TOOLS FOR ASSESSING VINE NITROGEN STATUS; ROLE OF NITROGEN UPTAKE IN THE "TERROIR" EFFECT

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INTRODUCTION

Among the numerous nutrients vines extract from the soil, nitrogen is the one that interferes most with vine vigor, yield, berry constitution and wine quality. Many studies relate on the influence of various levels of nitrogen fertilization on vine growth, yield and berry constitution (KLIEWER, 1971; BELL et *al.*, 1979; DELAS et *al.*, 1991; SPAYD et *al.*, 1993; SPAYD et *al.*, 1994). Other papers deal with the depressive effect of cover crop on vine nitrogen supply, which can partly explain the quality-improving effect of this technique (SOYER et *al.*, 1996).

Vine nitrogen uptake is likely to vary to a considerable extend with soil parameters, even when no nitrogen fertilization or cover crop occurs. Figuring among those parameters are: soil organic matter content, C/N ratio of soil organic matter and soil organic matter turnover. The latter depends mainly on soil temperature, soil aeration, soil pH and soil moisture content. Despite considerable empirical evidence, almost no literature is available on vine nitrogen status as a function of soil characteristics and the impact of this status on vine development, berry constitution and wine quality. This might be explained by the lack of accuracy of currently available indicators of vine nitrogen status, such as petiole or leaf blade nitrogen content, or their lack of accessibility, as is true for cane arginine content. In this paper we discuss the use of several forms of nitrogen in grape juice (must) as indicators of vine nitrogen status. The accuracy of these indicators provides the means to differentiate nitrogen offer by the soil in "terroir" studies and assess its impact on berry quality potential.

II- MATERIELS AND METHODS

II-1 Experimental plots

Two levels of mineral nitrogen supply, 0 kg/ha and 45 kg/ha, were compared in 1999 on a low nitrogen supplying soil in the Saint-Emilion region (France). Vines were 11 years old *Vitis vinifera* cv. Merlot noir, grafted on Riparia Gloire de Montpellier rootstock and planted at 6,000 vines per hectare. Both fertilization levels were replicated five times. Five techniques for vine nitrogen diagnosis were compared in this fertilization trial.

Nitrogen supply to vines depending on soil characteristics was studied in 1999 on three soils in the Saint-Emilion region: a gravelly soil, containing over 50% gravel, a heavy clay soil and a sandy soil, having a water table within the reach of the roots. On each soil, 100 vines of *Vitis vinifera* cv. Merlot, *Vitis vinifera* cv. Cabernet franc and *Vitis vinifera* cv. Cabernet-Sauvignon were grafted in 1996 on adult vines. Vine density was 6,000 vines per hectare and rootstocks were Riparia x Rupestris (3309C on gravel and clay; 101-14 Mgt on sand). No mineral nitrogen had been applied to any of these plots for several years.

II-2 Techniques for assessing vine nitrogen status

In the 1999 Saint-Emilion fertilization experiment, vine nitrogen status was assessed by petiole total nitrogen content, leaf blade color intensity measured by a device called "N-tester", grape juice total nitrogen content, grape juice assimilable nitrogen content and grape juice NH_4^+ content.

Pétiole total nitrogen content

On each replicate, 100 petioles were sampled randomly. Petioles were dried and ground to powder. The powder was mineralized by a Kjeldahl attack. Liberated NH_4^+ was analyzed by colorimetric method (autoanalyser).

Leaf blade color intensity

Leaf blade color intensity was measured by means of a "N-tester". N-tester is an appliance which is commercially used to adjust nitrogen fertilization during the growing season of rice and wheat. After having pinched 30 undamaged, adult, primary leaves, the N-tester displays a value ranging between 400 and 650. High values indicate dark colored leaves and hence high nitrogen supply; low values indicate pale green leaves, resulting from low nitrogen supply.

Must nitrogen content.

Three forms of nitrogen in must were tested for their accuracy as vine nitrogen status indicators: total nitrogen, assimilable nitrogen and NH_4^+ nitrogen.

Must total nitrogen content is obtained by mineralizing 5 ml of must with 15 ml of concentrated sulfuric acid at high temperature (400°C, Kjeldahl attack). All forms of nitrogen (proteins, amino acids....) are broken down into NH_4^+. NH_4^+ is transformed to NH_3 by addition of NaOH 10N. NH_3 is distilled and measured out by titration. Mineralizing must is difficult, mainly because of the presence of large amounts of sugar. This operation can take more than 12 hours on an electric burner or over open fire and it can fail because of caramelization and the appearance of foam.We tested must mineralization by means of microwave. This clean, rapid technique (1 hour), which prevents foam formation and caramelization, is presented in "results".

Must assimilable nitrogen was measured out by the formol method (also called: Sörensen method). 25 ml of must is taken to pH 8.5 by the addition of NaOH. 10 ml of formol at pH 8.5 is added. Formol reacts with NH4+ and with the -NH3+ function of amino acids. Liberated H+ is proportional to the assimilable nitrogen content in the sample and can be titered with NaOH 0.05N.

Must NH4+ nitrogen was measured out by means of an enzymatic kit (Boehringer, Mannheim, Germany).

III- RESULTS

III-1 Measurement of must total nitrogen content by means of microwave mineralization.

Instead of heating the mixture of must and sulfuric acid over an open fire or on an electric burner, the Kjeldahl attack was performed in a Maxidigest MX 4350 (Prolabo, 94126 Fontenay-Sous-Bois, France) mineralizator. In this device, heating is carried out very quickly by means of microwave. H2O2 is added for oxidation. The maxidigest MX 4350 can treat four samples simultaneously. Complete mineralization is obtained in only one hour. No foam or caramelization was observed on any of the samples mineralized.

Trials were performed with a standard lysine solution, containing 2800 mg of lysine per liter. In four replicates, an average of 98,6% of the lysine was measured (table I).

replicate	lysine mg/l in sample	lysine mg/l measured	% of lysine measured
1	2800	2800	100
2	2800	2702	96,5
3	2800	2772	99
4	2800	2772	99
average	2800	2761,5	98,6
SE		32,38	1,16

Table I: Measurement of lysine in a standard lysine solution by dosing NH_4^+ after mineralyzation by MAXIDIGEST MX4350 (microwave)

Nine replicates were carried out on a standard grape juice (table II) An average of 589,87 mg/l of nitrogen was found, with a standard error of 12,6 mg/l of nitrogen. Thus, the least significant difference of total nitrogen measured with this protocol is ± 25.2 mg/l (at 5%).

replicate	total nitrogen (mg/l)
1	574
2	616
3	599
4	594
5	582
6	588
7	582

8	580
9	594
average	589,9
SE	11,3

Table II: Measurement of total nitrogen in a standard grapejuice by dosing NH_4^+ after mineralization by MAXIDIGEST MX4350 (microwave)

III-2 Comparison of five indicators of vine nitrogen status

Petiole total nitrogen content, leaf blade color intensity measured by N-tester, must total nitrogen, must assimilable nitrogen and must NH4+ nitrogen were compared for their accuracy in differentiating vine nitrogen status in the previously described fertilization trial.

Differences in vine nitrogen status between the two levels of nitrogen fertilization (0 kg N/ha and 45 kg N/ha) were best shown by must total nitrogen ($\alpha<0{,}0001$, table III). Must assimilable nitrogen and must NH4+ nitrogen were also powerful indicators for differentiating vine nitrogen status depending on fertilization levels ($\alpha<0{,}001$).

Leaf blade color intensity, measured by N-tester, also revealed differences in vine nitrogen uptake depending on the fertilization level. Nevertheless, this indicator was less accurate than any of the three forms of must nitrogen ($\alpha<0{,}01$, table III).

In the Bordeaux area, vine nitrogen status diagnosis is usually carried out by measuring petiole total nitrogen content. In our experiment, petiole total nitrogen content made to differentiating the fertilization levels possible, but only at $\alpha< 0{,}05$ (table III). Therefore, it was the least powerful indicator of vine nitrogen status among the five tested. Of the other elements tested in the petiole analyses, vine phosphorus uptake seems to be inversely correlated with nitrogen uptake. Similar interactions between nitrogen and phosphorus have already been observed in grassing experiments in the Bordeaux area (Soyer and Molot, unpublished data).

In this fertilization experiment, 45 kg of nitrogen/ha had a clear effect on vine vigor (table IV). Pruning weight increased by 35%. No negative effect on berry constitution was observed (data not shown), but yield was homogenized on the tested plots by grape thinning.

		total N	must nitrogen content (14/9/99) assimilable N	NH4+ N	N-	Tester			petiole analysis (veraison)		
	replicate	(mg N /l)	(mg N /l)	(mg NH3 /l)	30/07/99	17/09/99	Total N%	P%	K%	Mg%	Ca%
no	1	448	208	29	465	460	0,62	0,10	2,71	0,69	1,54
mineral	2	423	190	31	469	451	0,52	0,11	3,36	0,67	1,59
nitrogen	3	462	194	38	525	501	0,49	0,10	3,01	0,64	1,71
fertilization	4	420	193	30	526	546	0,42	0,10	2,93	0,71	1,63
(0 N/ha)	5	428	183	37	529	514	0,57	0,09	3,03	0,70	1,56
	average	**436**	**194**	**33**	**503**	**494**	**0,52**	**0,10**	**3,01**	**0,68**	**1,61**
	SE	18,09	9,13	4,18	32,7	39,2	0,08	0,01	0,23	0,03	0,07

mineral	1	535	247	42	555	535	0,61	0,09	2,87	0,81	1,79
nitrogen	2	496	224	48	547	556	0,77	0,08	2,72	0,77	1,85
fertilization	3	532	223	49	594	583	0,62	0,08	2,59	0,86	1,72
applied	4	518	228	47	593	609	0,59	0,08	2,96	0,82	1,54
(45 Kg/ha)	5	552	227	48	595	601	0,70	0,08	2,79	0,79	1,49
	average	**527**	**230**	**47**	**577**	**577**	**0,66**	**0,08**	**2,79**	**0,81**	**1,68**
	SE	20,95	9,83	2,77	23,7	31,0	0,08	0,00	0,14	0,03	0,16
	F in table at 5%	5,05	5,05	5,05	5,05	5,05	5,05	5,05	5,05	5,05	5,05
	calculated F	0,78	0,89	0,45	0,55	0,66	0,98	0,40	0,35	0,71	0,13
The variances of the treatments can not be dissociated, so a Student test can be carried out											
	probability	0,000084	0,000312	0,000275	0,0035	0,0062	0,0234	0,0013	0,1072	0,0002	0,3729
	student number	7,302	6,033	6,147	4,092	3,685	2,794	4,811	1,814	6,533	0,944
Significant difference at α:		0,0001	0,001	0,001	0,01	0,01	0,05	0,01	-	0,001	-

Table III: Three forms of must nitrogen content at ripeness, leaf colour intensity measured by N-tester and petiole analyses at veraison

for two levels of nitrogen fertilization (N=0 and N=45 kg/ha)

		pruning weight
	replicate	(g/vine)
no	1	166,4
mineral	2	150,6
nitrogen	3	197,2
fertilization	4	216,4
(0 N/ha)	5	193,4
	average	**184,8**
	SE	26,15
mineral	1	228,3
nitrogen	2	288,6
fertilization	3	214,4
applied	4	266,2
(45 Kg/ha)	5	253,4
	average	**250,2**
	SE	29,59
	F in table at 5%	5,05
	calculated F	0,82
The variances of the treatments can not be dissociated,		
so a student test can be carried out		
	probability	0,006
	student number	3,702

Significant difference at α:	0,01

Table IV: Pruning weight for two levels of nitrogen fertilization (n=0 and N=45 kg/ha)

III-3 The use of must nitrogen content as an indicator of vine nitrogen uptake in "terroir" studies.

Three forms of must nitrogen (total nitrogen, assimilable nitrogen, NH4+ nitrogen) were used in a Saint-Emilion terroir study in 1999 to assess vine nitrogen uptake as a function of soil conditions (see also: materials and methods). All forms of must nitrogen tested, allowed differentiating nitrogen uptake level, which was high in the gravelly soil, low in the heavy clay soil and intermediate in the sandy soil (figure 1a, 1b and 1c: Merlot). Differentiation was possible at any date between veraison and ripeness. Similar results were found for Cabernet franc and Cabernet-Sauvignon, although absolute levels of must nitrogen depended on the grapevine variety (Cabernet-Sauvignon > Merlot > Cabernet franc, data not shown).

Must total nitrogen of the three cultivars was very well correlated with must assimilable nitrogen (r^2= 0.76, figure 2a); must total nitrogen was less well correlated with must NH4+ nitrogen (r^2= 0,29, figure 2b). Given the fact that the percentage of must assimilable nitrogen compared to the must total nitrogen content depends on the grapevine variety (table V), the correlation between must total nitrogen and must assimilable nitrogen was even better for one cultivar (r^2= 0,95 for Merlot, figure 3).

Figure 1a: Evolution of total nitrogen in grapejuice on three soils of the Saint-Emilion region, in 1999, from veraison until ripeness

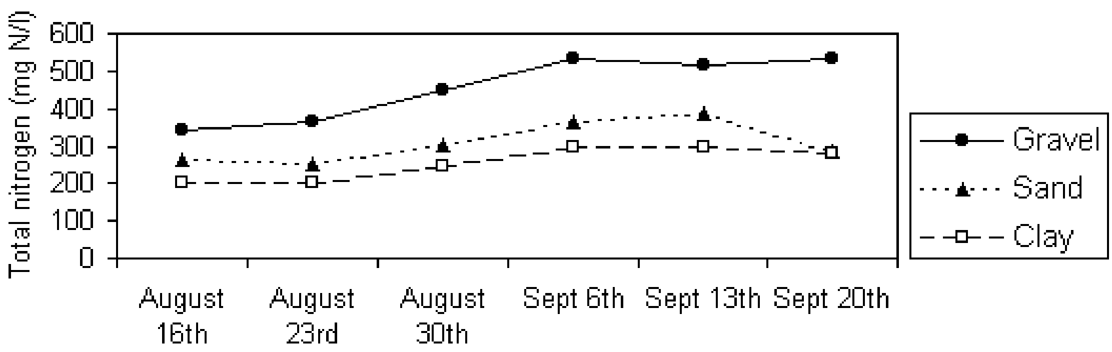


Figure 1b: Evolution of assimilable nitrogen in grapejuice on three soils of the Saint-Emilion region, in 1999, from veraison until ripeness

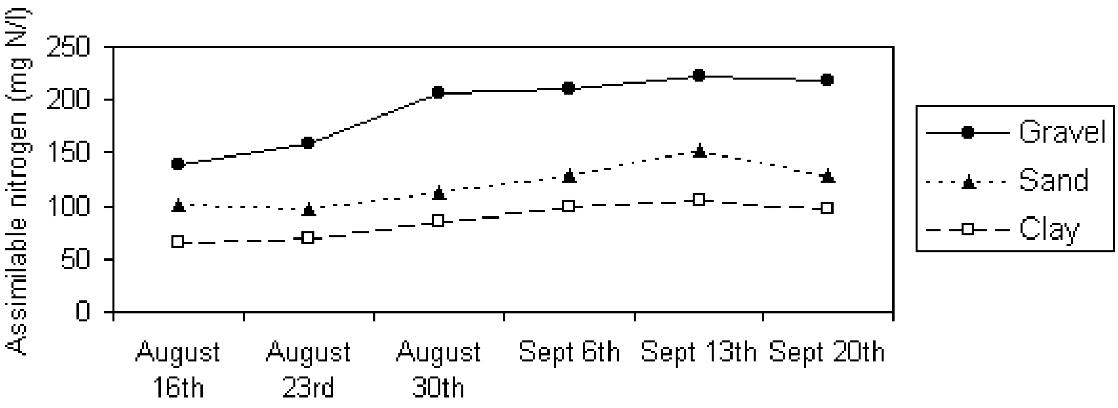


Figure 1c: Evolution of NH4+ nitrogen in grapejuice on three soils of the Saint-Emilion region, in 1999, from veraison until ripeness

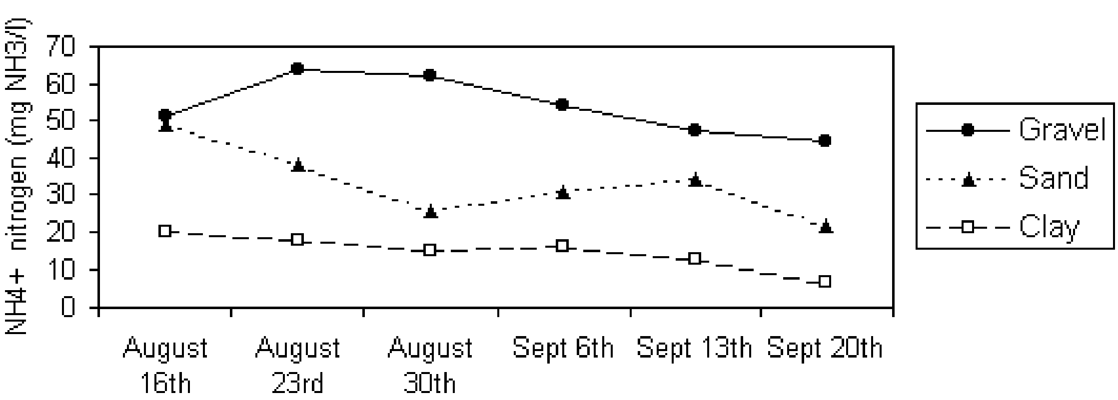


Figure 2b: Correlation between must total nitrogen content and must NH4+ nitrogen content (data from three cultivars: Merlot, Cabernet franc, Cabernet-Sauvignon)

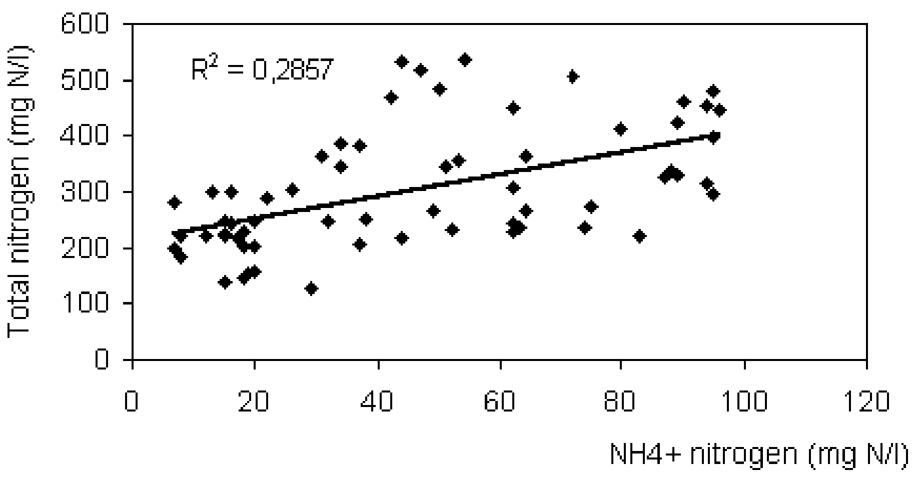
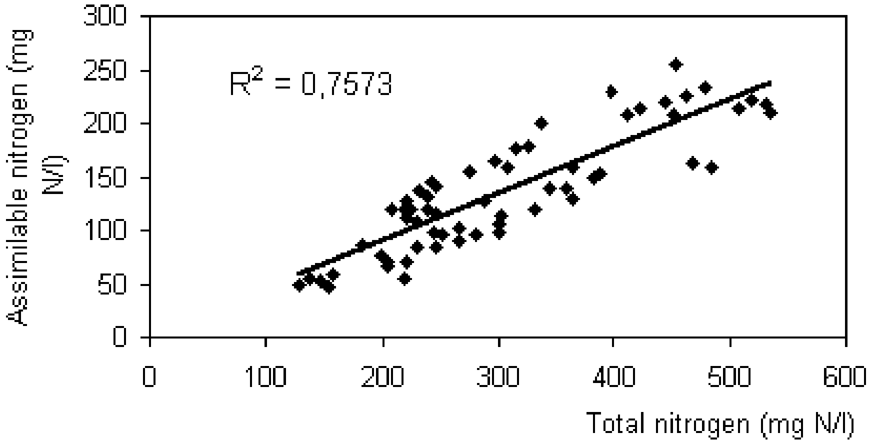
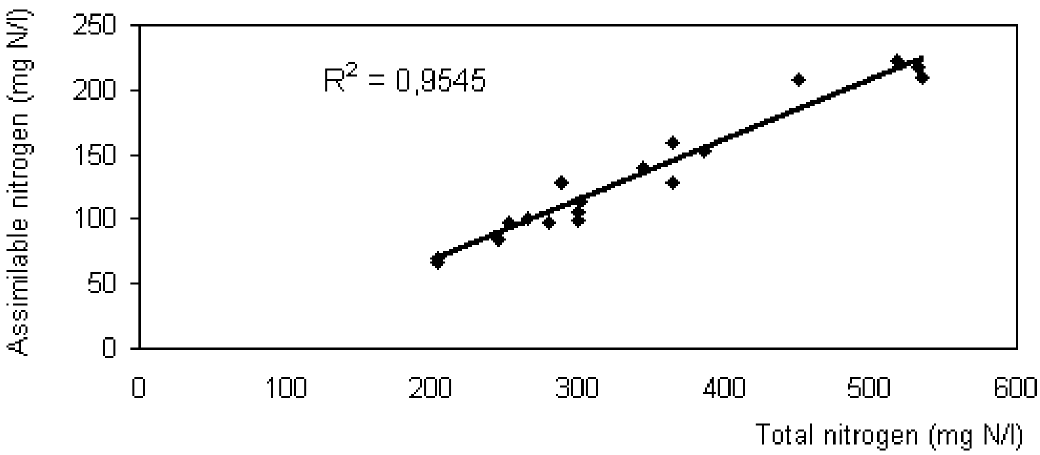


Figure 2a: Correlation between must total nitrogen content and must assimilable nitrogen content (data from three cultivars: Merlot, Cabernet franc, Cabernet-Sauvignon)



In another "terroir" study, carried out in 1997 in Saint-Julien (Médoc, France) we showed that limited nitrogen uptake, as a result of particular soil conditions, can limit vine vigor and thus be a quality enhancing factor (VAN LEEUWEN *et al.,* 1999). Vine nitrogen status was measured by must total nitrogen content. In this survey, based on six plots established on various soils, the best wines were produced on two plots, of which one was affected by mild water stress (1G: minimum pre-dawn leaf water potential = -0.30 MPa) and another by low nitrogen supply (4H: berry total nitrogen content = 161 mg/l). This emphasizes the role of moderate environmental stress in the production of high quality-potential grapes. Some results of the 1997 Saint-Julien experiment are shown in table VI.

Figure 3: Correlation between must total nitrogen content and must assimilable nitrogen content (Merlot)



	% of total nitrogen:	
variety	assimilable N	NH4+ N
Merlot	38% ± 4%	10% ± 4%

Cabernet franc	40% ± 8%	11% ± 6%
Cabernet-Sauvignon	53% ± 6%	23% ± 7%
average	44%	15%

Table V: Ratio of assimilable N / total N and NH_4^+ N / total N for three red grapevine varieties in Saint-Emilion (1999)

IV- DISCUSSION AND CONCLUSION

Few studies are devoted to nitrogen supply to vines in relation to soil conditions or to the role of nitrogen supply in the "terroir" effect, mainly because of the difficulties in establishing vine nitrogen status diagnosis. We show that must total nitrogen is a powerful indicator to assess vine nitrogen supply. Must mineralization can easily be carried out by a Maxidigest MX 4350, in only one hour. The main disadvantage of this technique is the high cost of the Maxidigest device.

Must assimilable nitrogen content is very well correlated with must total nitrogen content and thus can also be used for vine nitrogen diagnosis. The main

	4H	1G	3A	5P
Total nitrogen (mg/l)	161	306	229	380
Minimum pre-dawn leaf water potential (Mpa)	-0,23	-0,30	-0,20	-0,16
Significant difference at 5%	a	b	ac	c
Leaf area (m²/vine)	0,80	1,01	0,96	1,46
Significant difference at 5%	a	a	a	b
Pruning weight (kg per vine)	0,19	0,24	0,31	0,43
Significant difference at 5%	a	a b	b	c
Yield (kg per vine)	0,765	0,935	1,090	1,103
Significant difference at 5%	a	a b	b	b
Berry weight (g)	1,1	1,26	1,34	1,46

Sugar content (g/l)	214	200	198	185
Total acidity (meq/l)	72	77	84	85
Ripening speed (of the pulp) : k	0,80	0,72	0,66	0,56
Malic acid content (meq/l)	25	18	27	31
Berry anthocyanin content (mg/l)	1231	989	823	748
Tasting (sum of preferences)	58	50,5	39	21
(highest sum is the best preference)				
Significant difference at 5%	a	a	a	b
Berry weight and berry constitution are measured on the day of harvest: 27/09/97				

Table VI: Effects of nitrogen uptake as measured by berry total nitrogen content, water status as measured by minimum pre-dawn leaf water potential, on vine vigor, yield, berry constitution and wine quality (Saint-Julien, 1997)

advantage of this technique is its low equipment requirement. Furthermore, must assimilable nitrogen content is useful information for enologists and winemakers needing to assess must fermentiscibility. It is often measured routinely on must before alcoholic fermentation. Many authors cite 130 mg N/l as a threshold for possible fermentation problems (MASNEUF *et al.*, 1999). This does not mean that the same figure can be used for vine nitrogen status diagnosis. More research is needed to establish normal values for must assimilable nitrogen content in relation to a balanced vine nitrogen supply. Levels of most forms of nitrogen in grapevines vary to a considerable extent, depending on the cultivar (KLIEWER, 1991). This is probably also be the case for assimilable nitrogen, so normal values have to be set for each cultivar. Limited nitrogen supply to vines can be a devigorating, quality-enhancing factor. This means that values of must assimilable nitrogen, reflecting optimum vine nitrogen supply conditions, might for some cultivars, be under 130 mg N/l. In those cases, must nitrogen supply should be recommended, rather than vine nitrogen supply.

Vine nitrogen status diagnosis by must nitrogen content is carried out after veraison and a possibly necessary change in fertilization practices can only be recommended for the year following. For early-season nitrogen supply diagnosis, "N-tester" proves to be an easy-to-handle device. More research is needed though to test its accuracy in different climatic conditions (year effect). The effect of copper sprayings on the response of the N-tester also has to be assessed.

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