Better late than never: *the formation of distinctive pepper aromas in cool-climate Shiraz*

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Many cool-climate Australian Shiraz wines exhibit a distinct 'black pepper' character which is caused by the very potent sesquiterpene aroma compound rotundone. In a recently completed study, AWRI and CSIRO researchers explored how the interplay of site, climatic conditions and harvest decisions influence the formation of this character in cool-climate Shiraz.

IN BRIEF

- There are only a few major flavour compounds in wine that originate directly from the grape berry and are not formed during fermentation or wine ageing.
- One of these is rotundone – the potent peppery aroma compound that is a feature of some cool-climate Shiraz styles.
- The odourless compound α-guaiene has been identified as the precursor to rotundone in grapes.
- Both α-guaiene and rotundone typically only form very late in the growing season and preferentially in seasons with cool conditions and later harvest dates.
- From a practical perspective, a late harvest date is key to maximising the chances of pepper character in wine.

INTRODUCTION

Australia has the world's second largest area of Shiraz vineyards, with the variety accounting for nearly 30% of Australia's vineyard area. Many cool-climate Australian Shiraz wines exhibit a distinctive yet variable 'black pepper' aroma and flavour, caused by the aroma compound rotundone. AWRI researchers identified rotundone as the compound responsible for black pepper aroma in 2008 (Wood *et al*. 2008) and, to date, it remains the only compound identified as contributing to this distinctive aroma in wine. Rotundone is very potent — detectable by the human nose at just 16ng/L in red wine. It can also be a polarising compound, as around 25% of wine consumers are 'blind' to it and cannot smell it even at very high concentrations. Because of this phenomenon, two people tasting the same Shiraz wine might perceive it very differently.

In contrast to most wine aroma compounds, which are formed during fermentation and ageing, rotundone is extracted directly from grapes into wine. Consequently, its concentration has potential to be influenced by both vineyard practices and seasonal conditions. The amount of rotundone in grapes is known to vary widely from year to year, across vineyards, and even within a single bunch of grapes (Bramley

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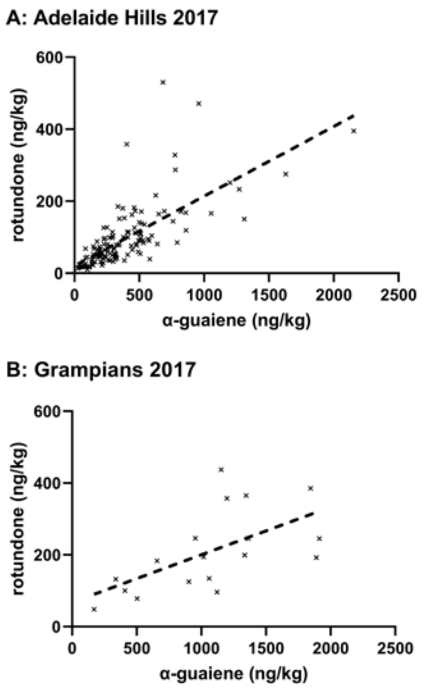

Figure 1. The relationship between α-guaiene and rotundone in grapes (ng/kg) from Shiraz vineyards in (A) the Adelaide Hills and (B) the Grampians, sampled at harvest maturity.

et al. 2017, Scarlett *et al*. 2014, Zhang *et al*. 2015). Given its elusive occurrence, this research aimed to investigate the origins of rotundone by identifying its precursor(s) and understanding the factors that contribute to its observed variability.

α-GUAIENE AS PRECURSOR OF ROTUNDONE IN GRAPES

The 2017 Australian growing season and vintage were generally marked by good rainfall and cool to mild temperatures — conditions likely to lead to elevated rotundone levels in Shiraz. This was confirmed through analysis of grapes collected from collaborators' vineyards in the Adelaide Hills and Grampians in April 2017, which found rotundone at average concentrations of 93ng/kg and 209ng/kg, respectively — around 10-fold higher than in a typical 'low pepper' season. With a relatively 'high pepper' season confirmed, investigations of a proposed precursor, α-guaiene, could be conducted by analysing large numbers of samples from the sites in the Grampians and Adelaide Hills. This compound (which is odourless) can convert to rotundone by simple aerial oxidation, which can be sped up in the laboratory by increased temperature or with light exposure (Huang *et al.* 2014, Siebert *et al*. 2016). In addition, an enzyme found in Shiraz grapes, α-guaiene 2-oxidase (CYP71BE5), is capable of oxidising the α-guaiene to rotundone (Takase *et al*. 2016). Specifically, the team wanted to learn whether α-guaiene is always present in Shiraz grapes and if its oxidation reaction might be responsible for determining the rotundone concentration in grapes and ultimately 'peppery' attributes in wine.

At both vineyard sites, grape rotundone concentrations were varied but closely corresponded with the concentrations of α-guaiene (Figure 1). In other words, grapes lacking α-guaiene did not contain rotundone either. Also, the conversion rates of α-guaiene to rotundone were remarkably similar between the two sites, as indicated by the regression lines (Figure 1 A, B).

The specific location of all the vines sampled in the Adelaide Hills vineyard had been previously georeferenced using GPS. Therefore, it was possible to determine the patterns of spatial variability across this vineyard for rotundone, α-guaiene and other volatile grape metabolites. The maps in Figure 2 show that the spatial distribution of grape rotundone at the Adelaide Hills site, planted with the Shiraz clones 2626 and 1127, was again closely matched with α-guaiene. This lends further support to the essential role of α-guaiene in the formation of rotundone and suggests that the 'peppery' character in wine could be optimised by controlling the levels of α-guaiene in grapes. It was also noted that the concentration bands of rotundone and α-guaiene crossed the border between the two clonal plantings, showing that environmental conditions had greater impact on α-guaiene and rotundone formation than any clonal effect (if indeed there was one).

Furthermore, analysis of grape samples collected during berry ripening across multiple trial sites and growing seasons established that the formation of elevated α-guaiene and rotundone were synchronised and typically only initiated very late during berry ripening (Barter *et al*. 2019). The importance of a delayed harvest date for achieving elevated rotundone concentrations in Shiraz grapes has also been independently observed in a recent study by researchers from CSIRO (Böttcher *et al*. 2021). In this study, harvest maturity was delayed by three to four weeks through the pre-veraison application of an auxin plant hormone, 1-naphthalene acetic acid (NAA). NAA-treated Shiraz grapes contained a 3.6-fold higher rotundone concentration than untreated grapes at the same harvest ripeness. This study highlights the potential for viticultural management tools that delay ripening to perhaps enhance the pepper character of Shiraz wine.

UNLOCKING THE SENSORY POTENTIAL OF GRAPE SESQUITERPENES: CAN WE TAKE ADVANTAGE OF THESE FLAVOUR PRECURSORS THROUGH WINEMAKING?

Sesquiterpenes have been known to be present in grape berries for many years (Schreier *et al*. 1976). During the study of α-guaiene in Shiraz grapes in this project, a further 13 sesquiterpene compounds were found, including α-ylangene, guaia-6,9-diene, γ-cadinene and δ-cadinene. Surprisingly though, only limited reports note the presence in wine of these commonly found grape sesquiterpenes. To gain a better understanding of typical sesquiterpene concentrations in wine, 14 commercial Shiraz wines from warm and cool climates across Australia (with vintages ranging from 2013 to 2018) were analysed for any sesquiterpenes present. The results showed only traces of a single sesquiterpene, α-muurolene, in these Shiraz wine samples. Model experiments established that the rather lipophilic (i.e. lipid or fat-loving) sesquiterpenes common in grapes are largely absent from wine (other than a trace of α-muurolene) because they are either not extracted at all into wine and/or can bind back to solids from grapes and are subsequently removed from juice or fermenting must. Rotundone differs from these other sesquiterpenes in that it is somewhat hydrophilic (i.e. more water-loving) due to its additional oxygen atom. Consequently, some rotundone present in grapes can be extracted into wine during fermentation (Caputi *et al*. 2011, Siebert and Solomon 2011). In other words, α-guaiene needs to be oxidised to form rotundone in grapes prior to crushing and fermentation for rotundone to make it into wine. Or more generally, sesquiterpene compounds can act as flavour precursors in grapes, but are not substrates for flavour formation during fermentation.

TYING IT ALL TOGETHER

Overall, the observations made in this work indicate that the onset of rotundone formation in grapes is favoured in seasons with cooler temperatures during ripening and coincides with the formation of a range of sesquiterpenes which are otherwise absent or present at very low concentrations in grapes. The odourless grape sesquiterpene α-guaiene acts as a direct precursor to rotundone and is converted in grapes by photochemical or enzymatic oxidation to rotundone, which is then extracted into wine during winemaking. What is yet to be established are the biological effector(s) required to trigger the rotundone formation in cool-climate Shiraz. Ultimately, such knowledge could enable more consistent vintage-to-vintage production of 'peppery' Shiraz grapes and wine. It could also potentially enable growing Shiraz grapes with 'cool-climate-like' flavour attributes in warmer regions, perhaps helping to mitigate risks from climate change, biodiversity loss or viticultural management decisions which might otherwise lead to unintended flavour consequences.

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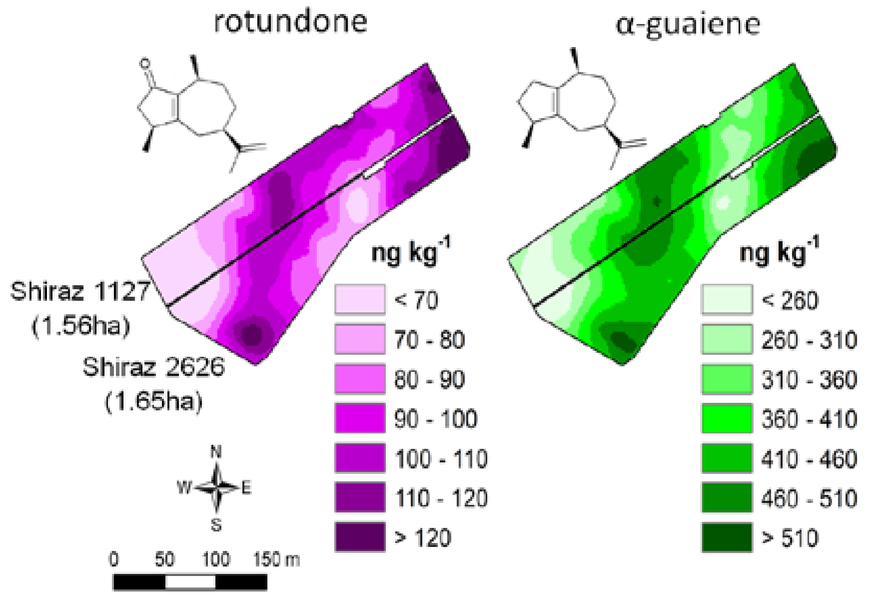

Figure 2. Variation of rotundone and α-guaiene concentrations in grape samples collected at maturity in 2017 from 141 vines across a 3.21 ha vineyard in the Adelaide Hills planted with two Shiraz clones, 1127 and 2626 (adapted from Barter *et al.* (2019)).

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