How ripe are my grapes?

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Introduction
The single most important decision a grower/winemaker can make in the vineyard which will ultimately affect the quality of the wine produced is the harvest date. In deciding harvest date the grower/winemaker is trying to optimise the compositional characteristics of the fruit for winemaking purposes, i.e. sugar, acid, colour, flavour and aroma. This is usually achieved by regular sampling and testing during the weeks leading up to harvest. While the usual interest is in the mean value of each of the quality parameters, it is important to appreciate how each of these quality parameters varies within the vineyard. Also, because winery payment schedules are being increasingly linked to grape quality specifications it is essential that sampling and measurement techniques accurately reflect the results for the entire vineyard or ‘harvest unit’.

The development of a statistically sound sampling strategy involves having a detailed understanding of all potential sources of variation. Variability may be either temporal (season-to-season) or spatial (vine-to-vine within a vineyard, bunch-to-bunch within a vine or berry-to-berry within a bunch). Spatial variability may enter a vineyard system through differences in soil type, crop load, vine size, cluster position and exposure to sunlight (Wolpert et al. 1980). A similar approach has been utilised in Michigan for Vidal Blanc (Wolpert and Howell, 1984) and Concord (Wolpert et al. 1980) and in Central California (Fresno) for Thompson Seedless (Kasimatis et al. 1975). The aim of this research was not to explain the viticultural causes of this variability, but to quantify the random effects of variability as a means of determining the number and allocation of samples required to deliver estimates of each wine grape quality parameter within particular confidence and error limits. Similar sampling strategies have been developed and successfully applied to yield forecasting in grapes (Dunn and Martin, 1998; Wolpert and Vilas, 1992).

Statistical terminology
Before launching into statistics it is important to be familiar with some commonly used statistical terminology.

The mean is described as the sum of all the measurements divided by the total number measurements recorded. The variation within a sample is described by a standard deviation, which is the square root of the sample variance. The reliability of the sample mean as an estimator of the true population is described by the 95% confidence interval, which is calculated from the Student’s t-distribution and using the standard error of the mean (standard error is calculated by dividing the standard deviation by the square root of the number of samples in the mean). The coefficient of variation is simply the ratio of the standard deviation to the mean expressed as a percentage. The percentage doubt is defined as the ratio of the 95% confidence interval to the mean, expressed as a percentage.

The best sample size may be calculated using the following formula:

Best sample size = $t^2 \times (CV^2 / TD^2)$

Where:
- $t$ – is a value from the t-distribution (usually sufficient to assume $t=2$ at 95% confidence level).
- $CV$ – is the percent coefficient of variation (%CV)
- $TD$ – is the tolerance of doubt (%doubt), i.e. the maximum % doubt that will be tolerated.

Sampling berries or bunches: The great debate?
When discussing issues related to sampling with viticulturists and winemakers, the discussion often pauses on what do you sample in the vineyard? Many people believe that berry sampling is easier and provides accurate results, while others advocate bunch sampling for exactly the same reasons. Whatever method is employed it is important to ensure that the sampling method is representative of the whole population and that it doesn’t influence the result through potentially introducing bias. In the case of berry sampling, and particularly in tight bunched varieties like Chardonnay, Cabernet Sauvignon, etc, it would be hard to give all berries equal chance of being selected. Therefore some sampling bias may be introduced when considering berry sampling as opposed to bunch sampling.

To examine this further an experiment was conducted to compare berry and bunch sampling for measurements of sugar (°Brix). In this experiment a Cabernet Sauvignon vineyard located at Irymple, north west Victoria was sampled either using random bunch sampling techniques (random vine and position on vine techniques used) or random berry sampling techniques (random vine and bunch on vine techniques to select bunch and then berries removed carefully from rachis and random berries selected using detached berries). Results in Table 1 indicate that there were small differences in the means (not statistically significant at $p<0.05$ level, results not shown), but all the different measures of variation around the mean were higher in the berry measurements compared with bunch...
In summarising this work, it probably doesn’t matter whether or not you decide to choose berries or bunches as sampling units as long as the sample is representative, that is no bias introduced, and that approximately twice as many berries as bunches are sampled. This could be further refined by viticulturists or winemakers by conducting similar experiments to determine the levels of variation within their particular vineyards.

Post-veraison variation observed in °Brix and anthocyanins

Before deciding on a sampling strategy it is important to fully understand all sources of both temporal and spatial variability. In Figure 1, differences in temporal variability between veraison and harvest can be observed in both °Brix and total anthocyanins for Shiraz grown in north-west Victoria. The tendency is for both of these parameters to decrease throughout berry maturation, however, the variation observed in total anthocyanins is much greater than that observed in °Brix (Figure 1). This has important implications if accurate assessments of berry maturity are required at lower °Brix levels. This may mean that more samples may need to be collected when attempting to estimate maturity in vineyards designated for sparkling wines compared with full-bodied dry table wines, if a similar tolerance of doubt is required.

Comparing the variation in °Brix and total anthocyanins to the variation in yield within a vineyard

While Figure 1 does illustrate the typical differences observed in the variation between °Brix and total anthocyanins, it is important to appreciate how they vary in comparison to yield. While the yield per vine can vary 5–6 fold in a single vineyard (i.e. 4–22 kg/vine) the relative change in °Brix can be very small (i.e. 22–25.5 °Brix) (Figure 2a). Also, there may be a tendency for higher yielding vines to have a slightly lower °Brix (Figure 2a).

In examining total anthocyanins, while the yield per vine can again vary 5–6 fold in a single vineyard (i.e. 4–22 kg/vine) the relative change in total anthocyanins can be 3–4 fold, i.e. 0.8–2.4 mg/g berry weight (Figure 2b). There is also a strong tendency for higher yielding vines to have a lower total anthocyanin content (mg/g berry weight basis). Therefore, it is important to realise that the sample sizes required to estimate °Brix, total anthocyanins or yield to a similar tolerance of doubt can be very different.

Typical variation in °Brix and total anthocyanins across a range of vineyards

While different quality parameters differ with respect to their variability, it is also important to understand how these vary between different vineyards. The differences in soil

**Table 1 – A comparison of mean and variation in °Brix between berry and bunch samples collected from a Cabernet Sauvignon vineyard in Irymple, north-western Victoria (n=60). Best sample size was calculated, where the tolerance of doubt was 4%**.

<table>
<thead>
<tr>
<th></th>
<th>Berries</th>
<th>Bunches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean °Brix</td>
<td>17.83</td>
<td>18.1</td>
</tr>
<tr>
<td>Std Deviation</td>
<td>2.26</td>
<td>1.64</td>
</tr>
<tr>
<td>95% confidence limit</td>
<td>0.58</td>
<td>0.42</td>
</tr>
<tr>
<td>% coefficient of variation</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>% doubt</td>
<td>3.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Best sample size (4% tolerance of doubt)</td>
<td>40</td>
<td>21</td>
</tr>
</tbody>
</table>
type, vineyard area, crop load, vine size, cluster position and exposure to sunlight, all may contribute to the variability observed in vineyards (Figures 3a and 3b). In examining 16 different vineyards across southeastern Australia (four Shiraz, nine Cabernet Sauvignon, one Merlot, one Chardonnay and one Riesling vineyard) where 50 random bunches were sampled and °Brix recorded, the percentage coefficient of variation ranged from 2.9–9.1% (Figure 3a). In examining 14 different vineyards across southeastern Australia (four Shiraz, nine Cabernet Sauvignon, one Merlot vineyard) where 50 random bunches were sampled and total anthocyanins recorded, the percentage coefficient of variation ranged from 9.7–33.5% (Figure 3b). Again, this highlights the difference observed between the variability of °Brix compared to that observed with total anthocyanins.

Effect of vineyard size on variation

One parameter which is often thought to influence sampling intensity is vineyard size or area. This may arise due to the perceived increase in variability associated with increases in vineyard area or the need to adjust sampling intensity proportionally to the vineyard area. In studies of a large number of vineyards across southeastern Australia, vineyard area does not appear to influence the degree of variation in both °Brix and total anthocyanins (Figures 4a and 4b). This suggests that sampling intensity does not need to be adjusted or modified according to the area of an individual vineyard.

Separating vineyard and laboratory variation

It is also important to appreciate that the variability observed may actually come from either inherent variability in the vineyard, or through the sub-sampling and measurement process in the laboratory. It is possible to separate these sources of variability and determine where the variability may enter the system. In examining °Brix measurements, approximately 75% of the variation (CV=3%) appears to come from the vineyard, while only 25% (CV=1%) appears to come from the sub-sampling and measurement process (Table 2). However, in the case of total anthocyanins, approximately 44% (CV=7%) of the variation appears to be associated with the vineyard and 56% (CV=9%) can be apportioned to the sub-sampling and measurement process (Table 2). This highlights the potential to improve the laboratory and analytical techniques associated with the measurement of total anthocyanins as conducted using the Iland et al. (2000) technique.
Table 2. Apportioning the total variance between the vineyard and laboratory components for a Shiraz vineyard located in southwestern New South Wales.

<table>
<thead>
<tr>
<th>Source</th>
<th>°Brix</th>
<th>Total anthocyanins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vineyard component (%CV)</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Laboratory component (%CV)</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Total %CV</td>
<td>4</td>
<td>16</td>
</tr>
</tbody>
</table>

Implications for sampling

In deciding on a sampling regime, it would be ideal to know exactly the variation of the parameter to be sampled. This way sampling can be adjusted on an individual case so that error and waste can be minimised. However, in practice it is difficult at present to adjust sampling according to the variability of each vineyard and each particular wine grape quality parameter. It is probably better to design a sampling regime that is widely applicable, but incorporates an allowable level of waste and error. This may be achieved by determining the mean level of variation about each quality parameter and determining the best sample size, as mentioned earlier in this paper, for a given tolerance of doubt. However, in this case approximately 50% of all vineyards would be under-sampled, which will result in error and unreliability in the sampling system for those particular vineyards. Some of these vineyards will also be over-sampled. While this too is considered wasteful in the generic sense, it will only mean greater confidence in the result of the sampling process.

Another approach may be to analyse the variation in the percent coefficient of variation of each quality parameter and decide on how much of the population to include when designing the sampling regime. In this study, the standard deviation of the coefficient of variation (%CV) was calculated. Knowledge of statistics indicates that adding the standard deviation to the mean can account for approximately 84% of the population. In this case approximately 16% of all vineyards would be unde-sampled, which will result in error and unreliability in the sampling system for those vineyards and over-sampling many of the other vineyards, which will only lead to more confidence in the results for those vineyards. This system may be more practical and reliable for industry, however, it is not the intention of this paper to ‘set the bar’ for industry or business and decide on how much of the population to include when designing the sampling regime.

In examining °Brix, the average percent coefficient of variation observed was 5.7%, with a standard deviation of 2.0% (Table 3). This means that 84% of the population had a %CV of approximately 7.7% or less. Therefore calculating best sample size with a 5% tolerance of doubt means that 45 bunches are required (Table 3). A 40 bunch sample will generate an estimate of the mean with approximately 7.6% doubt or less for 84% of vineyards (Table 3).

Finally, although appropriate sampling strategies can be designed to obtain an accurate estimate of a particular wine grape quality component, the post-harvest changes which can occur in pH, TA and colour need to be recognised and accounted for in discussions between viticulturists/growers and winemakers (Krstic et al. 2001).

Table 3. The mean, standard deviation and 84% of population level (mean plus one standard deviation) of %CV in °Brix, best sample size at 5% tolerance of doubt and the %doubt in a 20 bunch sample in 16 different vineyards across southeastern Australia (four Shiraz, nine Cabernet Sauvignon, one Merlot, one Chardonnay and one Reisling vineyard).

<table>
<thead>
<tr>
<th>%CV</th>
<th>% doubt with 20-bunch sample</th>
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<tbody>
<tr>
<td>5.70</td>
<td>2.5</td>
</tr>
<tr>
<td>2.00</td>
<td>1.0</td>
</tr>
<tr>
<td>7.70</td>
<td>3.5</td>
</tr>
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</table>

In examining total anthocyanins, the mean percent coefficient of variation observed was 17.6%, with a standard deviation of 6.5% (Table 4). This means that 84% of the population had a %CV of approximately 24.1% or less. Therefore calculating best sample size with a 7.5% tolerance of doubt means that 45 bunches are required (Table 4). A 40 bunch sample will generate an estimate of the mean with approximately 7.6% doubt or less for 84% of vineyards (Table 4).

Table 4. The mean, standard deviation and 84% of population level (mean plus one standard deviation) of %CV in total anthocyanins, best sample size at 7.5% tolerance of doubt and the %doubt in a 40 bunch sample in 14 different vineyards across southeastern Australia (four Shiraz, nine Cabernet Sauvignon, one Merlot vineyard).

<table>
<thead>
<tr>
<th>%CV</th>
<th>No. samples required with 7.5% doubt</th>
<th>% doubt with 40-bunch sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.6</td>
<td>25</td>
<td>5.6</td>
</tr>
<tr>
<td>6.5</td>
<td>20</td>
<td>2.0</td>
</tr>
<tr>
<td>24.1</td>
<td>45</td>
<td>7.6</td>
</tr>
</tbody>
</table>

In practice, this means that a 20 bunch sample will provide an estimate of °Brix within 3.5% doubt for most vineyards, i.e. if the mean was 24 °Brix, the real result may lie somewhere between 23.16 – 24.84 °Brix. Conversely, a 40 bunch sample will provide an estimate of total anthocyanins within 7.6% doubt for most vineyards, i.e. if the mean total anthocyanin content was 1.5 mg/g berry weight, then the real result may lie somewhere between 1.39–1.61 mg/g berry weight.

Conclusions

The development of a sampling strategy based on a detailed understanding of the variability in each wine grape quality parameter is important in delivering accurate estimates within particular confidence and error limits. In sampling for °Brix, a 20 bunch sample can provide an estimate of the mean for most vineyards with a 3.5% doubt or less. In sampling for total anthocyanins, a 40 bunch sample can provide an estimate of the mean for most vineyards with a 7.6% doubt or less.

Finally, although appropriate sampling strategies can be designed to obtain an accurate estimate of a particular wine grape quality component, the post-harvest changes which can occur in pH, TA and colour need to be recognised and accounted for in discussions between viticulturists/growers and winemakers (Krstic et al. 2001).
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References