VARIETY SPECIFIC THRESHOLDS FOR PLANT-BASED INDICATORS OF VINE NITROGEN STATUS

Agnès Destrac-Irvine, Elisa Marguerit, Mark Gowdy, Bruno Suter, Julien Fort, Francesco Rinaudi and Cornelis van Leeuwen

EGFV, Bordeaux Sciences Agro, INRAE, Univ. Bordeaux ISVV, F-33882 Villenave d'Ornon, France

*Corresponding author: agnes.destrac-irvine@inrae.fr

Abstract

Aim: Several plant-based indicators of vine N status are reported in the literature. Among these, yeast assimilable nitrogen in grape must (YAN) and total N concentration of petiole and leaf blades are considered to be reliable indicators and so is the chlorophyll index, measured with a device called N-tester. The N-tester index is used to measure the intensity of the green colour of the leaf blade, and therefore to estimate its chlorophyll content. The aim of this study is to measure the nitrogen content of various grapevine organs (petiole, leaf blade, grape must) and the intensity of the green colour of leaf blades, in order to establish variety specific thresholds for the interpretation of plant-based indicators of vine nitrogen status.

Methods and Results: To study the varietal effect on indicators of vine N status, the latter were measured during 4 years on 35 grapevine varieties grafted on the same rootstock and planted with replicates in an experimental vineyard in the Pessac-Léognan appellation in Bordeaux. The results of N-tester measurements carried out at mid-flowering and mid-véraison were compared with the nitrogen content of leaf blades and petioles at véraison and the concentration of yeast assimilable nitrogen (YAN) in the must at maturity.

Conclusions: Strong varietal and year effects were observed for all indicators. Leaf blade nitrogen showed the lowest variability and YAN the highest. The N-Tester values recorded at mid-flowering were more consistent than those at mid-véraison.

Significance and Impact of the Study: Among the nutrients required by the vine, nitrogen is one of the most important. It is an essential factor in vegetative and reproductive development. Vine nitrogen status influences grape composition and wine quality. In addition, a low concentration of assimilable nitrogen in the must causes fermentation problems because N is one of the essential substrates for yeast growth. Vine N status depends on environmental factors (soil and climate) but can be managed through fertilisation and vineyard floor maintenance. Hence, plant-based indicators for vine nitrogen status are of utmost importance to optimize management practices for obtaining high wine quality and sustainable yields. The data generated by this experiment can help to take into account varietal specific responses to nitrogen availability when establishing thresholds for plant-based indicators of vine N-status. An example is provided for N-tester values at mid-flowering.

Keywords: Vine nitrogen status, petioles, leaf blades, must

Introduction

Nitrogen is an important nutrient for vines. Nitrogen is absorbed over the whole season, from budbreak to leaf fall. Although it is generally considered that most intense nitrogen uptake occurs in spring (between budbreak and flowering) and post-harvest, Conradie (1986) reports that the period with the highest absorption rate is situated between the end of active shoot growth and veraison. Translocation of nitrogen between perennial parts (roots, trunks), shoots and grapes is also an important active process by which the vine ensures its internal nitrogen balance (Keller, 2020; Verdenal *et al.,* 2020). Hence, nitrogen content in vine organs varies over the season.

Grapevine nitrogen status is a major driver of vigour, yield, primary and secondary metabolites in grapes, as well as sensitivity to fungal diseases (Keller, 2010; Verdenal *et al.,* 2020). Vine nitrogen status is determined by the soil type, the climatic conditions which impact organic matter turnover and cultural practices like fertilisation and vineyard floor management (van Leeuwen *et al.,* 2000). Vine nitrogen status has a major effect on wine quality, in particular through its impact on secondary metabolites. Low vine nitrogen status increases phenolic compounds in grapes (Hilbert *et al.,* 2003), but negatively impacts on aromas like those from the thiol family (Helwi *et al.,* 2016). Because phenolic compounds are important attributes for red wines, and aromas from the thiol family for many white wines (e.g. from Sauvignon blanc, Sémillon and Riesling), optimum vine N-status is lower for red varieties compared to white varieties. Because vine N-status is partly determined by soil and climate conditions, it can be considered as a terroir factor (van Leeuwen *et al.,* 2018). It can, however, also be easily modulated though cultural practices.

Assessment of vine N-status is of utmost importance for growing high quality potential grapes under sustainable yields. Measurement of nitrogen availability through soil analyses is not convenient. Neither soil total nitrogen, nor soil mineral nitrogen, are accurate indicators to account for the vine's nitrogen status. Soil total nitrogen does not reflect nitrogen availability, because it comprehends mainly nitrogen contained in organic matter, which first has to be broken down by the soil microflora before the released nitrogen (NO_3^- and NH_4^+) can be absorbed by the vines. Soil mineral nitrogen varies over the season, depending on the intensity of organic matter turnover (mineralisation), leaching, and absorption by the vines; it can only be used for nitrogen availability assessment when measurements are repeated several times during the season.

Plant based indicators perform much better. Several indicators are reported to be reliable, including petiole total N content, leaf blade total N content, yeast assimilable nitrogen (YAN) and leaf blade colour intensity measured with a device called "N-tester" (Yara, Oslo, Norway), also called "chlorophyll index". This tool is based on the technology of a Minolta SPAD (Minolta, Tokyo, Japan), but with a specific calibration (van Leeuwen *et al.,* 2000). Although these four indicators are easy to measure, it is very difficult to establish standard values, for several reasons. First, nitrogen content in plant organs varies over the season. This means that standards apply to a specific timing of measurement, ideally based on phenological stages. Flowering and véraison are key stages for measuring vine N status. Second, optimum vine N status is a relative concept, which depends on production objectives: optimum vine N status increases with yield objectives, and decreases with quality objectives, in particular for the production of red wines. Third, for a similar N availability to the vines, accumulation of nitrogen in petioles, leaf blades, grapes (YAN) and leaf blade colour (assessed with N-tester) is highly variable across varieties. This means that interpretation standards for plant-based indicators of vine N status need to be variety specific.

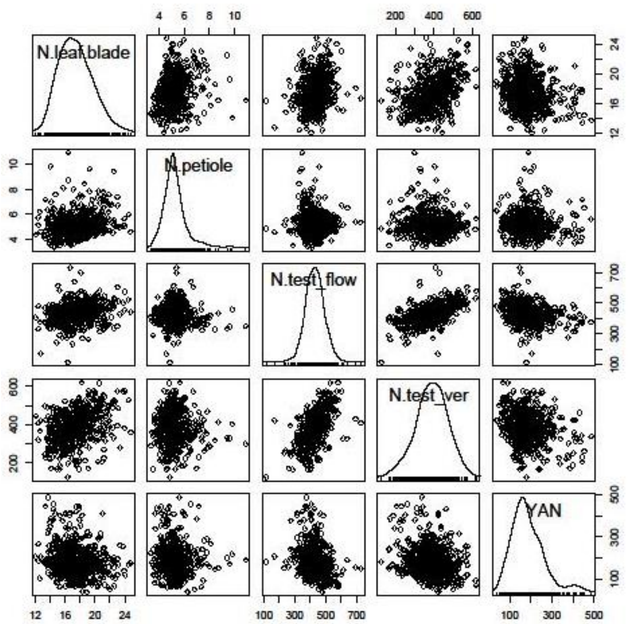
The objectives of this research are to compare five plant-based indicators of vine N status across a wide range of varieties. Petiole total N content at véraison, leaf blade total N content at véraison, grape YAN at sugar ripeness and leaf blade colour intensity measured by N-tester at 50% flowering and 50% véraison were assessed on 35 varieties cultivated in a common garden over four years. The range of variability of each indicator is measured and year effect versus variety effect is discussed. For N-tester values at 50% flowering, corrections are proposed to take into account the variety effect when establishing standards for interpretation.

Materials and Methods

Measurements were carried out in the VitAdapt common garden, located in F-33140 Villenave d'Ornon, close to Bordeaux (France) at 44°47'23.83 N'', 0°34'39.3'' W (Destrac *et al.,* 2016). VitAdapt encompasses 52 varieties (31 red, 21 white). Ten vines of each variety are planted in five blocks (replicates), summing up to a total of 50 vines per variety. All varieties are grafted on SO4 rootstock. Five plants based indicators (petiole total N at mid-véraison, leaf blade total N at mid-veraison, YAN at 95% of maximum sugar accumulation and leaf blade colour intensity measured at mid-flowering and mid-véraison) were measured during four years (2015, 2016, 2017 and 2019) in four blocks of the VitAdapt common garden on 35 varieties (21 reds, 14 whites). 16 values (four years, four replicates each year) were available for statistical analyses. A possible effect of soil variability was neutralized by taking the measurements for each variety in four locations (blocks) in the field. Petiole and leaf blade total N was measured on oven dried petioles and leaf blades (Dumas method). YAN was measured in a WineScan^{TM} by Fourier Transform InfraRed Spectroscopy (FTIR). The WineScan^{TM} YAN measurements were calibrated by colorimetric method for alpha amino nitrogen and enzymatic method for ammonium, where YAN = alpha amino N + NH_4^+. N-tester measurements were carried out according to operating procedure provided by the constructor (Yara, Oslo, Norway). For each measurement in each replicate block, 30 leaves were sampled and values were averaged.

Results

Values for the four plant based vine N status indicators showed great dispersion, depending on year, variety and block (Figure 1A). Determination coefficients between indicators were weak (Figure 1). Highest correlation coefficient was observed between N-tester measured at mid-flowering and mid-veraison (0.58; Figure 1B).



A

	N leaf blade	N petiole	Ntest_flo	Ntest_ver	YAN
N leaf blade		r = 0.2120 p-value: $4.125e^{-07}$	r = 0.2554 p-value: $8.604e^{-10}$	r = 0.3951 p-value: $2.2e^{-16}$	r =-0.1916 p-value: $4.981e^{-06}$
N petiole	r = 0.2120 p-value: $4.125e^{-07}$		r = -0.0709 p-value: 0.09387	r =-0.0681 p-value: 0.1074	r = -0.0507 p-value: 0.2314
Ntest_flo	r = 0.2554 p-value: $8.604e^{-10}$	r = -0.0709 p-value: 0.09387		r = 0.5831 p-value: $2.2e^{-16}$	r = -0.2766 p-value: $2.717e^{-11}$
Ntest_ver	r = 0.3951 p-value: $2.2e^{-16}$	r =-0.0681 p-value: 0.1074	r = 0.5831 p-value: $2.2e^{-16}$		r = -0.2449 p-value: $4.332e^{-09}$
YAN	r =-0.1916 p-value: $4.981e^{-06}$	r = -0.0507 p-value: 0.2314	r = -0.2766 p-value: $2.717e^{-11}$	r = -0.2449 p-value: $4.332e^{-09}$	

B

Figure 1: A - Scatterplot between YAN at 95% of maximum sugar accumulation, petiole total N at mid-véraison, leaf blade total N at mid-véraison and leaf blade colour intensity measured at mid-flowering and mid-véraison. B - Pearson coefficients. Green boxes indicate significant correlations at 0.05 or higher.

Dispersion of the values across varieties was assessed by the coefficient of variation (Cv%), which ranged from 5.5% (Ntot leaf blade) to 22.9% (YAN) (Table 1).

Table 1: Average, standard deviation and coefficient of variation for the five indicators of vine N-status across 35 varieties.

	N petiole (%DW)	N leaf blade (%DW)	YAN (mg/L)	Ntest_flow	Ntest_ver
average	5.22	17.43	185	430	389
standard deviation	0.51	0.96	42	41	54
Cv%	9.8%	5.5%	22.9%	9.6%	13.9%

The total variance of the measured values is the result of a variety effect (its assessment is the objective of this research), a year effect (vine nitrogen status varies from year to year depending on climatic conditions during the spring, which impact mineralisation speed of organic matter in the soil) and a block effect. In this trial, the block effect, although statistically significant, was very small compared to the variety and year effect and can be neglected. Values were in a similar range in each block and can be considered as non-limiting vine nitrogen status conditions. The variety effect was greater than the year effect, although the contribution to the total variance varied according to the indicator considered (Table 2). Grapevine variety explained 17.6% (N leaf blade) to 40.6% (N tester véraison) of the total variance. Grapevine variety × year interaction was always significant, which means that the order of the grapevine varieties was different for each indicator in each year. This interaction was, however, relatively low (although significant) for YAN and N-tester at mid-flowering and veraison.

Table 2: Percent of variance explained by grapevine variety, year, interaction and residuals (2-way ANOVA).

	N petiole (%DW)	N leaf blade (%DW)	YAN (mg/L)	Ntest_flow	Ntest_ver
Grapevine variety	31.2%	17.6%	30.6%	39.9%	40.8%
Year	9.4%	16.4%	29.7%	25.0%	27.3%
Grapevine variety × year	25.0%	32.0%	16.0%	11.8%	12.8%
Residuals	34.4%	34.0%	23.7%	23.3%	19.1%

Values for YAN and N-tester at mid-flowering are presented as boxplots in Figure 2. Dispersion of values is greater for YAN compared to N-tester at mid-flowering, which confirms the data presented in Table 1.

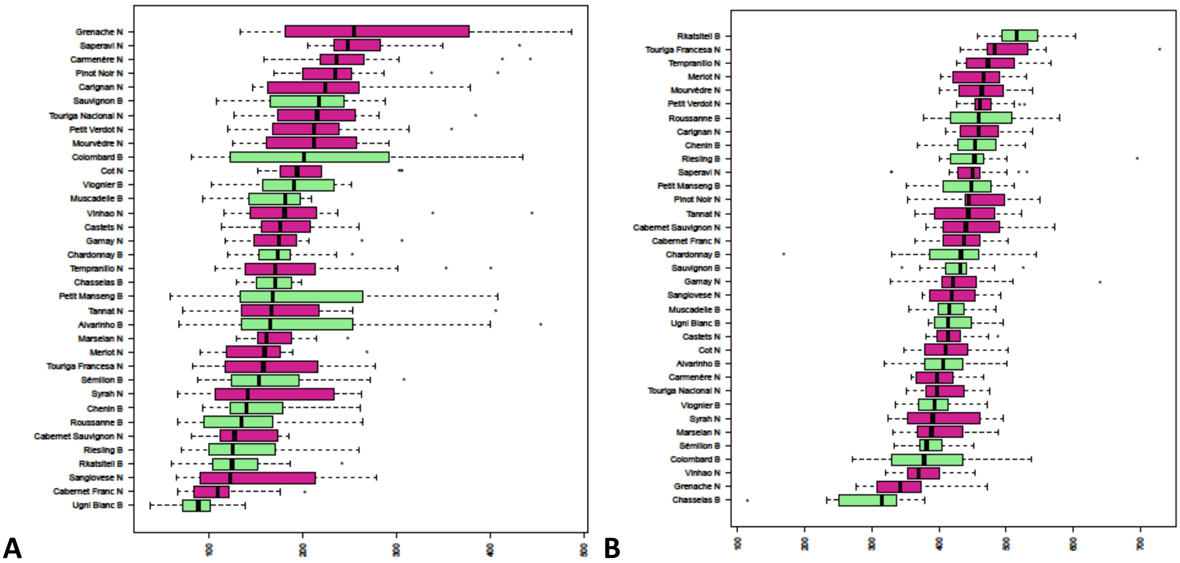


Figure 2: Boxplot of YAN in mg/L (**A**) and N-tester values at mid-flowering (**B**) across 35 varieties. Each box represents 16 values (4 years, 4 replicate blocks in each year).

Figure 2 clearly shows the variety effect on the physiological indicators investigated. Ugni blanc, Cabernet franc and Sangiovese accumulate very little YAN in their grapes, while Grenache, Saperavi, Carmenère and Pinot noir heavily accumulate YAN in their grapes in the same soil and climate conditions. Chasselas, Grenache and Vinhao have pale green leaves, resulting in low N-tester values at mid-flowering, while under the same soil and climate conditions the green colour of the leaves of Rkatsiteli, Touriga franca, Tempranillo and Merlot is much darker.
These observations can be used for the interpretation of standard values. In this trial varieties were cultivated in the same climate and soil conditions and nitrogen availability can be considered similar for each variety. The observed differences result from variety specific behaviour under similar conditions. For N-tester measurements at flowering, Sauvignon blanc scored a value similar to the average value across all varieties and can thus be considered as a reference variety for this indicator. If N-tester values are to be compared across varieties, a correction relative to Sauvignon blanc can be proposed based on our results (Table 3). Varieties were grouped according to an Ascendant Hierarchical Classification (AHC) and the proposed corrections are rounded for each group. If the proposed correction is positive it means that the values of the variety considered had a lower value than Sauvignon blanc whereas, if the proposed correction is negative, it means that the values of the variety considered had a higher value than Sauvignon blanc.

Table 3: Average N-tester values at mid-flowering for 35 cultivars (n = 16). Different colours indicate different groups according an Ascendant Hierarchical Classification (AHC). Relative differences to Sauvignon blanc (reference variety) are indicated. Rounded corrections can be used when N-tester values are compared across different varieties.

Variety	Average N-tester at flowering	Correction for comparison with reference variety (Sauvignon blanc)	Rounded correction
Chasselas B	319	+111	+70
Grenache N	348	+82	
Vinhao N	376	+54	
Colombard B	385	+45	
Sémillon B	386	+44	
Viognier B	395	+35	Varieties with intermediate N-tester index (no correction)
Carmenère N	399	+31	
Syrah N	400	+30	
Marselan N	403	+27	
Touriga nacional N	408	+22	
Alvarinho B	409	+31	
Cot N	415	+15	
Chardonnay B	417	+13	
Muscadelle B	421	+9	
Castets N	422	+8	
Sangiovese N	422	+8	
Ugni blanc B	425	+5	
Sauvignon blanc B	430	0	
Gamay N	435	-5	
Cabernet franc N	436	-6	
Saperavi N	443	-13	-30
Petit Manseng B	445	-15	
Cabernet Sauvignon N	452	-22	
Tannat N	453	-23	
Chenin B	454	-24	
Riesling B	459	-29	
Pinot noir N	460	-30	
Merlot N	461	-31	
Carignan N	463	-33	
Roussanne B	464	-34	
Mourvèdre N	465	-35	
Petit Verdot N	471	-41	
Tempranillo N	481	-51	
Touriga franca N	508	-78	-80
Rkatsiteli B	520	-90	

Conclusion

Plant-based indicators are widely used to assess vine nitrogen status in grapevines. Several indicators are reliable and widely used to compare soil effect, climate effect, or cultural practices (fertilisation or vineyard floor maintenance) for a given variety cultivated under different conditions. Until now, it was not possible to use these indicators when two or more varieties were considered, because of variety-specific behaviour. Our results provide a tool to correct raw data acquired on different grapevine varieties for comparison purposes

References

Buckee, GK., 1994. Determination of total nitrogen in barley, malt and beer by kjeldahl procedures and the Dumas combustion method collaborative trial. Journal of the Institute of Brewing, 100(2): 57-64.

Conradie, WJ., 1986. Utilisation of nitrogen by the grape-vine as affected by time of application and soil type. South African Journal of Enology and Viticulture, 7(2): 76-83.

Destrac-Irvine, A., van Leeuwen, C., 2016. VitAdapt: an experimental program to study the adaptation of a large range of Vitis vinifera varieties for Bordeaux vineyards. In: *ClimWine 2016: Sustainable grape and wine production in the context of climate change*, pp. 155-171.

Helwi, P., Guillaumie, S., Thibon, C., Keime, C., Habran, A., Hilbert, G., Gomès E., Darriet P., Delrot S., van Leeuwen, C., 2016. Vine nitrogen status and volatile thiols and their precursors from plot to transcriptome level. BMC Plant Biology, 16(1): 173.

Hilbert, G., Soyer, JP., Molot, C., Giraudon, J., Milin, M., Gaudillere, JP., 2015. Effects of nitrogen supply on must quality and anthocyanin accumulation in berries of cv. Merlot. Vitis, 42(2): 69.

Keller, M. (2020). *The Science of Grapevines*. Academic Press: Cambridge, Massachusetts, USA.

Soyer, JP., Choné, X., Friant, P., Molot, CH., van Leeuwen, C., Dubordieu, D., 2000. L'intéret du dosage de l'azote total et de l'azote assimilable dans le moût comme indicateur de la nutrition azotée de la vigne. Journal International des Sciences de la Vigne et du Vin, 34(2): 75-82.

van Leeuwen, C., Roby, JP., de Rességuier, L., 2018. Soil-related terroir factors: A review. OENO One, 52(2): 173-188.

van Leeuwen, C., Destrac-Irvine, A., Dubernet, M., Duchêne, E., Gowdy, M., Marguerit, E., Pieri, P., Parker A., de Rességuier, L., Ollat, N., 2019. An update on the impact of climate change in viticulture and potential adaptations. Agronomy, 9(9): 514.

Verdenal, T., Dienes-Nagy, A., Zufferey, V., Spring, J-L., Viret, O., Marin-Carbonne, J., van Leeuwen, C., 2020. Understanding and managing nitrogen nutrition in grapevine : a review. OENO One, 54(4), in press.