Nitrogen nutrition and grape and wine production

BRUNO HOLZAPFEL (NSW DPI, WAGGA WAGGA), JOHN BLACKMAN (CSU, WAGGA WAGGA) and MARKUS MÜLLER (Formerly CSU, WAGGA WAGGA)

Bruno Holzapfel, Research Scientist – Viticulture
Email: bruno.holzapfel@dpi.nsw.gov.au
Phone (02) 69334023

In a nutshell

- Vineyard nitrogen (N) supply elevates berry N and yeast assimilable N (YAN)
- N mobilisation from other annual and reserve tissues is an important contributor to N in grapes
- Petiole N and early YAN concentrations can predict final YAN levels at harvest
- Assessment of must YAN levels prior to fermentation is important to determine additional yeast requirements
- The final wine flavour characteristics are influenced by amounts and composition of YAN in the must

Introduction

The nitrogen supply in the vineyard affects the N status of vines, which affects the form and concentration of nitrogenous compounds in must. During berry ripening, mobilisation of N from vine storage organs often moves to the bunches and alters the amount of N in the grapes The total berry N content increases during maturation, with the majority of N and amino acids present in the flesh and the skins. Arginine and proline are often the most dominant amino acids in the must (Figure 1A), with the later usually accumulated at the end of the berry ripening process and can not be utilised by the yeast. Ammonium (NH_4) is another important N containing compound in the must, but the concentration declines during berry maturation. Free assimilable amino N (FAN) increases during this period and is therefore influenced by harvest date (Figure 1B).

Primary amino acid N or α amino (FAN) and ammonium N (NH_4) are classified as yeast assimilable N (YAN), having a critical role in the fermentation process. Low YAN levels result in slow (or stuck) fermentation, while high levels lead to fast or boiling ferments. In addition, YAN levels and composition influence aroma and flavour characteristics, this is due to the production of desired/undesired compounds during the fermentation and consequential presence in the wine. A range of 250 to 350 mg/L is seen as the optimum YAN level in the must and required additions are often made with di-ammonium phosphate (DAP) in the winery when YAN levels are insufficient to sustain fermentation. However, it is important to know the YAN levels prior to the addition of DAP, to avoid rapid fermentations and the development of undesired compounds in the wine.

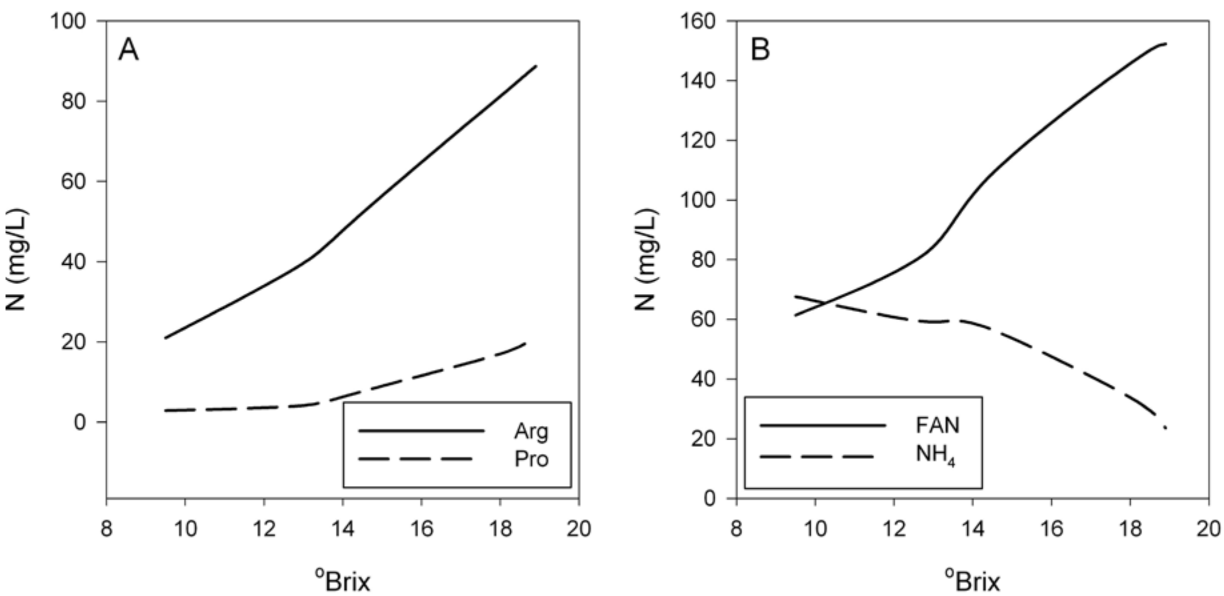

Figure 1. *Changes in Riesling juice N concentration of arginine (Arg) and proline (Pro), from veraison to harvest (A). The changes of yeast assimilable N (B) in free assimilable amino acid N (FAN) and ammonium (NH4) from veraison to harvest (Müller et al. unpublished data)*

Vineyard N supply and must N

The main factors, other than seasonal and environmental, for N uptake are the amount of water and N fertiliser applied to a vineyard, both of which play a major role in the growth and development of vines. The amount applied is usually based on petiole N Levels (or N status) at flowering and by considering the production level (N removal). The N status of the vine does relate to berry N, but low N supply can also reduce cluster initiation, fruit set and berry size. The timing of N application is also critical for influencing vine growth and yield and on the N directed towards the berries. An N application between bloom and veraison results in higher berry N compared to an application after harvest (Figure 2A). Application during the post-harvest period usually enhances early development and yields in the following season. Reduced water supply in the form of partial rootzone drying (PRD) can lower berry YAN concentrations, while regulated deficit irrigation (RDI) can lead to a slight increase of must YAN (Figure 2B).

The application method is also important, foliar application and fertigation being the most effective. Urea sprays applied at rates between 5 to 50 kg/ha (concentration less than 4%) around veraison can considerably increase berry YAN levels at harvest. N applied through the fertigation system from bloom to veraison has also shown to be very effective in elevating juice amino N. Soil applied N is less effective, with the N form in the fertiliser or N to C ratio in organic fertilisers determining the rate of uptake.

Assessment and prediction YAN

The YAN levels could be predicted by petiole levels measured at veraison, to allow for adjustments of N supply in the period of berry development, also considering the potential yield (Figure 3A). However, an application might be necessary during early berry development and this would be indicated by N measures at flowering. Testing the level of N in the petiole at veraison provides the opportunity for further adjustments that might be required to reach an optimum YAN level at harvest.

Veraison petiole values of about 0.5N %DW for red grapes correlate with about 100mg N/L FAN. The contribution of NH_4 in the must has also to be considered, since YAN levels of 100mg N/L for red and 150 mg N/L for white musts are seen as a minimum requirement for low risk fermentation. However, the optimum concentrations for white grape must are in the range of 250 to 350 mg N/L. The range for red grape must is not defined, but is likely lower since the YAN in the skins can be utilized during the wine making process. Because of the connection between petiole N and must FAN, the petiole N concentrations also have a strong relationship with the sugar consumption during fermentation (Figure 3B).

Final YAN levels can also be predicted by assessing those more than a month prior at harvest, by direct determination in the juice during berry maturation. Such a prediction will provide early information on N addition requirements in the winery, but this would make it difficult to make adjustments in the vineyard. The use of veraison petiole N can provide additional information for optimising YAN levels in the vineyard; these assessments are more feasible with cheaper techniques available to determine the N concentrations.

Must YAN and fermentation

It is most reliable to determine yeast assimable amino (YAN) levels close to harvest, as harvest maturity influences the nitrogenous compounds in the berry. In addition, the harvest procedure and the transport

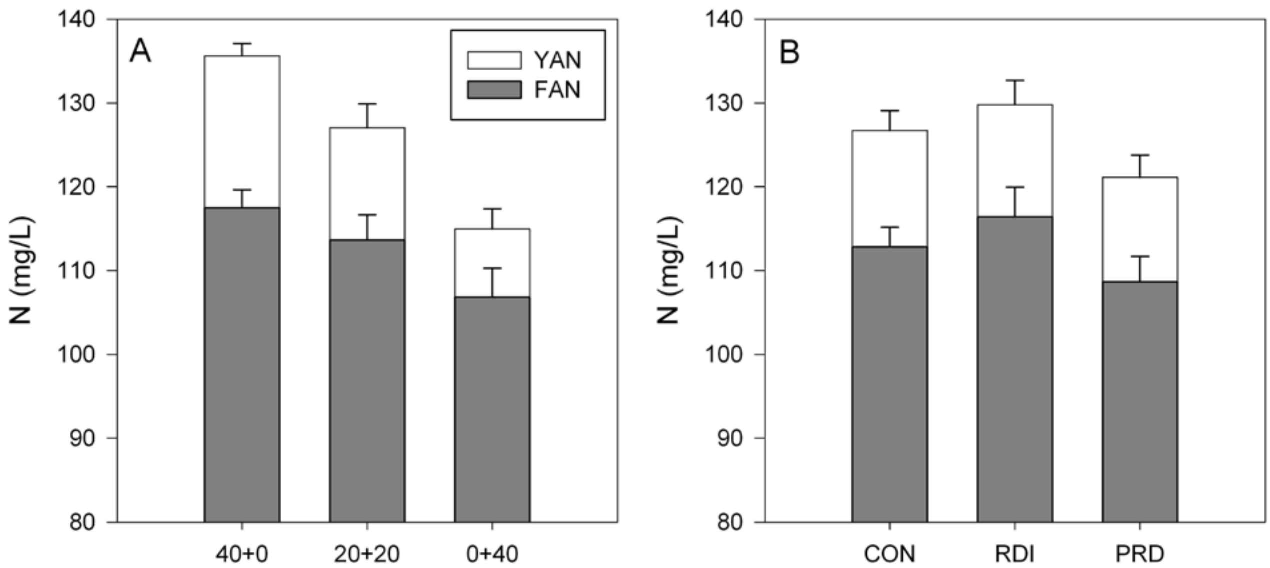

Figure 2. *The influence of N timing with an application rate of 40 kg/ha (A) and irrigation management (B) in a Shiraz vineyard on free assimilable amino (FAN) and total yeast assimilable N (YAN) (Holzapfel et al. unpublished data).*

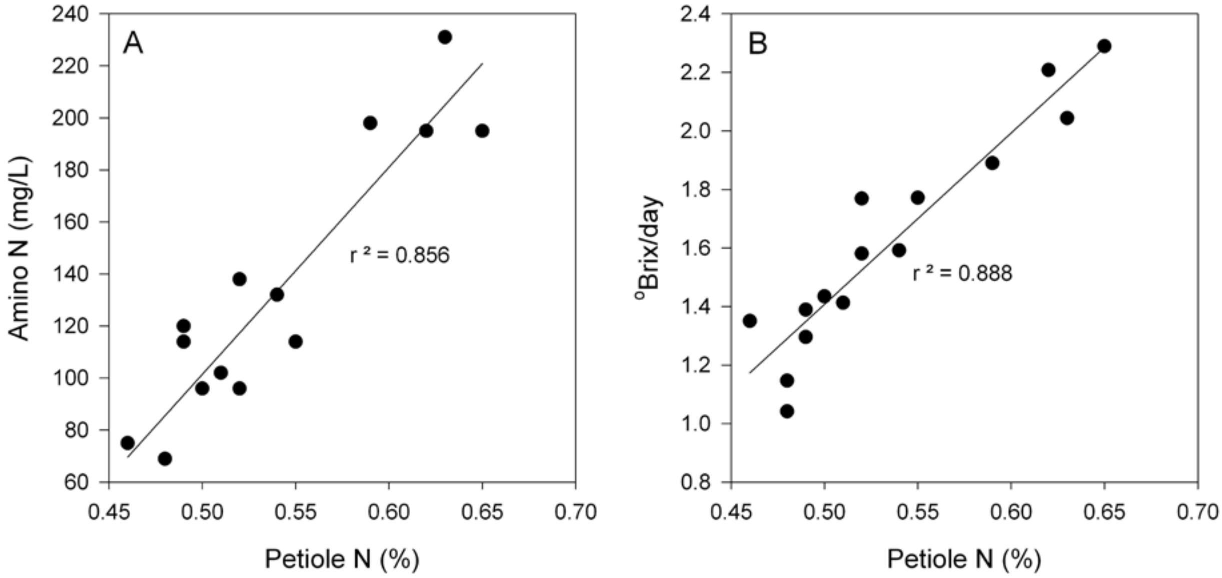

Figure 3. *Relationship between petioles N at veraison and assimable amino N at harvest (A) and daily sugar consumption in the fermenting Shiraz must (B) (adapted from Holzapfel and Treeby 2007 and 2013).*

can reduce must YAN concentrations due to microbial growth. YAN in the must is composed of free a amino N (FAN) and ammonium (NH_4), with the minimum requirements for yeast being about 100 or 150 mg N/L of must for the completion of fermentation, for red and white grapes respectively. The yeast utilised first the NH_4 and then the primary amino N, with the preference of utilisation also varying between the various amino acids. The amount of YAN (and also sugar levels) in the must influences the fermentation rate; musts with low levels are associated with sluggish and stuck ferments, while musts with high levels ferment too fast (Figure 4).

The amount of YAN present in the must impacts on the compounds produced during the fermentation process. Low N must results in elevated levels of thiols in the wine and higher alcohols and lower amounts of esters and long chain volatile fatty acids. In contrast, high N must leads to an increase in ethyl acetate, acetic acid and volatile acidity. Higher concentrations of urea, ethyl carbamate and biogenic amines in the wine have also been observed in wines produced from high YAN musts. In addition, the composition of the FAN is also important for wine composition, since the various amino acids provide C-skeletons for certain flavour compounds produced during the fermentation process, impacting on the sensory profiles of the wines produced.

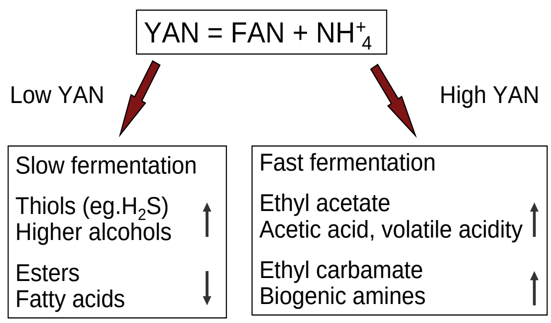

Figure 4. *Juice yeast assimilable N (YAN) levels and the wine making process.*

Impact on wine characteristics

The aromas and flavours in wine are influenced by four major sources, with the compounds formed by the yeast during the fermentation being one of them. The yeasts vary in their requirements for N, ranging from very low to extreme. Therefore it is important to measure the YAN levels in the winery to determine the requirements for N additions to the must prior fermentation. These levels can be assessed for instance enzymatically for NH4 and with the o-phthaldaildehyde/N-acethyl-cysteine reagent (NOPA) for α-amino N.

The amount of YAN is not only important for the fermentation rate, but also for compounds produced during the process. For instance, deficiencies results in undesired thiols (hydrogen sulfide), being produced from S containing amino acids, while must with balanced YAN levels are high in desired aroma compounds such as terpenes and esters. To optimise fermentation and the produced compounds during the process inorganic or organic N is added to the must, being in from of DAP or α-amino N or a mixture of both. The issue with the inorganic N is that that the yeast is using this form with preference to the amino acid N. Therefore it is better to add a complete nutritional supplement to musts that are YAN deficient.

The required N additions to the must should only be based on must YAN levels, but also should take into account the fermentation conditions (e.g. yeast and temperature) and consider wine style targets. As indicated above, the N compounds present in the must influence the volatile compounds and therefore the aroma profile of the wine (fermentation bouquet). The flavour is also affected by the production of non-volatile compounds (eg. polyols). In addition, the remaining N after the alcoholic fermentation has an important role for the secondary fermentation (malolactic) and microbial stability of the wine.

Conclusions and recommendations

N supply and N status is closely related to must amino acid composition and amounts, being crucial for the formation process and wine aroma and flavour. The vineyard adjustments to achieve an optimum YAN level at harvest can be based on petiole N status at bloom and veraison. The later can be particularly helpful in making prediction of YAN levels at harvest, allowing additional N applications if required during berry ripening. However, it is important to monitor the YAN levels in the incoming must to the winery, for determining required adjustments for the wine making process. Insufficient YAN must levels can be enhanced by adding inorganic or organic N in the winery. However, it is best to use DAP and other supplements only for fine tuning the must, while the grape amino-N content should be optimized in the vineyard for maximising wine flavour and aroma.

Acknowledgments

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