# Technical notes

## Mapping the origins of high rotundone and pepper flavour in Shiraz

Shiraz is Australia's most important red grape variety and the 'black pepper' flavour in some Shiraz wines, especially those from cooler regions, is very distinctive. Understanding the origin of the spicy/pepper aroma in wine has been a focus of attention at the AWRI for a number of years. The compound responsible was identified at the AWRI in 2007 as rotundone (Siebert et al. 2008, Wood et al. 2008). It is a sesquiterpene, related chemically to the monoterpenes found in floral varieties such as Riesling.

Rotundone is very potent with as little as 16 parts per trillion able to be detected by the human nose in red wine (Wood et al. 2008). Interestingly, approximately one in five people cannot perceive it, even at levels several orders of magnitude above the average odour detection threshold. This phenomenon of so-called 'specific anosmia' where certain sections of the population are highly insensitive to a particular compound, is not very common, although some other wine flavour compounds can show this behaviour, such as the raspberry/violet compound β-ionone. Thus two people tasting the same wine may perceive it very differently.

Results from early investigations into rotundone revealed that it is a grape-derived aroma compound, and is located within the grape skin (Siebert and Solomon 2011). It is quite water soluble and easily extracted from the skins even before fermentation, so the rotundone concentration rapidly increases in a must during the early stages of fermentation, and from then on remains stable and unaltered throughout the winemaking process (Siebert and Solomon 2011) and subsequent bottle ageing (Jeffery et al. 2009).

A study of commercial wines covering a range of varieties, vintages and regions showed that the majority that contained rotundone were in fact Shiraz (Jeffery et al. 2009). This survey also revealed that rotundone is more commonly found in wines from cooler climates and that levels can vary greatly year to year for the same vineyard, being more prevalent in cooler vintages. During grape ripening, rotundone concentration remains low until well after veraison before increasing late in the season (Siebert and Solomon 2011).

Since the discovery of rotundone as a key aroma compound, collaborations have been formed across Australia and the world to identify the factors that influence rotundone concentration in grape berries, whether environmental or related to viticultural management practices.

## Mapping variation across a single vineyard

In 2012, a collaboration with CSIRO and Mount Langi Ghiran sought to investigate the variation of rotundone concentration across a single vineyard, resulting in a 'pepper map' (Scarlett et al. 2014). This project, the first of its kind, compared spatial variation in rotundone to a number of other variables within the vineyard in order to pinpoint possible factors that may be influencing the biosynthesis of the compound.

The vineyard chosen for the 'mapping' was in the Grampians region of Victoria, which has a cooler climate, and is well known for producing 'peppery' wines. Grape samples were collected from 177 vines across a 6.1 ha commercial Shiraz vineyard along with data relating to topography, soil chemistry and vine vigour. The vineyard was a good candidate for the study with varying levels of vine vigour, variations in vineyard soils and a difference in elevation of 15.3 m from the lowest vine to the highest.

The variation in rotundone concentration was surprisingly large across the grape samples, with the highest being 15 times greater than the lowest. A plot of the concentrations across the vineyard revealed that the levels of rotundone were not random; a clear distribution pattern emerged (Figure 1). This plot was then overlayed with the distribution maps of the other vineyard measurements for comparison. Very high concentrations were seen in the south and south-eastern regions of the vineyard, whereas the grapes collected from the north-western section contained much less rotundone.

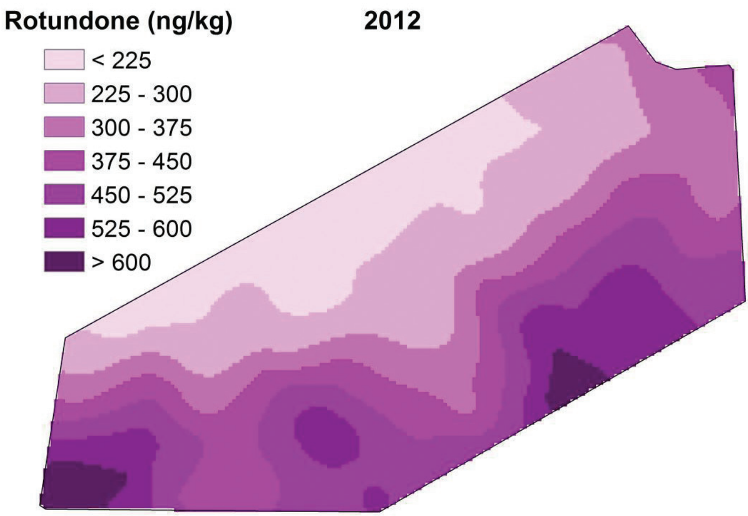


**Figure 1.** Spatial variation of rotundone concentration in berries across a 6.1 ha Shiraz vineyard in the Grampians region of Victoria (This figure was originally published in Scarlett et al. (2014) Aust. J. Grape Wine Res. 20(2): 214–222 and is reproduced with permission.)

In 2013 the experiment was repeated and samples from the same 177 positions were collected and analysed, and although it wasn't a very peppery vintage (with concentrations just one thirtieth of those from the 2012 vintage) the same pattern of spatial variation was evident.

From this study, there was no obvious correlation between rotundone concentration in the berries and vine vigour alone. A link between rotundone concentration and the bulk electrical soil conductivity ($EC_a$) was seen, showing a negative correlation between the two, as well as an effect from the slope or aspect within the vineyard. Overall, the results suggested that vine vigour and soil moisture played a less important role in the formation of rotundone than factors related to the vineyard's topography, such as aspect. This effect might be attributed to the associated differences in temperature and/or sunlight exposure because the vines subject to less light and/or cooler temperatures were found to have the highest concentration of rotundone.

## From within vineyard variation to within bunch variation

In a subsequent study Pangzhen Zhang from the University of Melbourne characterised in great detail the rotundone variation within grape bunches as part of his PhD research working in collaboration with the AWRI, Mount Langi Ghiran and Rathbone Wine Group (Zhang et al. 2013). For this extension of the 'pepper map', three different vineyard zones that consistently yield low, medium and high grape rotundone concentrations were identified based on the vine vigour, slope and soil characteristics as determined previously. In samples collected in 2012, grape rotundone concentrations averaged at approximately 225, 360 and 450 ng/kg in grapes from these three zones, while no notable differences were observed for the compositional parameters, pH and titratable acidity, anthocyanins and total phenolics, and total soluble solids (°Brix).

Furthermore, bunches of grapes harvested from each of three different vineyard zones were divided into four segments: the top sun-exposed, bottom sun-exposed, and the top not-exposed and bottom not-exposed. Notably, rotundone concentrations were significantly higher in the tops of the bunches rather than the bottom parts, and were also enhanced on the side shaded from direct sunlight compared to the exposed side. In all instances, the grapes situated at the top back of a bunch had the highest concentration of rotundone.

## Leaf removal lowers rotundone concentration

In a separate study, a French group of wine researchers working in collaboration with the AWRI examined the effect of viticultural practices on rotundone concentrations in the red variety, Duras (Geffroy et al. 2014). The different parameters studied included leaf removal, grape thinning, irrigation and the application of a potential rotundone elicitor, jasmonic acid. A research vineyard in the Gaillac region was selected, based on its reputation for the production of 'peppery' wines and the experiment was conducted over two years: 2011 and 2012. The grapes were collected from both sides of a row, and an equal amount from the top, middle and bottom of the bunches was sampled. Each treatment was performed in triplicate. Due to quarantine and logistical restrictions on the transfer of grape material from France to Adelaide, only the wines produced from these grapes were sent to the AWRI for analysis.

Leaf removal at veraison resulted in a large effect, with rotundone concentration in the resulting wine reduced by a massive 69% in 2011 and 52% in 2012, compared to the control where no leaves were removed from the fruit zone. Another experiment involving bunch thinning of 40% at mid-veraison showed little effect, as did the application of jasmonic acid.

**Summary**

The accumulation of rotundone at the top back of a bunch, the correlation between location of higher 'pepper' vines and the topography data from the 'pepper map', and the leaf removal study by Geffroy et al. all strongly support the hypothesis that sunlight exposure and/or heat have a negative effect on rotundone accumulation. This augments earlier observations where higher rotundone concentrations were typically reported for grapes from certain vineyards located in cooler regions, with cooler growing seasons further enhancing grape rotundone concentrations.

A recently published paper showed that a potential sesquiterpene precursor compound very similar in structure to rotundone (α-guaiene) can be readily converted to rotundone by simple oxidation through exposure to air in a laboratory (Huang et al. 2014). While this is a very interesting finding, it appears unlikely that autoxidation in air is a major mechanism for rotundone formation in grapes as in that case one would expect a greater concentration on the warmer, sun-exposed sides of grape bunches, because oxidation reactions are generally favoured by higher temperatures. Further studies are currently being performed at the AWRI investigating other possible mechanisms of rotundone formation. One such theory involves the presence of fungi such as *Botrytis*, and the associated activity of fungal oxidase enzymes, which would be aided through colder, less exposed and more shaded grapegrowing conditions. Such enzymes have been used in the fragrance and flavours industry for rotundone synthesis from *α*-guaiene, so it is quite possible that they may also be making a contribution to the formation of rotundone on grapes.

Overall, the results from recent experiments show that a few viticultural practices and environmental factors can have a major impact on rotundone concentration. Beyond grape ripeness and harvest date, key variables are sunlight and/or temperature in the bunch zone, which can be related to the topography of a vineyard; bunch exposure; soil properties; vine vigour; and water status. These results should allow grapegrowers and winemakers to obtain greater control over the style of Shiraz wine they produce.

## References


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