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To: Grape and Wine Producers

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Subjects:

1. Nitrogen

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- **b. Nitrogen Uptake**
- **c. Yeast Assimilable Nitrogen (YAN)**
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1. Nitrogen.

In the Old World, the conviction has been held for centuries that wine is, at its core, the reflection of a place. While there is no single-word translation for *terroir* into English, the French often use this one word to explain why a wine tastes the way it does, as a result of its place. As discussed in *Enology Notes* #168, nitrogen availability to the vine can be considered a *terroir* factor. Nitrogen is one of the most important elements impacting vine growth, fruit chemistry and, therefore, ultimate fruit and wine potential. The following is a review of some important nitrogen issues.

Wines made from fruit with adequate nitrogen generally have superior aroma and overall quality (Sinton et al. 1978, Bell and Henschke 2005). While it is a universal New

World practice to add supplemental nitrogen to the fermentor, there is ample evidence to suggest that such additions fail to enhance the fermentation as much as does natural, grape-produced nitrogen (Sinton et al. 1978, Treeby et al. 1996).

Even in soils with limited nitrogen concentrations, there can be large differences in the amount of nitrogen taken up by the vine due to soil type and composition, depth, moisture, microbial content, etc. Shallow soils are often reported to be superior in wine potential than deeper soils, due to lower water holding capacity and possibly lower nitrogen, both contributing to a reduction in vigor. Nitrogen availability increases with the organic content and organic turnover. Organic matter turnover is increased under the following conditions (van Leeuwen 2013):

- Low carbon/nitrogen ratio
- High soil pH
- Low temperature
- High soil moisture

a. Red vs. White Grapes. Red grape potential for quality wines has been correlated to vine nitrogen status, particularly when water is not limiting (van Leeuwen 2013). Low vine nitrogen reduces vine vigor in general, and increases tannins and anthocyanins (Chone et al. 2001). Thus, in many respects, red grape quality is increased by limited vine nitrogen status (van Leeuwen et al. 2013) and feature referred as regulated deficit nutrition (Keller 2012). Vegetatively-vigorous varieties may need less nitrogen and more moisture stress to attain balance (Gladstone 2011).

In white grape production, the considerations for the nitrogen status of the vine may be quite different. In whites, low nitrogen reduces the concentration of important aroma/flavor precursors. Additionally, as discussed in *Enology Notes* #168, low nitrogen can produce a low concentration of glutathione, an important antioxidant in white wine production (see *Enology Notes* # 98, 101, 102, 112, 127, 129, 134, 144, 159 for a review of glutathione).

b. Nitrogen Uptake. Nitrogen is the mineral nutrient for which the plant has the highest demand and which most limits growth. Although nitrogen makes up 80% of the atmosphere, grapevines cannot directly use N_2 (gaseous nitrogen). Vines rely on the uptake by roots, mostly in the form of nitrate (NO_3^-) dissolved in the soil. Nitrate ions are reduced by the nitrate reductase system to ammonium (NH_4^+), transported, and stored as amino acids (Sponholz, 1991).

c. Yeast Assimilable Nitrogen (YAN). Nitrogen compounds act as nutrients for microorganisms involved in winemaking and wine spoilage. The nitrogenous components of grapes and juice that are metabolically available to yeasts include organic (amino) acids and inorganic ammonium (NH_4^+) salts. Some important points are reviewed below:

- Ammonium nitrogen plus the yeast utilizable amino acids ("free *alpha*-amino acid nitrogen," or FAN) are referred to as YAN (yeast assimilable nitrogen).
- YAN analysis, therefore, requires measurement of ammonium nitrogen and the utilizable amino acids.
- In grapes, the YAN ranges from near 30 to more than 400 mg/L.
- Ammonia is used by yeasts prior to amino acids.
- The minimum level of YAN required for successful completion of alcoholic fermentation is reported to be 120-140 mg/L for musts with sugar concentrations of up to 22°Brix. Higher Brix concentrations require higher YAN levels.
- A low YAN level may signal a low level of the micronutrients that are needed for yeast growth.
- The presence of NH_4^+ delays both the timing and extent of amino acid incorporation into the yeast cell.
- NH_4^+ is not only incorporated preferentially to FAN, but also alters the established pattern of amino acid uptake (Jiranek et al. 1990).
- Timing of nitrogen supplementation (and the form of supplement) may play a crucial role in successful completion of alcoholic fermentation and volatiles produced.

d. Free Alpha-Amino Acid Nitrogen (FAN). All 20 of the commonly occurring *alpha*-amino acids are found in grapes and wine. Their total concentration is 0.4-6.5 g/L (Wurdig and Woller 1989). FAN nitrogen includes assimilable primary or free *alpha*-

amino acids. Collectively, this group comprises 75-85% of the total amino acids (Wurdig and Woller 1989). Of the FAN amino acids, arginine is typically present at levels 5-10 times that of the other amino acids, and represents 30-50% of the total nitrogen utilization (Henschke and Jiranek 1993).

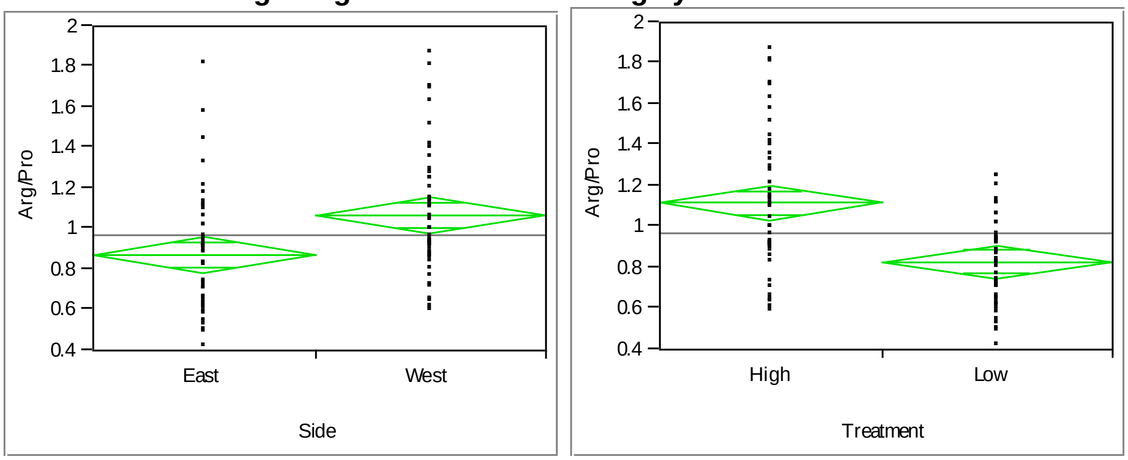
Most grape growing and winemaking decisions can influence the relative proportion of FAN/NH_4^+ fraction and total YAN, including the following:

- climate
- season
- grape variety
- rootstock
- soil type
- soil moisture
- irrigation practices
- cover crops, mulch
- fertilization
- vine diseases
- nutrient and/or mineral deficiencies
- maturity
- processing methodology

Arginine is frequently the most abundant FAN amino acid in fruit, while proline is often the most abundant non-FAN amino acid, and cannot be used by fermenting yeast. With nitrogen fertilization (> 3g N/plant), the amino acid profile can change (Sponholz 1991). YAN and YAN components (FAN and ammonia) can also be different among vineyard blocks and growing regions, and can be impacted by vineyard management practices.

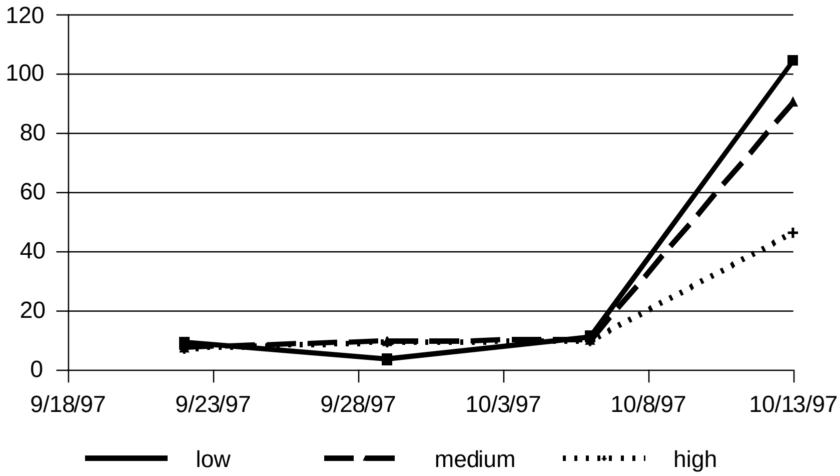
As discussed in *Enology Notes* # 167 certain vineyard management activities can influence fruit nitrogen. Figures 1 and 2 show results from several of our studies evaluating the effect of canopy side and crop load on mature VSP-trained Cabernet Sauvignon grapes grown on N-S facing rows in Virginia (de Bordenave and Zoecklein 1999). Both the crop load and canopy side impacted the ratio of arginine (a FAN amino acid) and proline (a non-FAN amino acid). The line across each diamond plot below represents the group mean, while the vertical span of each diamond indicates the 95% confidence interval for each group. Both the canopy side (fruit from east vs. west) and crop load (2.5 kg/vine vs. 4.5 kg/vine) demonstrated differences in arginine-to-proline ratios.

Figures 1 and 2. Effect of canopy side and crop load on the arginine/proline ratio of Cabernet Sauvignon grown on VSP training system for one season.



Grape maturity and crop load are important issues influencing the concentration of YAN. As seen in Figure 3, the concentration of FAN nitrogen (a component of YAN) in Cabernet Sauvignon grapes changes as a function of maturity and crop load.

Figure 3. FAN nitrogen (mM) in Cabernet Sauvignon grape clusters thinned to low (2.6 kg/vine), medium (4.9 kg/vine) and high (5.3 kg/vine) crop level in 1997. (de Bordenave and Zoecklein 1999)



e. Monitoring Plant Nitrogen. As reported in *Enology Notes* #168, many French winemakers determine vine nitrogen status using juice analysis of YAN at harvest.

Using YAN analysis as a plant barometer is not a new concept. It may represent a simple way of evaluating vine nitrogen status and may allow for the assessment of parcel or block variations.

f. Yeast Strains and YAN. The following growth requirements may be significantly different among strains of *Saccharomyces* spp.:

- nitrogen requirements
- oxygen requirements
- time of uptake and release of specific amino acids during fermentation
- ability to ferment to dryness
- concentration of H_2S and other sulfur-like off-odor compounds produced
- magnitude of response to environmental conditions

FAN amino acids are not incorporated equally by yeast, and preferences may vary significantly among strains (Manginot et al. 1998). Some are utilized at the beginning of the growth cycle, some later, and some not at all. Ammonia, on the other hand, is consumed preferentially to amino acids in growing populations.

g. Processing and YAN. Pre-fermentation maceration (cold soak) and native fermentations (both yeasts and lactic acid bacteria) may deplete FAN and vitamins required by *Saccharomyces* spp.

- Native yeast and bacteria, present initially at relatively low population densities, require significant amounts of YAN and vitamins to build biomass.
- By the time *Saccharomyces* spp. populations become established, levels of available nitrogen may be too low for complete fermentation or to optimize desirable aroma production.

Winemaking practices coupled with juice clarification may impact nutritionally important substrates including YAN (Guitart et al. 1998; Houtman and duPleissis 1981). Important processing conditions that impact YAN include the following:

- whole cluster pressing vs. crush and drain
- saignée or bleeding (removal of a portion of the red juice after a short exposure to the skins) vs. non-dejuiced
- short vatting vs. extended post-fermentation maceration

Amino acids, including FAN amino acids, are not equally distributed in the grape. For example, in mature Cabernet Sauvignon, about 8.5% of the total is in the seeds, 15% in the skins, and 77% in the pulp. Separation of the pulp juice from the skins, as occurs with bleeding, has a significant qualitative influence and quantitative impact on FAN.

The two amino acids present in the greatest concentration in the fruit are usually proline and arginine. Proline cannot generally be used by the yeast, while arginine can and is a very important FAN component.

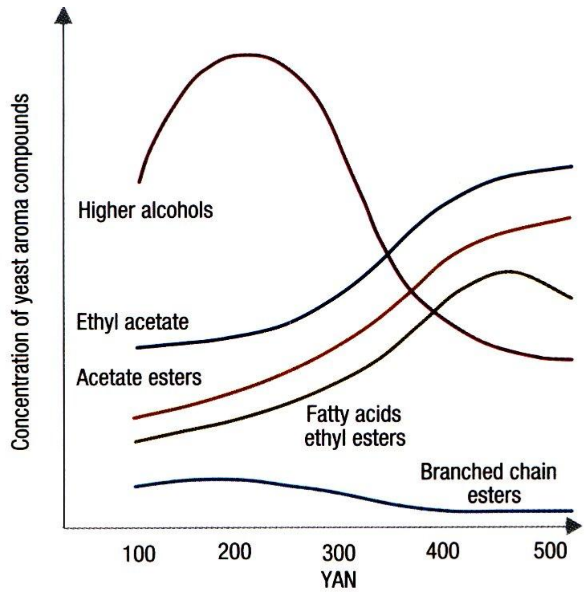
In the case of Cabernet Sauvignon (and likely most other red varieties), the ratio of arginine to proline is much greater in the skins than the pulp. Pulp juice, which is removed during bleeding or saignée, has a relatively high concentration of proline (approximately 55%) which cannot be used by the yeast, and a small concentration of the more potent amino acid arginine and others needed to carry out a healthy fermentation. The lower incidence of incomplete fermentation in red musts, compared with white, supports the concept that the slow release of nitrogen from grape skins during fermentation is important.

- Wines produced by bleeding may require a higher concentration of supplemental nitrogen due to the relatively high proline versus arginine in the pulp juice. This is a very important rosé production consideration.
- Nitrogen concentrations in white juice can be reduced by 10-15% following cold settling.
- Fining agents may serve to further deplete vital nitrogen sources.

h. YAN Impact on Aroma/Flavor Compounds. Due to the importance of fermentation-derived volatiles in the aroma character of a wine, yeast metabolic pathways that are influenced by YAN levels are of particular interest and importance (Ugliano et al. 2007).

Various studies have demonstrated that the amount of available nitrogen can affect the production of different groups of volatile compounds. Figure 4 (Ugliano et al. 2007) shows a summary of general trends. These trends are conditional on yeast strain and fermentation conditions, but do show potential consequences of indiscriminate YAN supplementation of a must, particularly with DAP (25.8% ammonia).

Figure 4. Relationship between initial YAN concentration and final concentration of volatile compounds after fermentation. (Ugliano et al. 2007)



The following summarizes the relationships between YAN and aroma compounds:

- Higher alcohols have fusel oil-like characters that may contribute to complexity, but may also mask fruity character at higher levels.
- Fatty acid ethyl and acetate esters generally contribute to fruity character.
- High levels of ethyl acetate can give nail-polish-remover or solvent characters.
- Branched chain esters can be significant contributors to the red-berry fruit character of some red wines.
- Assimilable nitrogen availability can contribute significantly to wine aroma and flavor, and, as a result, supplementation should be done with caution after determining YAN concentrations.

i. Supplement Products vs. Native YAN. Many winegrowers in Europe profess to have adequate native YAN, avoiding the need to supplement, even if they accepted such practices. In the New World, many assume additional nitrogen is needed for fermentation and that there is no difference between native YAN produced by the plant and addition products, an unwarranted assumption. There is evidence that additions fail to enhance the fermentation as much as does natural, grape-produced nitrogen (Sinton et al. 1978, Treeby et al. 1996).

Nutritional supplementations are frequently conducted using either ammonium salt, diammonium phosphate (DAP: 25.8% ammonia, 74.2% phosphate), or proprietary blends containing amino acids, minerals, and vitamins. In the U.S., the legal limit of DAP is 960 mg/L, which corresponds to 203 mg N/L. But what is the relationship between these compounds and native, plant-derived YAN? There is evidence that additions fail to enhance the fermentation as much as does natural, grape-produced nitrogen (Sinton et al. 1978, Treeby et al. 1996).

Those using supplements generally advocate the use of balanced nutritional formulations in lieu of DAP. In addition to a readily-available form of nitrogen, many propriety products may contain amino acids, minerals, vitamins, and/or other ingredients important for yeast growth, including some or all of the following:

- inorganic N (DAP)
- organic N (*alpha*-amino acids)
- unsaturated fatty acids
- sterols, thiamine, folic acid, niacin, biotin, and calcium pantothenate
- magnesium sulfate
- inactive yeast cell walls
- peptides, including glutathione
- micro-crystalline cellulose
- other yeast products

Other yeast products include a wide variety, including inactivated yeast, yeast autolysates, extracts and yeast hulls/ghosts. Yeast hulls can have very good adsorbing capacities, depending on how they were produced. Their main role during fermentation is to bind to toxic medium-chain fatty acids secreted by the fermenting yeasts, thereby detoxifying the environment and allowing the fermenting yeast to ferment to dryness. If yeast cell walls contain parts of the cell membrane, they can also be a source of sterols and lipids. Yeast cell walls may be added to enhance fermentation rates, as a source of nutrients, and to restart stuck fermentations.

- Yeast hulls stimulate yeast populations by providing a source of C_{16} and C_{18} unsaturated fatty acids, which act as oxygen substitutes under long-term fermentative conditions (Ingledew 1996).
- Hulls may provide a source for some amino acids, as well as surface area to facilitate release of potentially inhibitory levels of CO_2.
- Because yeast hull preparations contain lipids (fats) that oxidize upon exposure to oxygen, they may degrade and develop a "rancid" character upon extended storage. They should be evaluated sensorially prior to each use.

There are inactivated yeast-based products recommended for enhancing white wine longevity. These are not really nutrients, but rather are a source of glutathione (see *Enology Notes* Index).

2. Dehydration Research Review

Producers around the world use fruit dehydration as both a means of increasing wine quality potential from fruit harvested early and as a stylistic tool. Procedures involve the use of dehydration sheds, converted tobacco barns or simply dehydration trays in the field. Procedures can be time consuming, result in variable quality and, in the case of heated sheds, energy intensive.

Last season, Dr. Molly Kelly and I began an evaluation of dehydration techniques. We hope to lower the cost of production and improve quality by evaluating a proprietary compound shown to cause the microscopic wax cuticle platelets of the grape to essentially realign.

The wax cuticle of grapes is comprised mostly of even-numbered carbon-chain alcohols, aldehydes, acids, esters, and hydrocarbon chains, with oleanolic acid as the main constituent. These are produced in the fruit and are responsible for controlling cuticular transpiration. We were able to increase the dehydration rate by a factor of two or three by making the wax platelets hydrophilic, allowing water to more easily transfer directly into the atmosphere.

Preliminary data was collected during the 2013 season using Cabernet franc harvest and dehydrated in a tobacco barn. We compared non-dehydrated fruit, with fruit dried for 48 hours, and with fruit treated with a dehydrating agent for 48 hours. Results demonstrated significant changes in all berry parameters (Table 1).

Table 1. Effect of 48-Hour Dehydration in Air and Using a Dehydrating Agent on Cabernet Franc Grape Parameters.

ASSAY	CONTROL	DEHYDRATED	DEHYDRATED+TREATMENT
BERRY WEIGHT (g)	1.95	1.84	1.59
YAN (mg/L N)	288	323	505
DEGREES BRIX	21.8	28.5	35.5
MALIC (g/L)	3.82	3.00	5.23
pH	4.11	4.03	4.21
TA (g/L)	4.77	6.53	8.64

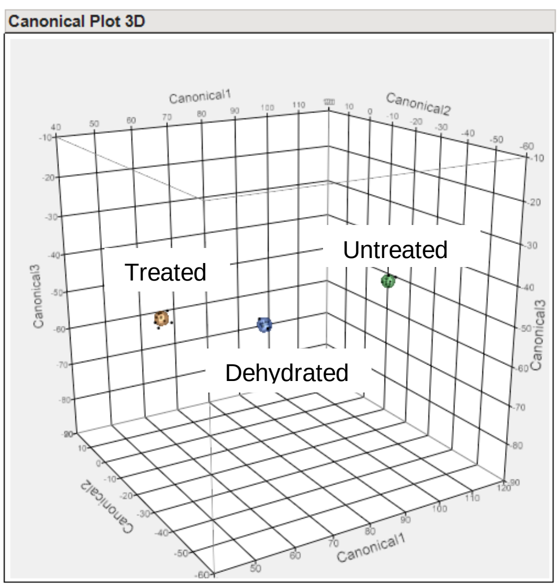
As can be noted, there was a considerable increase in the extent of dehydration as a result of cuticle modification, resulting in changes in the concentrations of fruit components.

We are particularly interested in the impact on fruit and subsequent wine volatiles. From the research of several groups, we know that some of the important positive red fruit volatile components include β-damascenone, β-ionone, benzyl alcohol, phenethyl alcohol and various terpenes, while negative components may include methoxypyrazines, hexanal, and some 6-carbon compounds. This is essentially the balance of fruity-type compounds vs. green and underripe components.

In previous studies, we have used electronic nose technology to determine relative changes in these and other important fruit and wine volatiles, including the evaluation of fruit maturity (Athamneh et al. 2008, Martin et al. 2008), vineyard management practices (Devarajan et al. 2011, Zoecklein et al. 2011, Kelly et al. 2013), and wine processing (Gardner et al. 2011).

The canonical plots below demonstrate the ability of a conducting polymer electronic nose to distinguish among treatments (Figure 5). Differences are indicated by non-intersecting circles. This suggests that the volatile compositions of the berries are different. Current GC/MS analysis will aid in our ability to define those differences. More to follow.

Figure 5. Effect of 48-Hour Dehydration in Air and Using a Dehydrating Agent on Cabernet Franc Grape Volatiles, as Measured by a Conducting Polymer Electronic Nose.



3. In Memory of Dr. Justin Morris

Dr. Justin Morris, an internationally recognized grape and wine leader, passed away recently at age 77.

Few people have accomplished the level of consistent excellence in teaching, research, and leadership as did Dr. Justin Morris. He was a friend and mentor who possessed a rare combination of intellect and personality that made him a very popular and an effective leader.

He was a Fellow in the American Society of Horticultural Sciences and the Institute of Food Technologists, received the American Society for Enology and Viticulture Merit Award in 1996, the Food Industry's Forty-Niner's Service National Award in 1998, and the American Wine Society Award of Merit in 1999. He was inducted as a Supreme Knight by the Order of Knights of the Vine in 2004, to the Arkansas Horticulture Hall of Fame in 2005, and to the Arkansas Agriculture Hall of Fame in 2009. These are just a few of his many awards. Justin published more than 410 research and trade articles, 30 book chapters and two books! His students are in positions of prominence in wine regions around the world!

Additionally, Justin Morris was a pioneer in mechanized agriculture research. However, I am convinced that his most outstanding accomplishment was to convince a young, green, brash Californian to move east and to channel his efforts from commercial winemaking to a career in academics. For this I am forever grateful!

Fortunately for all that knew Justin Morris, someone who is not completely forgotten never completely dies. His legacy will live on within his extensive scientific contributions and the countless lives he has touched!

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