 aromatic families, including a spicy family. These spicy notes (licorice/anize, black pepper and clove) imply a varietal origin clearly dissociable from the woody notes.

TABLE 1. Main volatile compounds identified in pepper (*Piper nigrum*).

Monoterpenes	Sesquiterpenes	Other compounds
α-Thujene	*α*-Copanene	Eugenol
α-Pinene	*β*-Caryophyllene	Methyleugenol
Sabinene	*β*-Bisabolene	Myristicine
β-Pinene	Caryophyllene oxide	Safrole
1,8-Cineole	*α*-Cis-Bergamotene	Benzaldehyde
Limonene	*α*-Trans-Bergamotene	Trans-Anethole
Linalol	*B*-Bisabolene	Piperonal
Camphene	*δ*-Cadinene	2-Methylpropanal
δ^3-Carene	*γ*-Cadinene	2-Methylbutanal
Myrcene	Calamenene	3-Methylbutanal
Cis-Ocimene	*α*-Copaene	m-Methyl acetopherone
α -Phellandrene	*α*-Cubenene	p-Methyl acetopherone
β-Phellandrene	*β*-Cubenene	n-Butyropherone
α-Tepinolene	α -Curcumene	Phenylacetic acid
γ-Terpinene	*β*-Elmenes	Cinnamic acid
Terpinolene	*α*-Selinenes	Piperonic acid
	Rotundone	Butyric acid
		3-methylbutyric acid

Adapted from Jagella et Grosch (1999), Plessi *et al.* (2002), Wood *et al.* (2008) and Chen *et al.* (2011)

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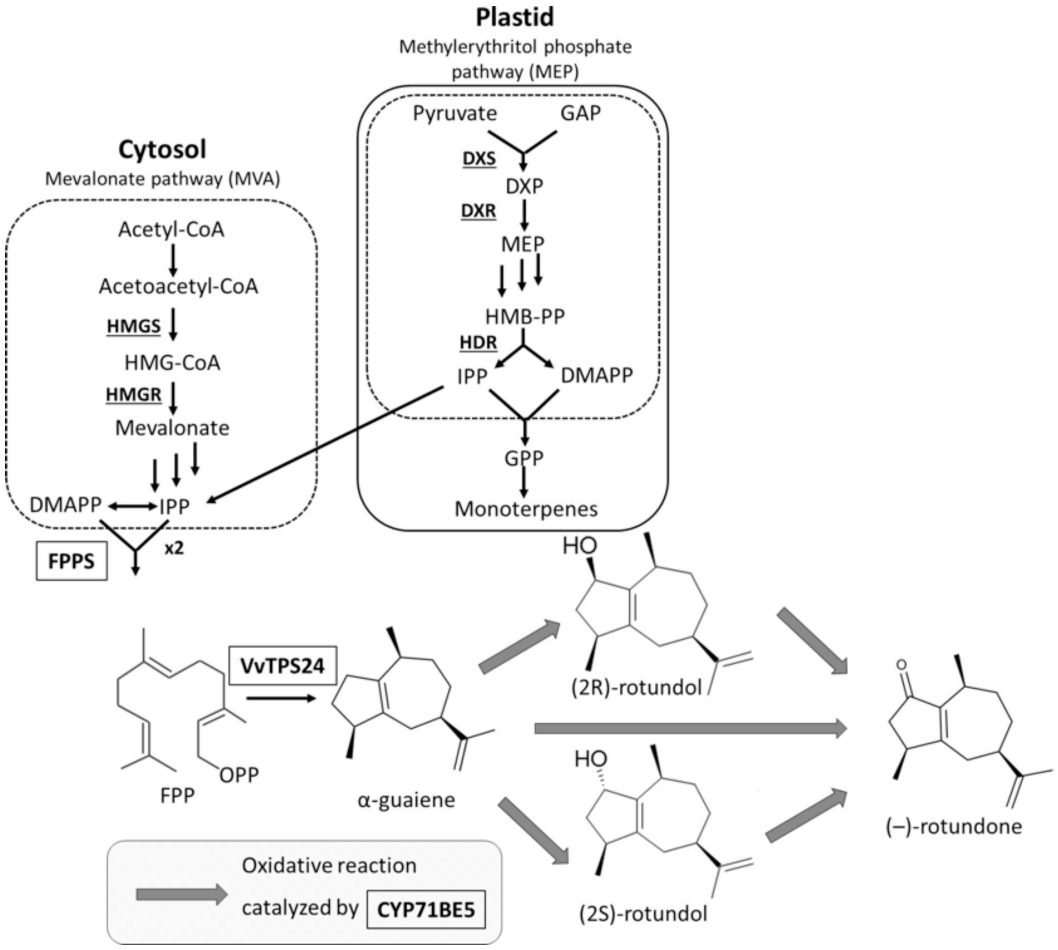


FIGURE 1. Biosynthetic pathway for (−)-rotundone proposed by Takase *et al.* (2016a).
GAP, glyceraldehyde 3-phosphate; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; DXP, 1-deoxy-D-xylulose-5-phophate; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; MEP, 2-C-methyl-D-erythritol-4-phosphate; HMB-PP, HDR, (E)-4-hydroxy-3-methylbut-2-enyl diphosphate reductase; (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; HMGS, 3-hydroxy-3-methylglutaryl-CoA synthase, HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; FPPS, farnesyl diphosphate synthase; FPP, farnesyl diphosphate; OPP, diphosphate in the structure of FPP; CYP71BE5, cytochrome P450 enzyme which can transform α-guaiene to (−)-rotundone

2. Toward the discovery of rotundone, the pepper aroma compound

It is well known that some grape varieties can develop spicy or peppery notes. Notably, this is the case for red wines made from international cultivars, such as Syrah grown in the northern Rhône Valley or in the state of Victoria in Australia, or Gamay from the Massif Central and some Beaujolais crus from France or Switzerland. Other French regional grape varieties such as Duras, Mondeuse or Mourvèdre planted within the Protected Designation of Origins Gaillac, Savoie and Bandol, respectively, may also exhibit particularly pronounced peppery notes. These notes often appear more pronounced in fresh vintages or in cool areas and there are 'peppery' vineyards that consistently produce 'peppery' wines (Iland and Gago, 1995). Despite the importance of Syrah in France (grown on more than 65,592 ha in 2011 according to http://plantgrape.plantnet-project.org), the aroma of this variety has received little attention from the French researchers. The most consistent work was conducted on Syrah wines produced under hot climatic conditions. This work highlighted that glycosidic precursors responsible for fruity and black olive aromas were the main contributors to the aromatic character of Syrah wines from the southern Rhône Valley (Segurel *et al.*, 2004; Segurel, 2005). These observations are consistent with other studies held 15 years earlier on the role of these precursors in the earthy, tobacco/cigar notes of Syrah wines (Abbott *et al.*, 1991).

The earlier research on the molecule responsible for the pepper character in wine was conducted in Australia. As part of a specialization thesis, Brightman (2000) studied the volatile composition of Syrah bunches using gas chromatography (GC) coupled with olfactometry (O) and mass spectrometry (MS). This work was unable to identify a single spectral zone associated with peppery or spicy notes. A few years later, Parker *et al.* (2007) used an untargeted metabolomic approach. Samples of Syrah bunches, whose intensity of peppery character was assessed by an expert panel, were analyzed using headspace and GC-MS methods. Multivariate analysis of 13,000 mass spectra per bunch identified α-ylangene, a sesquiterpene, as the best marker/predictor of these peppery notes. This work also highlighted that this marker, which could not be detected in the wines, did not have any aromatic impact.

It was only in 2008 that the main compound responsible for the peppery notes of red wines was formally identified in an Australian Syrah wine (Wood *et al.*, 2008). This molecule is an oxygenated sesquiterpene (SQT) of molecular formula $C_{15}H_{22}0$, (3S, 5R, 8S) -5-isopropenyl-3,8-dimethyl-3,4,5,6,7,8-hexahydro-1 (2H) -azulenone, better known as rotundone (Figure 1). One of the most surprising discoveries is that this compound had never been detected in pepper despite the substantial number of publications dealing with the volatile composition of *P. nigrum* (Jagella and Grosch, 1999; Plessi *et al.*, 2002). Until then, the distinctive aromatic character of pepper was attributed to the complex interaction of several odorants (Jagella and Grosch, 1999). The fact that rotundone was identified so late deserves some explanation. First, the molecule is present at concentration levels in the ng/L range and is likely to co-elute with other SQT, which complicates its detection. In addition, rotundone has a strong affinity with the stationary phase in GC and a high retention time. This means that rotundone appears late during GC-O sessions, at a time when judges are usually less attentive because no molecule of interest is expected (Herderich *et al.*, 2012). Finally, the fact that a specific anosmia has been reported for this compound (Wood *et al.*, 2008) must have complicated its detection.

3. Analysis methods of rotundone in wines and grapes

Since 2008, several research groups around the world have developed analytical methods for the quantitative determination of rotundone in grapes and wines. These methods are based on SPE-GC-MS (Culleré *et al.*, 2016), SPE-SPME-GC-MS (Siebert *et al.*, 2008; Mattivi *et al.*, 2011; Geffroy *et al.*, 2014; Nauer *et al.*, 2018) or SBSE-GC-MS (Takase *et al.*, 2015, Escudero *et al.*, 2019) and mostly on stable isotope dilution analysis (SIDA). SIDA is a quantification method for which the internal standard is an analog of the analyte labeled with stable isotopes. As the physicochemical properties of the standard and the analyte are very similar, biases related to preparation and injection can be avoided. In the case of rotundone analysis, *d5*-rotundone, in which five hydrogen atoms have been replaced by five deuterium atoms, is used as an internal standard. In these quantification methods, sample preparation essential for increasing the trace amount of rotundone (ng/L) is achieved by solid phase extraction (SPE) and/or solid phase microextraction (SPME) or stir bar sorptive extraction (SBSE). SPE is a preparation method in which compounds in solution in a liquid phase are selectively adsorbed onto a solid phase, depending on their physicochemical properties (Simpson, 2000). The stationary phase is contained in a cartridge in the form of a syringe often made of a polymeric resin. Then, the analytes are recovered by elution, usually using dichloromethane. SPME is a more recent SPE technique (Arthur and Pawliszyn, 1990) and is easy to implement, powerful and does not require any solvent. This method relies on the use of a silica or molten glass fiber disposed inside a removable hollow needle. A stationary phase, generally a polymer film determining the extraction capacity, is grafted onto this fiber. SBSE is a variant of SPME introduced in the late 1990s (Baltussen *et al.*, 1999). The extraction support is a magnetic bar coated with a polymer acting as an absorbent resin, generally polydimethylsiloxane (PDMS). This technique, which is slightly harder to implement than SPME, makes it possible to increase the quantity of polymer, to decrease the phase ratio and thus to increase the theoretical recovery.

In order to limit co-elution phenomena and optimize separation, two-dimensional gas chromatography (GC-GC) has also been used for the determination of rotundone (Geffroy *et al.*, 2014, Takase *et al.*, 2015; Escudero *et al.*, 2019). This approach consists in coupling two capillary columns with distinct polarity properties. The second column allows the separation of a poorly resolved part of the first chromatogram. The transfer of the fraction of the chromatogram to the second column is called ‘heart cutting’.

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Several solvents were used for the extraction and quantification of rotundone in grapes: ethanol (Siebert *et al.*, 2008); acetone (Mattivi *et al.*, 2011); and n-pentane or acetate of ethyl (Takase *et al.*, 2015). The maximum rotundone levels were found in Vespolina grapes (6.13 µg/kg) and in Schioppettino wines (561 ng/L) (Table 2).

4. Occurrence of rotundone in plants, wines and spirits

Rotundone owes its name to the plant species in which it was first discovered, *Cyperus rotundus,* more commonly known as the Asian nutgrass (Kapadia *et al.*, 1967). The molecule has also been detected in many Mediterranean aromatic species (Wood *et al.*, 2008), such as rosemary (*Rosmarinus officinalis*), thyme (*Thymus vulgaris*), marjoram (*Origanum majorana*), basil (*Ocimum basilicum*) and in *Atriplex cinerea*, a persistent shrub found in Australia. Rotundone has been shown to significantly contribute to the aroma of roasted chicory (Wu and Cadwallader, 2019) and many fruit juices such as grapefruit, orange, apple and mango (Nakanishi *et al.*, 2017a, Nakanishi *et al.*, 2017b). In these fruit juices, 3-epi-rotundone, a novel stereoisomer of rotundone has been identified and characterized (Nakanishi *et al.* 2017c). This stereoisomer has a much higher odor threshold determined at 19,100 ng/kg. Recently, two hydroxylated forms of rotundone, (+)5,14-dihydroxyrotundone-9-(2'-methybut-2'-enoate) and (-)5,14-dihydroxyrotundane-9-benzoate, were isolated from the roots of *Croton hirtus*, a species of *Euphorbiaceae* distributed in Mexico and the Caribbean (Rosandy *et al.*, 2019).

Rotundone is a rather ubiquitous aroma compound that has been found in many grape varieties (Table 2). Researches carried out in Australia has shown rotundone at concentrations exceeding its perception threshold in Syrah, Durif (the Californian 'Petite Syrah' which results from the cross of Syrah and Peloursin), Mourvèdre and Graciano, a variety widely planted in the Spanish vineyard of La Rioja (Herderich *et al.,* 2012).

In Italy, rotundone has been found in Vespolina, Schioppetino and in Grüner Veltiner, a white cultivar widespread in the northeast of the country and in Austria (Caputi *et al.*, 2011; Nauer *et al.*, 2018). More recently, rotundone has been identified in wines made from Duras, Fer, Négrette and Prunelard from south-west France (Geffroy *et al.*, 2014, *Geffroy et al.*, 2019b; Geffroy, 2018) and from Gamay from cool climate vineyards (Culleré *et al.*, 2016; Geffroy *et al.*, 2016a). It has also been detected in Cot (Malbec), Abouriou and Castets (Maturana tinta) (Culleré *et al.*, 2016; Geffroy, 2018) and Mondeuse and Arani (Geffroy, 2018). Takase *et al.* (2015) showed that Japanese Syrah wines had remarkable levels of rotundone, while grapes from Koshu, Muscat Bailey-A, Cabernet-Sauvignon and Merlot contained negligible concentrations. Similarly, wines made from Tannat, Manseng noir and Touriga Nacional showed rotundone levels below the odor threshold of the compound (Geffroy, 2018).

The clone, which is defined by the International Office of Vine and Wine (OIV) as the certified vegetative descent of one vine chosen for its identity, its phenotypic characteristics and its sanitary condition, also has an impact on rotundone concentration in wines. In Australia, clone 2626 is renowned for producing spicy Syrah wines and this empirical knowledge was confirmed analytically. Indeed, this clone showed greater rotundone concentrations at harvest in comparison with clone 1127 (Siebert and Solomon, 2011). Clonal variations have also been described in Italy for Grüner Veltliner (Caputi *et al.*, 2011). In south-west France, higher rotundone concentrations were reported during two consecutive seasons in Duras wines made from clones 554 and 654, compared with 555 (Geffroy *et al.*, 2015a).

In addition to wine, rotundone has also been detected in many spirits (bourbon, tequila, rum, whiskey and brandy) and notably those aged in oak barrels (Genthner, 2014). Rotundone concentrations usually increase with the duration of aging.

5. Sensory aspects and appreciation of rotundone by consumers

Rotundone is an extremely potent aroma compound with an odor threshold of 8 ng/L in water and 16 ng/L in red wine (Wood *et al.*, 2008). A specific anosmia has been reported for this compound, as during the first sensory tests 20–25 % of the panelists were unable to detect rotundone in water, even at very high concentrations (>4000 ng/L). In another study conducted in France, anosmic respondents represented 31 % of the panelists (Geffroy *et al.*, 2017b). The molecule has also been described as one of the most impactful aroma compounds in wine (Ferreira, 2012). Its sensory contribution to the peppery character of cool Margaret River Syrah wines has been proved through aroma reconstitution studies (Mayr *et al.*, 2014). In this study, the omission of rotundone resulted in a lower rating for the 'pepper' attribute.

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TABLE 2. Rotundone concentration in grapes at harvest and in commercial (C) or experimental (E) single-variety wines.

Grape variety	Reference	Region from where the grapes/wines were sourced	Range of concentration	
			Grape (ng/kg)	Wine (ng/L)
Arani	Geffroy (2018)	Armenia	–	68 (C)
Arrat	Culleré et al. (2016)	South-west France	–	47 (E)
Abouriou	Culleré et al. (2016)	Côtes du Marmandais, France	–	58 (C)
Cabernet Franc	Logan (2015)	Hawke's Bay, New Zealand	243	–
Cabernet-Sauvignon	Takase *et al.* (2015)	Koshu, Japan	21	–
Castets (Maturana Tinta)	Culleré *et al.* (2016)	La Rioja, Spain	–	8-112 (C)
	Geffroy *et al.* (2017b)	La Rioja, Spain	–	50 (C)
Cot (Malbec)	Culleré et al. (2016)	Cahors, France	–	16 (E)
	Geffroy (2018)	Cahors, France	–	51 (E)
	Logan (2015)	Hawke's Bay, New Zealand	7	
Duras	Geffroy *et al.* (2014)	Gaillac, France	–	7-59 (E)
	Geffroy *et al.* (2015a)	Gaillac, France	–	60-390 (E)
	Culleré et al. (2016)	Gaillac, France	–	162 (E)
	Geffroy *et al.* (2016b)	Gaillac, France	–	75-197 (E)
	Geffroy *et al.* (2017a)	Gaillac, France		2-30 (E)
	Geffroy *et al.* (2019a)	Gaillac France	–	25-239 (E)
Durif	Herderich *et al.* (2012)	Several regions of Australia	–	2-35 (C)
Fer	Geffroy *et al.* (2019b)	Marcillac, France	–	7-69 (E)
Gamay	Culleré et al. (2016)	Central France	–	19-85 (C)
	Geffroy *et al.* (2016a)	Several regions of France	–	12-109
Graciano	Culleré et al. (2016)	La Rioja, Spain	–	< 0.6-17 (C)
	Herderich *et al.* (2012)	Canberra district, Australia	–	38 (C)
Grüner Veltliner	Mattivi *et al.* (2011)	Several regions of Austria	–	63-266 (C)
	Nauer *et al.* (2018)	Several regions of Austria	–	9-85 (C)
Koshu	Takase *et al.* (2015)	Nirasaki, Japan	60	–
Manseng noir	Geffroy (2018)	Saint Mont, France	–	4-13 (E)
Merlot	Takase *et al.* (2015)	Ueda, Japan	62	–
	Logan (2015)	Hawke's Bay, New Zealand	18	–
Mondeuse	Geffroy *et al.* (2017b)	Savoie, France	–	54-62 (C)
Mourvèdre	Culleré et al. (2016)	Unknown	–	11 (C)
	Geffroy *et al.* (2017b)	Bandol, France	–	32 (C)
Muscat Bailey-A	Takase *et al.* (2015)	Fuefuki, Japan	16	–
Négrette	Geffroy (2018)	Fronton, France		38 (E)
Noiret	Homich *et al.* (2017)	North-east USA	1280 -3450	118 -224 (E)
	Harner *et al.* (2019)	North-east USA	109 -1176	–
Pineau d'Aunis	Geffroy (2018)	Loire valley, France	–	66-200 (C)
Pinotage	Logan (2015)	Hawke's Bay, New Zealand	6	–
Pinot noir	Herderich *et al.* (2012)	Several regions of Australia	–	3-11 (C)
	Geffroy (2018)	Central France	–	38 (C)
Prunelard	Geffroy (2018)	Gaillac, France	–	85 (E)
Riesling	Herderich *et al.* (2012)	Frankland river, Australia	–	9 (C)
Sangiovese	Logan (2015)	Hawke's Bay, New Zealand	16	–
Sauvignon	Takase *et al.* (2015)	Ueda, Japan	27	–
	Logan (2015)	Hawke's Bay, New Zealand	n.d.	–
Schioppettino	Culleré et al. (2016)	Friuli, Italy	–	19-35 (C)
	Mattivi *et al.* (2011)	Friuli, Italy	–	457-561 (C)
Syrah	Wood *et al.* (2008)	Several regions of Australia	620	150 (C)
	Herderich *et al.* (2012)	Several regions of Australia	–	7-161 (C)
	Scarlett *et al.* (2014)	Grampians, Australia	73-1082	–
	Drew *et al.* (2016)	Adelaide Hills, Australia		2-29 (E)
	Zhang *et al.* (2015a)	Grampians, Australia	15-493	–
	Zhang *et al.* (2015b)	Grampians, Australia	–	2-116 (C)
	Logan (2015)	Hawke's Bay, New Zealand	13-300	17-90 (C)
	Takase *et al.* (2015)	Northern Rhône Valley	–	39-142 (C)
	Takase *et al.* (2015)	Several regions of Japan	1033 -2342	152 -232 (C)
	Culleré et al. (2016)	Southern France, Australia	–	2-18 (C)
Tardif	Geffroy (2018)	Saint Mont, France	–	88 -204 (E)
Touriga Nacional	Geffroy (2018)	Douro, Portugal	–	9 (C)
Tannat	Geffroy (2018)	Madiran, France	–	6-8 (E)
Vespolina	Mattivi *et al.* (2011)	Northern Italy	–	278 -560 (C)
	Caputi *et al.* (2011)	Northern Italy	1420 -6130	101 -503 (E)
Zinfandel	Logan (2015)	Hawke's Bay, New Zealand	30	–

n.d., non-detected; –, data not available

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Several research works highlighted a significant positive correlation between perceived pepper intensity at tasting and rotundone concentration in wine (Herderich *et al.*, 2012; Geffroy *et al.*, 2016a; Homich *et al.*, 2017).

Studies have been conducted to assess the appreciation of peppery notes by consumers. While comparing the preferences of Chinese and Australian consumers, Williamson *et al.* (2012) identified three distinct clusters of consumers for both populations. The main differences between countries concerned the number of panelists and the distribution within the different clusters. While two out of the three clusters preferred wines with sweet and red fruit notes, the third cluster particularly appreciated wines with great color intensity and intense black fruit and peppery characteristics. This finding is consistent with other work conducted in Australia on assessing consumer scores for a Merlot wine spiked with several aroma compounds (guaiacol, eucalyptol and rotundone) (Herderich *et al.*, 2012). Overall, rotundone addition was positively perceived by one-third of the consumers.

As part of a research project aiming at investigating the peppery typicality of Gamay wines from cool climate vineyards (Geffroy *et al.*, 2016a), four wines showing distinct sensory profiles (amylic, lactic, peppery and green) were selected for a consumer study. While no significant differences in terms of preference were identified between the wines, a strong opposition was noticed between French consumers preferring wines with amylic and peppery sensory profiles. It also emerged from this study that consumers appreciating peppery wines were generally those connoisseurs who pay more for a bottle of wine than the average consumer. Another study sought to establish, in a Duras wine, the consumer rejection threshold (CRT) for rotundone concentration at which the molecule becomes undesirable (Geffroy *et al.*, 2017b). No CRT could be determined and four clusters of French consumers were identified: a cluster composed of anosmic panelists; a cluster of mainly young panelists preferring the control wine without rotundone addition; a cluster preferring moderate levels of rotundone; and a cluster preferring wines with high rotundone concentrations. The response of consumers to rotundone appear complex but in most cases rotundone was perceived neutrally or positively, apart from by the young panelists. In this study, a 25 ng/L-rotundone addition induced a stimulus within the anosmic population, leading to the rejection of the spiked sample. This indicates that rotundone might induce a trigeminal stimulus. However, it remains unclear why no sensation was perceived by anosmic panelists at a higher rotundone concentration. It was shown that 2,4,6-trichloroanisole (TCA), a lypophilic compound, could attenuate olfactory transduction through partitioning into the lipid bilayer of plasma membranes (Takeuchi *et al.*, 2013). As rotundone is also a lipophilic compound (Caputi *et al.*, 2011), such molecular mechanisms of flavor reduction might also be involved.

6. Biosynthetic pathway, localization in berries and herbaceous organs

The rotundone biosynthetic pathway has not yet been completely elucidated. However as with other SQT, this non-glycosylated molecule could be synthesized in the plastid through the methylerythritol phosphate pathway (MEP) and/or in the cytosol via the mevalonate pathway (MVA), in response to herbivore attacks (May *et al.*, 2013). These two processes are mutually exclusive in most living organisms but coexist in plants. While monoterpenes are produced in grapevine leaves exclusively through the MEP pathway, the biosynthesis of SQT involved both pathways, with possible exports between chloroplast and cytoplasm (Hampel *et al.* 2005). Indeed, D'Onofrio *et al.* (2009) showed that ethyl jasmonate (MeJa) and jasmonic acid (JA) had the ability to stimulate SQT synthesis in cells of Cabernet Sauvignon and Riesling. The regulation of SQT synthesis is ensured by the genes of the *VvTPS* family (*Vitis vinifera* terpenoid synthase), of which 45 out of 89 are located on chromosome 18 (Martin *et al.*, 2010). As part of a program aiming at breeding new grape varieties that are resistant to cryptogamic diseases and that express peppery notes, an Austrian research team demonstrated that the heredity associated with rotundone production was supported by chromosomes 5 and 9 (Regner *et al.*, 2016). For rotundone, the biosynthetic pathway presented in Figure 1 has been proposed by Takase *et al.* (2016a).

α-guaiene has been identified as the precursor of rotundone (Huang *et al.*, 2014). In grapevines there are at least two alleles of the *VvTPS24* gene (Drew *et al.*, 2016) that encode distinct SQT synthetases (*VvGuaS* and *VvPNSeInt*) and the *VvGuaS* enzyme has the ability to convert farnesyl diphosphate (FPP) to α-guaiene (Figure 1) (Drew *et al.*, 2016). This polymorphism could explain why rotundone production only occurs in the berries of some grape varieties. Rotundone can be synthesized

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from α-guaiene by simple oxidation (Huang *et al.*, 2014; Huang *et al.*, 2015) or enzymatically in the grapes using an α-guaiene 2-oxidase (CYP71BE5) belonging to the cytochrome P450 family (Figure 1) (Takase *et al.*, 2016b). The same authors highlighted that transcription levels of CYP71BE5 were greater both in berry skin than in the flesh and in Syrah than in Merlot. Other work has shown that the expression of the *VvTPS24* and *CYP71BE5* genes did not differ between two vineyards of Syrah exposed to different environmental conditions and exhibiting distinct rotundone production levels (Takase *et al.*, 2016a). In this study, the main factor that allowed discrimination between the two vineyards was the level of transcription of the FPP synthetase-associated gene (FPPS). The same authors also reported different levels of expression for this gene between Syrah and Merlot. The regulation of rotundone synthesis in berries seems complex and is dependent on the *VvTPS24*, *CYP71BE5* and *FPPS* genes. If these three genes are likely to explain differences in genotype predisposition, the expression of the *FPPS* gene could also be regulated by environmental conditions.

Rotundone synthesis exclusively occurs in grape skins. It has been shown that the compound is absent from the flesh and seeds (Caputi *et al.*, 2011; Siebert and Solomon, 2011), but has been detected in leaves and stems, which suggests that rotundone concentration in red wines could be influenced by the presence of these herbaceous organs within the crop (Capone *et al.*, 2012). Other studies have shown that the molecule was also present in flower buds and that petioles, pedicels and shoots contained larger rotundone concentrations than those measured in berries (Zhang *et al.*, 2016a). However, in the same study these authors excluded the hypothesis of rotundone translocation from these organs to the berries through the phloem. Geffroy *et al.* (2016b) came to the same conclusion, as cutting the fruit-bearing cane 18 days before harvest did not stop rotundone accumulation. This *in situ* production is consistent with other works highlighting that the modulation of source-sink relationship did not significantly impact rotundone levels in wines (Geffroy *et al.*, 2014). Indeed, rotundone concentration is not affected by grape thinning and crop load (Geffroy *et al.*, 2014).

7. Kinetics of accumulation

Contrasting results have been obtained for the kinetics of rotundone accumulation, from inflorescence to harvest. While rotundone is detected at high concentrations prior to veraison in floral caps on Syrah (Zhang *et al.*, 2016a), the molecule remains undetectable on Noiret at bunch closure (Homich *et al.*, 2017). While Zhang *et al.* (2016a) and Luo *et al.* (2019) reported a U-shaped accumulation pattern for rotundone, Homich *et al.* (2017) showed that the accumulation only started from veraison. However, there is a consensus for the last few weeks before maturity, which are characterized by a fast accumulation of the molecule in berries (Caputi *et al.*, 2011; Siebert and Solomon, 2011; Geffroy *et al.*, 2014; Logan, 2015). In south-west France, concentrations reached a plateau from 44 days after mid-veraison (Geffroy *et al.*, 2014). Thus, the date of harvest appears to be a significant lever to control rotundone level in wines. In Fer grapes from the PDO Marcillac, a 7-day delay in harvest resulted in a two-fold increase in rotundone (Geffroy *et al.*, 2019b). Trials were conducted in Australia to delay maturity by spraying a 50-ppm solution of naphthalene acetic acid, a synthetic auxin (Davies *et al.*, 2015). By delaying the maturity by 23 days, this practice proved to be beneficial for improving the rotundone concentration of the wines. Other studies have modeled the kinetics of accumulation of rotundone in grapes from veraison (Zhang *et al.*, 2015b). The best model looks like a sigmoid whose parameters (slope at inflection point, plateau height, etc.) depend on the climatic characteristics of the vintage.

8. Biological function and impact of biotic environment

The biological function of rotundone is still unknown, but like other SQT, the molecule could be involved in defense mechanisms, particularly in response to insect attacks (D'Onofrio *et al.*, 2009). The exogenous foliar application of JA, a phytohormone involved in the defense mechanisms against herbivores, did not increase the concentration of rotundone in wines during a field trial (Geffroy *et al.*, 2014). According to these authors, it remains difficult to draw firm conclusions, as the effectiveness of the spraying is highly dependent on the conditions of applications and notably the penetration of the canopy. In the same way, the physical or chemical activity of herbivores on grape leaves had a very limited impact on rotundone production (Zhang *et al.*, 2016b). Other results might have been observed on bunches.

A positive correlation was found between the severity of powdery mildew (PM caused by *Erysiphe necator*) on bunches and the concentration

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of rotundone in wines, which indicates that PM might provoke a defense response leading to the production of rotundone (Geffroy *et al.*, 2015a). According to the same authors, the clonal difference in PM susceptibility could also explain the differences in rotundone levels among the four certified Duras clones. Bunch rot and acid rot do not induce the same defense reactions. On the contrary, while modeling rotundone concentration within several Duras blocks (Geffroy *et al.*, 2019a), gluconic acid, a secondary metabolite of *Botrytis cinerea*, was identified as a key variable negatively correlated with rotundone. Other works highlighted that acid rot (*Drosophila melanogaster* and/or *D. suzukii)* may not have an impact on rotundone biosynthesis (Geffroy *et al.*, 2016b).

9. Inter-vintage and intra-block variability

As with other grape-derived aroma compounds, rotundone is strongly impacted by the climatic characteristics of the vintage. Cool and wet vintages enhance rotundone accumulation and are particularly favorable for obtaining wines with greater rotundone levels (Caputi *et al.*, 2011; Zhang *et al.*, 2015b). The same Duras block, harvested at the same time point after mid-veraison and monitored for rotundone between 2008 and 2015 (Figure 2), shows a concentration ranging from 7 ng/L to 179 ng/L (Geffroy and Descôtes, 2017). Similarly, Zhang *et al.*, (2015b) reported variations from 2 ng/L to 116 ng/L between 15 vintages (from 1996 to 2014) for a wine made from the same vineyard located in the Australian Grampians wine region (Figure 2). For grapes, a 40-fold inter-vintage variation in rotundone concentration was also established (Bramley *et al.*, 2017).

It has been shown that rotundone distribution within the same vineyard block was spatially organized and that structural patterns were stable from one year to the next (Scarlett *et al.*, 2014; Bramley *et al.*, 2017). The observed variations are related to soil properties and topography (Scarlett *et al.*, 2014), grape microclimate and especially the percentage of hours above 25 °C (DH25) (Zhang *et al.*, 2015a), the cool night index (Geffroy *et al.*, 2019c) and $\delta^{13}C$ (Geffroy *et al.*, 2014), a proxy for water constraint experienced by the vine plant over the maturation period. These findings make it possible to organize differential harvesting, with the aim of producing with distinct levels of rotundone concentration from the same vineyard block wines.

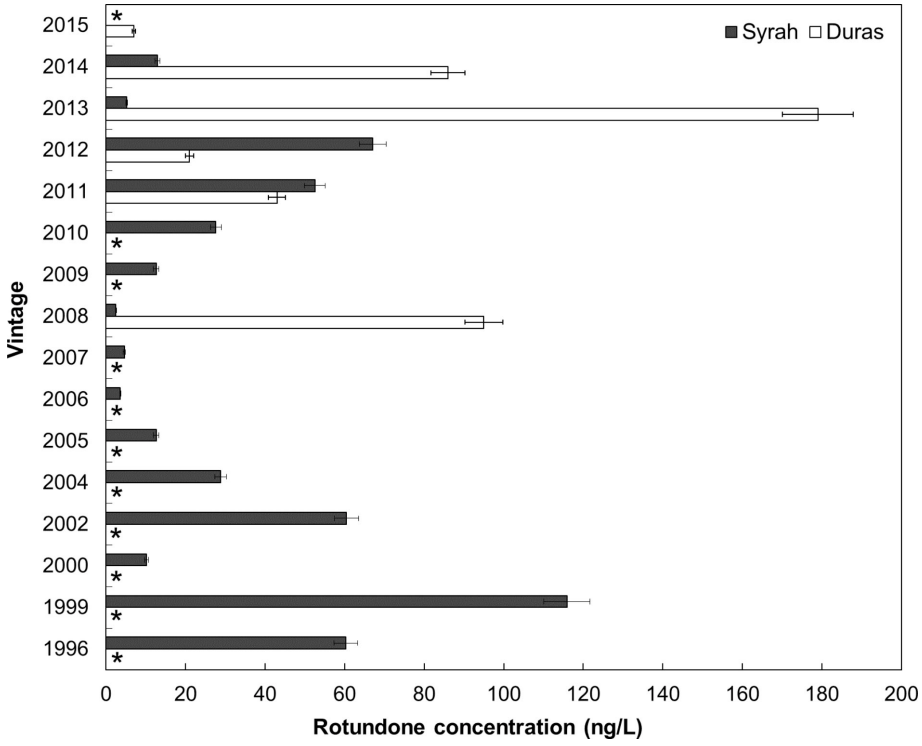


FIGURE 2. Impact of the vintage on rotundone concentration in Duras and Syrah wines made from two commercial vineyards located in the Gaillac (south-west France) and Grampians (Victoria, Australia) wine regions, respectively. Adapted from Geffroy and Descôtes (2017) and Zhang *et al.* (2015b). *, not determined. Error bars represent the 5 % uncertainty of the measurement.

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It has been demonstrated that trunk circumference (TC) could be used to approximate rotundone spatial distribution (Geffroy *et al.*, 2015b). Recent work has attempted to link soil microbial diversity with rotundone concentration in grapes (Vadakattu *et al.*, 2019). Without being able to prove the causality between the microbiome and the fruit composition, these authors have shown that, in areas favorable to high levels of rotundone, soils had a greater diversity of bacteria but a lower diversity of fungi.

10. Impact of defoliation, bunch surface temperature and exposure

Researchers have emphasized that berry temperatures above 25 °C (Zhang *et al.*, 2015a) or above 30 °C (Harner *et al.,* 2019) over the maturation period negatively affect rotundone accumulation. Beyond the maturation period, air temperature over the whole vine vegetative cycle, as reflected by the Huglin index or growing degree day (GDD), also appear to be a key variable to explain differences in rotundone concentrations between sites and vintages (Geffroy *et al.*, 2019a; Harner *et al.* 2019).

As a consequence, rotundone biosynthesis is in most cases negatively affected by defoliation. Indeed, defoliation performed on both sides of the row significantly penalized rotundone concentration in Duras wines from south-west France (Geffroy *et al.*, 2014) and in New Zealand defoliation led to a reduction in berry rotundone content (Logan, 2015).

However, contradictory results were obtained in north-east USA on Noiret, as exposure of the fruit had no effect on rotundone or led to an increase in the pepper aroma compound (Homich *et al.*, 2017). A similar trend was also observed on Fer produced in a temperate climate vineyard with very cool nights (Geffroy *et al.*, 2019b) which deserves further explanation. It is important to note that the most damaging impact of defoliation was observed when the technique was implemented on both sides of the row under warm climatic conditions (Geffroy *et al.*, 2014). It is known that bunch exposure to solar radiation can greatly increase berry surface temperature (Spayd *et al.*, 2002); we can assume that, in this warmer situation, leaf removal contributed to limiting, in exposed berries, rotundone production whose biosynthesis can be inhibited from 25 °C (Zhang *et al.* 2015a, Zhang *et al.*, 2015b). Under cooler climatic conditions (Homich *et al.* 2017; Geffroy *et al.* 2019b), it can be hypothesized that the rise in grape surface temperature was not enough to compromise rotundone biosynthesis.

It is also possible to assume that the physical injury caused by defoliation might have stimulated rotundone production through the MVA pathway, as discussed previously. However, this assumption seems implausible because the removal of lateral shoots did not produce the same effect on rotundone as defoliation (Geffroy *et al.*, 2019b). Furthermore, a significant gain in rotundone was observed when newly formed leaves were removed to maintain a good sun exposure until harvest (Homich *et al.*, 2017), which suggests that light and ultra-violet radiation might stimulate the production of rotundone. This hypothesis is consistent with other observations demonstrating a positive contribution of mean irradiation, hours of sunshine and cumulative solar exposure (CSEv) over the maturation period, to predict rotundone levels in Duras and Noiret wines (Geffroy *et al.*, 2019a; Harner *et al.* 2019). This is also in accordance with several studies emphasizing the light dependency of some sesquiterpene emission (Duhl *et al.*, 2008).

11. Impact of irrigation and water budget variables

Wines made from vines irrigated just before veraison have a greater rotundone concentration than those made from non-irrigated vines (Geffroy *et al.*, 2014; Geffroy *et al.*, 2016b). These findings are in accordance with other works highlighting an impact of cumulative rainfall (Geffroy *et al.*, 2019a) and water status (Geffroy *et al.*, 2014) on rotundone accumulation. Indeed, under the French context, the contribution of water budget variables appears to be greater than that of thermal variables, as over maturation in the hottest and rainiest vintages always mean higher levels of rotundone (Geffroy *et al.*, 2014; Geffroy *et al.*, 2016b; Geffroy *et al.*, 2019b). The importance of water supply was also emphasized in the north-east USA as calcium concentration in leaf petiole, a variable that correlated with $\delta^{13}C$, had a strong influence on rotundone accumulation (Harner *et al.*, 2019). In Australia, Zhang *et al.* (2015b) demonstrated that water balance explained inter-vintage differences in rotundone concentrations. However according to these authors, DH25 remains the main determinant of rotundone in wines. The fact that water budget variables did not have a larger contribution to rotundone models for Australian vineyards could be explained by a lower interannual coefficient of variation for these parameters, as vineyard blocks are usually

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irrigated. However, differences in topography and soil resistivity, some variables that explain variations in rotundone within a single Australian vineyard block (Scarlett *et al.* 2014), could lead to distinct level of vine water status. Vadakattu *et al.* (2019) recently proposed the hypothesis that soil microbiome could have a direct impact on berry rotundone concentration. We can also assume that variations in soil composition are responsible for differences in microbiome and vine water status and that this latter component may have an impact on rotundone production. It is also interesting to note that Geffroy *et al.* (2015b) showed rotundone correlated with TC, an indicator of the level of nitrogen and water constraints experienced by the vine since it was planted. As TC is likely to be temporally stable indicator over a few consecutive seasons, it is not surprising that rotundone spatial distribution patterns also exhibited a temporal stability in Australia between 2012 and 2015 (Bramley *et al.*, 2017).

However, it is not always possible to exclude that irrigation might have an indirect impact related to a cooler and shader bunch microclimate as a result of higher plant vigor. This hypothesis is unlikely as under the climatic conditions in south-west France water constraint is generally not limiting and vine vigor is often disconnected from water status (Geffroy *et al.*, 2019a). Measurements made on irrigated and non-irrigated vines grown in this viticultural area tends to strengthen this hypothesis, as irrigation had no impact on berry surface temperature (Geffroy *et al.,* 2016b).

Consequently, irrigation is more likely to have a direct effect on rotundone accumulation. By modifying the hormonal status of the berry, particularly the concentrations in indole 3-acetic acid, jasmonic acid, salicylic acid and abscisic acid (Niculcea *et al.*, 2014), irrigation could enhance the production of rotundone by stimulating the MVA pathway.

If irrigation appears to be an effective practice to promote rotundone accumulation in grapes, it could have a depreciative effect on wine phenolic compounds through dilution and limitation of biosynthesis (Ojeda *et al.*, 2002). A viticultural system combining pre-veraison irrigations equivalent to 60 mm of rainfall and 'Passerillage Eclaircissage sur Souche' (PES) was successfully investigated to enhance rotundone while mitigating this effect (Geffroy *et al.*, 2016b). The PES technique consists in cutting the fruit-bearing cane on a Guyot-trained vineyard two to three weeks prior to harvest. In comparison with the control, grapes and wines produced using this system showed a significant gain in grape sugar concentration (+3.0 °Brix) and in anthocyanins (+138 ppm), TPI (+15.4) and rotundone (+45 %) in the wine.

12. Impact of winemaking techniques and enological variables

Unlike other aroma compounds derived from odorless precursors or formed during fermentation, rotundone is directly extracted from berry skins during winemaking. Most of this compound is extracted between the second and fifth day of fermentation, when the yeast activity and the ethanol production are at a maximum (Siebert and Solomon, 2011). Around 10–12 % of the rotundone present in grapes is extracted during fermentation (Caputi *et al.*, 2011; Zhang *et al.*, 2017). The addition of ethanol or sugar during fermentation, to increase the alcohol content of the finished wine by 4 %, led to an extraction rate of 19 % (Zhang *et al.*, 2017).

Only 6 % of the grape rotundone is found in bottled wine (Caputi *et al.*, 2011). Indeed, significant rotundone losses are observed during racking, filtration and fining of the wine, likely in relation to its hydrophobic nature (predicted LogP = 4.98) and its likelihood to bind to other particles (Caputi *et al.*, 2011). LogP, also known as Log Kow, is a measure of the differential solubility of a molecule in octanol and in water. A positive and large value means that the molecule is more soluble in octanol than in water and therefore has a hydrophobic/ lipophilic character.

Given its localization in the grape pericarp, the final concentration of rotundone in wines is likely to be affected by the winemaking technique used. A study by Geffroy *et al.* (2017a) compared the impact of several winemaking techniques and fermentation variables on rotundone. None of the investigated techniques, including the addition of pectolytic enzymes during maceration or a cold pre-fermentation maceration performed at 4 °C for 72 h, made it possible to enhance rotundone in comparison with a control wine fermented at 25 °C for 8 days. On the contrary, a decrease of 20 % was observed for the semi-carbonic maceration treatment, for the wines fermented with *Saccharomyces uvarum* and when maceration was extended for 6 days after fermentation, indicating practical opportunities for reducing the pepper aroma in wine. We can suppose that the two latter treatments induced a greater rotundone absorption by the lees. The low concentrations of

rotundone observed for the thermovinified and the rosé wines reached 20 % and 13 % of that of the control wine, respectively, which can be explained by the pre-ferment removal of skins. This suggests that rotundone is not likely to have a large sensory contribution to the aroma of rosé wines, unless it is present in unusually high concentrations in sourced grapes.

It has also been demonstrated that rotundone was very stable in bottled wines even after several years of storage and was not 'scalped' by the closure (Herderich *et al.*, 2012).

13. What next?

Since the discovery of rotundone in 2008, information on this key and fascinating aroma compound has grown. Levers have been proposed to help winegrowers and winemakers modulate the rotundone levels of their wines. However, some aspects such as the molecular mechanisms involved in specific anosmia to rotundone or its biological function still deserve investigation. The hierarchy between abiotic and biotic factors affecting rotundone and particularly the influence of temperature, water supply and light, also remains unclear. All the trials referring to these factors were conducted under field conditions and studies on model plants in controlled conditions could shed new light. This model could be a microvine (Pellegrino *et al.*, 2019), a natural mutant that bears simultaneously, on the same plant, all the phenological stages of inflorescence development, or a fruiting cutting (Ollat *et al.*, 1998).

In the future, one of the main issues for researchers will be to provide the wine industry with sustainable strategies to enhance rotundone in the context of climate change. Climate change is already affecting wine production and induces a large variability in wine aroma composition between vintages (van Leeuwen and Darriet, 2016). In most cases, this may take the form of altered precipitation regimes and higher temperatures during maturation, which are conditions unfavorable to obtaining high levels of rotundone in wines. While irrigation and the spraying of synthetic auxin (Geffroy *et al.* 2014, 2016b; Davies *et al.*, 2015) have proved their effectiveness in enhancing rotundone in wines, these strategies are not sustainable in the long term due to the scarcity of water resources and the consumer demand for wines made without chemicals.

Adaptation to climate change is presently the subject of several researches and some strategies should be tested to enhance rotundone accumulation or limit its degradation. For example, the spraying of a white kaolin-based protective film could help reduce the grape surface temperature and limit heat stress (Shellie and King, 2013). Late pruning, a practice used more than 50 years ago (Antcliff *et al.*, 1957) and is increasingly popular (Friend and Trought, 2007; Gatti *et al.*, 2016), could shift the maturation period toward cooler conditions. However, as the phenology is only delayed by a few days, the expected impact of this practice on the average temperature during maturation should remain limited.

Plant material seems to be a more promising adaptation strategy. The rootstock, due to the vigor imparted to the scion and its impact on bunch microclimate (Koundouras *et al.*, 2008), is likely to affect rotundone levels. Indeed, preliminary work carried out on nine rootstocks showed that Fercal and 196-17 Castel tended to produce wines with enhanced rotundone concentrations (Olivier Geffroy, unpublished data, 2019). The scion transpiration is genetically regulated by the rootstock and quantitative trait loci (QTL) associated with these traits have been identified (Marguerit *et al.,* 2012). This paves the way for the development of programs aiming at breeding rootstocks adapted to drought. By enhancing vigor and limiting the level of water constraint, these rootstocks of the future should favor rotundone accumulation in grapes. The cultivar is another key lever of adaptation. Most of the research conducted worldwide on rotundone concerns early maturation grape varieties such as Syrah, Duras and Gamay. The maturation of such varieties generally occurs from late-July to mid-September in the northern hemisphere, under warm conditions not favorable to the production of rotundone. The identification of genotypes that can produce rotundone and ripen late (e.g. in October) should make it possible to manage the peppery potential by blending with more traditional cultivars. The example of Tardif, an almost extinct grape variety from south-west France recently registered in the French Catalogue Official, tends to reinforce this hypothesis. Wines made from Tardif express particularly high rotundone levels (Table 2), even during hot and dry vintages (Geffroy, 2018). Indeed, as its name suggests (Tardif means 'late' in French), its maturity occurs in mid-October, one month after that of the other 'peppery' varieties planted in the area.

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