Patterns of within-vineyard spatial variation in the ‘pepper’ compound rotundone are temporally stable from year to year

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Short title: Spatial variation of rotundone is temporally stable

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Abstract

Background and Aims: Within-vineyard variation in the concentration of grape berry rotundone has been shown to be spatially structured and related to variation in the land (soil, topography) underlying the vineyard, although its temporal stability has not been evaluated. Our aim here was therefore to ascertain whether patterns of rotundone variation were stable from year to year. This is an important aspect in understanding and exploiting terroir on a vineyard-scale as it informs how targeted management might take advantage of the variation to the benefit of both grapegrowers and winemakers. It also facilitates targeted research into the biophysical factors that may be critical to the formation of rotundone in grapes.

Methods and Results: Immediately prior to the commercial harvest of a 6.1 ha vineyard block in the Grampians region of Victoria, 177 geo-referenced samples of grapes were collected in each of three seasons (2012, 2013 and 2015) and analysed for their rotundone concentration. The mean berry rotundone concentration varied 40-fold between seasons, yet spatial analysis of maps of rotundone variation produced for each year indicated that the patterns of spatial variation were stable across seasons.

Conclusions: Irrespective of the seasonal factors which affect the mean concentration of berry rotundone, variation in the land (soil, topography) underlying the vineyard is a consistent driver of within-vineyard variation in this important grape-derived flavour and aroma compound.

Significance of the Study: This work suggests that targeted vineyard management strategies, including selective harvesting, may be used to manipulate wine style – in this case, the pepperiness of cool climate Shiraz wines. It also suggests that further study of the relationships between the environment and berry composition is warranted in pursuit of a more robust understanding of terroir.

Keywords: *Precision Viticulture, Vitis vinifera (cv. Shiraz), terroir, wine flavour and aroma*

Introduction

The 'peppery' flavour and aroma of some Shiraz wines is a characteristic which is favourably regarded in some markets and considered evocative of the *terroir* of the vineyards from which those wines derive. Shiraz wines produced in the cool Grampians region of Victoria, for example, tend to have a higher concentration of rotundone, the compound responsible for 'pepperiness' (Siebert et al. 2008, Wood et al. 2008), than that of Shiraz wines produced in other Australian regions (Jeffery et al. 2009).

Recent research (Scarlett et al. 2014) demonstrated that, for a 6.1 ha Shiraz vineyard in the Grampians region, variation in the concentration of rotundone in grape berries was spatially structured at the within-vineyard scale and was related to variation in the land underlying the vineyard. There appeared to be a clear soil effect, but of particular note, was the apparent control exerted by topography through its effects on slope and aspect (Scarlett et al. 2014), with the inference being that variation in temperature and/or incident solar radiation was driving variation in the concentration of berry rotundone. More recently, Zhang et al. (2015a) working at the scale of the vineyard, vine and bunch in the same vineyard as that used in the Scarlett et al. (2014) study, have similarly suggested the likely importance of temperature and light interception on rotundone accumulation, with modelling indicating that a temperature in excess of 25°C in the bunch zone negatively affects rotundone concentration. Meanwhile, an analysis of wines produced from this vineyard over 15 vintages showed that the highest concentration of rotundone was found in wines from cool and wet seasons (Zhang et al. 2015b). Whilst other work has started to unravel the genetic elements of the biosynthesis of rotundone (Drew et al. 2015, Takase et al. 2015), the question of how this understanding might be applied to the advantage of grapegrowers and winemakers remains uncertain.

The results of Scarlett et al. (2014) were important in that they were the first demonstration of within-vineyard variation in a grape-derived wine flavour and aroma compound. They also presented a cautionary note for viticulturists and winemakers as previous work on vineyard variability had tended to support a negative relationship between both vine vigour and yield and fruit composition (Bramley and Hamilton 2004, 2007, Bramley et al. 2005, 2011a) when assessed using 'industry standard' indices (juice pH, titratable acidity, TSS, colour and phenolic substances). By contrast, variation in vine vigour appeared to be of limited, if any, importance as a driver of variation in berry rotundone (Scarlett et al. 2014). However, the Scarlett et al. (2014) work was a single season study. For their

results to form the basis for targeting future vineyard management, such as pruning (Geffroy et al. 2014), the use of berry ripeners (Davies et al. 2015), and/or decisions around harvest management and product streaming (Bramley et al. 2005, 2011b), a key question is whether or not the pattern of rotundone variation is stable across different growing seasons. In this paper, we describe work which sought to evaluate the temporal stability of patterns of spatial variation in berry rotundone concentration with a view to providing a more robust basis for consideration of how knowledge of rotundone variation might be used for the optimisation of grapegrowing and winemaking.

Methods and materials

Site details and sampling

This study was undertaken in the same 6.1 ha block at the Mt Langi Ghiran vineyard in the Grampians region of Victoria (37°S, 143°E) used by Scarlett et al. (2014) who provide details of the vineyard soils and climate. The block was planted to Shiraz on own roots in 1968 with row and vine spacings of 3.0 and 1.8 m; the vines are trained to a vertically shoot positioned, single wire arched cane system.

During the season which culminated in the 2012 vintage, 177 ‘target vines’ were identified within the block for subsequent sampling at vintage and were geo-referenced using a differentially corrected global positioning system (dGPS; accurate to approximately ± 50 cm in the x and y planes). The target vines were sampled on 5 April 2012. The bi-laterally pruned vines were notionally divided into ‘left’, ‘right’ and ‘central’ zones and a single bunch was randomly picked from each. The resulting three bunches per vine were placed in an inert food grade plastic bag, which in turn was placed in a chest of dry ice. Following sampling, the samples were stored at -20°C until required for laboratory analysis. Commercial harvest followed over the period 11–18 April, with selected areas harvested by hand following sensory assessment by the winemaker. Any remaining fruit was finally machine harvested on 18 April..

An identical procedure was followed in 2013 using the same 177 target vines which were sampled on 2 April. A similar approach to the 2012 commercial harvest was followed in 2013 with hand harvesting between 9–12 April followed by machine harvesting on 15 and 16 April.

Vintage 2012 turned out to be a year of high mean berry rotundone concentration, whereas in 2013 the values were low (see below). Pre-harvest sampling in 2014 (31 March) indicated that the mean concentration of berry rotundone was similar to that in 2013, and so, given limited resources, samples were not collected in 2014. Pre-harvest sampling was not conducted in 2015 as the vintage was much shorter than usual. Instead, all 177 target vines were sampled on 24 March and then a subset of nine samples from different areas across the vineyard was analysed. The values of rotundone found indicated that 2015 was sufficiently different to both 2012 and 2013 to give useful complementary data to those collected in 2012 and 2013. Therefore, the remaining 168 samples were analysed. Note that our objective here was to examine spatial variation in berry rotundone in contrasting seasons. Commercial hand harvest in 2015 occurred in the period 1–15 April with a machine follow up on 14 and 15 April.

Laboratory analysis

Rotundone was determined by the method of Siebert et al. (2008) with the modifications described by Scarlett et al. (2014). Thus, each frozen sample of three bunches was destemmed by hand, the berries thoroughly mixed and a 100 g subsample was gently blended using a Breville Wizz Stick hand-held blender. The blended berries were then centrifuged to separate the juice and solids; the juice was removed and retained. Ethanol (30 mL), water (30 mL) and d_5 -rotundone (51.6 ng in 100 μ L of ethanol) as internal standard (IS) was added to the solids, and the mixture was shaken (24 h), sonicated and centrifuged. The supernatant was added to the retained juice and filtered (1.0 μ m glass fibre) with the total extracted volume made up to 200 mL with water. The berry extract (200 mL) was subjected to solid-phase extraction [SPE; Siebert et al. (2008)] and the SPE residue was reconstituted in ethanol (0.5 mL) and water (9.0 mL). Analysis was by solid-phase microextraction (SPME) with multidimensional-GC-MS (Geffroy et al. 2013).

Geostatistical analysis and mapping

The methods of spatial analysis used here are also as described by Scarlett et al. (2014) with all maps produced in the present study interpolated onto the same grid of 2 m pixels derived from the block boundary as used by Scarlett et al. (2014). Thus, for each year of the study, maps were interpolated in

VESPER (Minasny et al. 2005) using global point kriging of log-transformed berry rotundone concentration values and a spherical variogram. This was followed by back-transformation of the kriged data (Webster and Oliver 2007).

A possible consequence of the kriging process when comparing multi-year data is that the effects of between-year differences in the variogram may impact on the assessment of differences/similarities in the patterns of variation in different years. One solution to this problem is to add an offset to the easting and/or northing position of data from different years, equal to a distance that is greater than the range of spatial dependence in any given year and to then pool the data across years for generation of a single ‘across years’ variogram which can then be used to krig the data for each year individually. Thus, in addition to kriging using season-specific variograms (as above), a fourth variogram was generated for all years combined after applying a 1000 m offset in both a northerly and easterly direction to the 2013 data, and a 2000 m offset in both directions to the 2015 data. Season-specific maps were then generated using this common ‘across-years’ variogram when kriging the data for the individual years. For the sake of completeness, in the present work, the 2012 data of Scarlett et al. (2014) were re-mapped along with those for 2013 and 2015.

Similarity in patterns of spatial variation in the mapped data between years was examined using *k*-means clustering in JMP (v.11.0.0, SAS Institute, Cary, NC, USA) with map display using the ArcGIS software suite (v10.2.2; ESRI, Redlands, CA, USA).

Results

Maps of the variation in the concentration of berry rotundone across the 4 years of the study (2012–2015; no map was produced in 2014) are shown in Figure 1. The spatial patterns in the maps bear strong similarities across years irrespective of whether the maps were interpolated using ‘unique’ season-specific variograms, or a common variogram derived from the data for all years. The temporal stability in patterns of rotundone concentration is even more striking when the maps are classified in terms of percentiles and a common legend applied to each (Figure 2), especially given the marked differences between the years in the mean rotundone concentration (Table 1). Thus, even with a 40-fold difference in mean rotundone concentration between 2012 and 2013, a 13-fold difference between 2012 and 2015, and a threefold difference between 2013 and 2015, the patterns of within-vineyard

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Comment [T H2]: Figure 2

Comment [T H3]: Table 1

variation in concentration of berry rotundone appear strikingly similar. Comparison of Figures 1 and 2 shows a tendency for the use of the common variogram to lead to a small increase in the mean kriged rotundone concentration in 2012 and 2013 and a small reduction in 2015. Nevertheless, the spatial structure across the years is for all practical purposes the same, with between-year differences in the 'unique' variograms having negligible effect on the displayed spatial variation in the interpolated maps. Thus, when the maps for the 3 sampled years are clustered together using *k*-means (Figure 3), further evidence is provided in support of temporally stable patterns of rotundone variation in this vineyard. Based on the cubic clustering criterion (SAS 1983), Figure 3b is considered more statistically robust than Figure 3a, and is similar to the pattern which the vineyard manager had identified as matching his mental picture of the block (Scarlett et al. 2014). Thus, Figure 4 provides an update to Figure 6b of Scarlett et al. (2014) and, like Figure 3b, strongly suggests that, in spite of marked seasonal variation in the mean concentration of rotundone over the 4 years of the study, the patterns of spatial variation in the concentration of berry rotundone are temporally stable. This finding lends much weight to the role of variation in the underlying land supporting the vineyard in driving the pattern of rotundone variation (Scarlett et al. 2014).

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Discussion

Whereas selective harvesting strategies have tended to be driven by remotely sensed measures of vine vigour (Bramley et al. 2005, 2011b), which are readily commercially available in Australia, the analysis of Scarlett et al. (2014) provided scant evidence of any effect of variation in vine vigour on variation in the concentration of berry rotundone. Rather, the influence of variation in soils and especially in topography were highlighted, with the importance of these attributes underlined by Figure 4. Clustering (*k*-means) of remotely sensed imagery obtained at veraison in 2013 (not shown) with the 2010 image used by Scarlett et al. (2014) shows the patterns of variation in vine vigour during these 2 years to be similar. We infer, therefore, that patterns of variation in vine vigour were temporally stable over the 4 years of the present study, as would be expected (Bramley 2010, Bramley et al. 2011a). Thus, whilst variation in vine vigour, as assessed using remote sensing, can give a general indication of variation in fruit composition in terms of indices such as juice pH, titratable acidity, TSS and berry anthocyanins and phenolic substances (Bramley et al. 2011a,b), it clearly does not provide a useful guide for assessing or manipulating the pepperiness of Shiraz wines. In this case, indicators other than remotely sensed vine vigour are clearly needed. Both the present study and that of Scarlett et al. (2014) indicate the potential utility of a digital elevation model (DEM) from which the topographic data were derived. Arguably, other indicators may be needed for other specific elements of fruit composition, such as the concentration of methoxypyrazines in Sauvignon Blanc or Cabernet Sauvignon which, like rotundone, occur at a concentration that is too low for ready sensing in vivo or on-the-go during harvest (Bramley et al. 2011c).

In the case of the potential management of wine pepperiness through the selective harvesting of cool climate Shiraz, the present study highlights the importance of a further issue. Rather than simply relying on an understanding of topographic variation, any decision to selectively harvest must be supported by appropriate chemical analysis pre-harvest. In the present study, 2012 was a 'high' rotundone year (Table 1). One can imagine that a high concentration of berry rotundone in some vineyard areas could present the winemaker with an opportunity, through blending, to manipulate the pepperiness of final wines. In contrast, the low rotundone concentration observed in 2013 (Table 1) would suggest that selective harvesting would offer little, if any benefit, in optimising wine pepperiness. In this case, it would be more sensible to harvest the crop as a single parcel. Such a decision would be readily supported by pre-vintage analysis of berry rotundone. Arguably, it is in the more medium rotundone years that selective harvesting could yield greatest return, with areas of fruit

of higher rotundone harvested into wines intended to be of peppery style, with the remaining fruit allocated to other product streams. We also note here that aspects other than pepperiness will be of importance, which is presumably why, even in 2013, the commercial harvest of the study block proceeded in a similar manner to 2012 and was guided by sensory assessment of the fruit by the winemaker in different parts of the block.

The importance of temperature to rotundone concentrations in grapes (Zhang et al. 2015a) and wines (Zhang et al. 2015b) has been noted previously with Zhang et al. (2015b) also finding an effect of water balance on wine rotundone concentrations. It is beyond the scope of the present paper to further explore any effects of water balance variation. Suffice to say that Scarlett et al. (2014) noted a close relationship between the SAGA wetness index (Boehner et al. 2002) and vine vigour which, as indicated above, does not appear to play a role in controlling variation in rotundone. Table 1 summarises some temperature and rainfall data over the period of the study for the Australian Bureau of Meteorology weather station at Ararat Prison (www.bom.gov.au; station number 089085), the closest such station. Recognising that this weather station is approximately 15.5 km from the study site, is at approximately 60 m lower elevation than the vineyard site (~300 m compared to ~360 m AHD), and that the Langi Ghiran Park (elevation up to 800 m AHD) lies between, it nevertheless provides some general information of potential value in understanding seasonal differences. The data in Table 1 suggest that the 2011/12 growing season had a comparatively high growing season rainfall and was cool in the veraison to harvest period (Feb–Mar), observations consistent with the importance attached to these weather effects by Zhang et al. (2015b) in terms of rotundone concentration. Conversely, the 2013 season was both markedly drier and warmer. The 2014/15 growing season was also fairly dry, but was comparatively cool with its mean rotundone concentration (three times that of 2013, but 15 times less than in 2012) presumably reflecting the interaction between these factors. Zhang et al. (2015b) also drew attention to the importance of a bunch zone temperature over 25°C in the veraison to harvest period; their data were logged in the bunch zone at 30 min intervals. With daily observations such as those accessed here, it is more difficult to discern specific temperature effects, although the data in Table 1 do support the findings of Zhang et al. (2015b), albeit that 2012 and 2015 apparently had a similar occurrence of days with a temperature over 25°C, but with 2014 (a low rotundone year; data not shown) having the fewest. Interestingly, a discriminant analysis of weather parameters undertaken by Zhang et al. (2015b) grouped the 2012, 2013 and 2014 seasons together. In comparing the 2012–2015 seasons, note also the apparent unimportance of the mean January temperature (MJT) which is often regarded as an important classifier of viticultural regions and sites

(Smart and Dry 1980). In contrast, the lower mean concentration of rotundone in grapes harvested in 2015 might reflect the earlier harvest date compared to that of the 2012 vintage. A shorter hang-time from veraison to harvest under suitable climatic conditions might be another important factor contributing to the lower rotundone concentration, as model experiments have shown that rotundone can be formed by chemical oxidation in air from its precursor α -guaiene (Huang et al. 2014), with reaction time and light quality being critical factors that determine the final rotundone concentration (Zibi 2015).

In summary, a substantial 40-fold variation in the mean concentration of berry rotundone was observed between three seasons, yet spatial analysis of maps of rotundone variation produced for each year demonstrated that the distinctive patterns of spatial variation in grape rotundone were stable across the seasons. This remarkable observation also provides a starting point for future research aimed at differentiating between the influence of seasonal climatic conditions and the contribution of other terroir-related environmental, genetic and/or biological factors that could independently or in combination contribute to higher values of rotundone concentration and distinctively peppery red wines.

Conclusions

Consistent with the previous work of Scarlett et al. (2014), this work demonstrates that within-vineyard variation in the concentration of the 'pepper compound' rotundone is spatially structured and related to variation in the land supporting the vineyard. Importantly, this variability is seen to be stable between seasons, even when seasonal differences in mean rotundone concentration are as large as 40-fold. Thus, targeted vineyard management strategies, including selective harvesting, have the potential to enable wine producers to manipulate wine style; in this case, the pepperiness of cool climate Shiraz wines. Further studies aimed at better understanding the relationships between the environment and elements of berry composition and their importance to terroir at both vineyard and regional scales are warranted.

Acknowledgments

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Figure 1. Variation in the concentration of grape berry rotundone in a 6.1 ha Shiraz vineyard in the Grampians region of Victoria across three seasons, and comparison of the effect of using either ‘unique’ season specific, or common (across all seasons) variograms for map interpolation.

Figure 2. Variation in the concentration of grape berry rotundone in a 6.1 ha Shiraz vineyard in the Grampians region of Victoria across three seasons, and comparison of the effect of using either ‘unique’ season specific, or common (across all seasons) variograms for map interpolation when maps are classified on the basis of percentiles.

Figure 3. Results of clustering the maps of rotundone variation obtained in 2012, 2013 and 2015 using *k*-means; (a) two and (b) three cluster solutions are shown. Also shown in (c) is the result of clustering these three map layers with the other map layers used by Scarlett et al. (2014) to describe variation in the land underlying the vineyard (three- cluster solution). Here, (EC_a) denotes bulk electrical soil conductivity (mS/m), as measured using electromagnetic induction soil sensing, SI denotes slope (%) and fN denotes aspect, expressed in terms of deviation from north. The numbers in the legend are the cluster means; in (c), values for EC_a followed by different letters are significantly different [$P < 0.05$; Scarlett et al. (2014)].

Figure 4. Interactions between soil (EC_a), slope (SI), aspect, expressed as degrees from north (fN), and the concentration of berry rotundone over three seasons in a 6.1 ha vineyard in the Grampians region of Victoria. Note that the position of the north arrow is approximate only.

Table 1. Selected weather data at the Ararat Prison weather station[†] in the 2012–2015 seasons and mean berry rotundone concentration of Shiraz fruit from the vineyard in the Grampians region of Victoria.

Year of harvest	Mean rotundone concentration (ng/kg) [‡]	GSR (mm)	MJT (°C)	MFMT (°C)	GDD FM ^F (°C)	DH ₂₅ (%)
2012	399	223.6	21.0	18.0	475.6	0.8
2013	10	123.3	20.0	19.9	582.3	1.7
2014	10	137.9	21.7	18.9	522.5	0.6
2015	31	142.7	19.5	18.3	486.9	0.8

[†]Bureau of Meteorology Site No. 089085.

www.bom.gov.au/climate/averages/tables/cw_089085.shtml. [‡]Mean of 177 samples except in 2014 when only seven samples were taken across the low and high zones. DH₂₅, degree days warmer than 25°C (Zhang et al. 2015b); GDD FM, growing degree days (base of 10°C) in February–March; GSR, growing season rainfall (calculated as total rainfall in October to March); MJT, mean January temperature; MFMT, mean February–March temperature.

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