

THE PEDOCLIMATIC CONDITIONS IMPACT THE YEAST ASSIMILABLE NITROGEN CONCENTRATION IN THE GRAPEVINE MUST AND THE VALORISATION OF FOLIAR-NITROGEN FERTILISATION

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Introduction

In winemaking, a minimum of 140 mg/L of YAN – amino acids and ammonium – in the must is required for the proper completion of alcoholic fermentation (Agenbach 1977, Hannam et al. 2013). But a non-restrictive concentration of 200 mg/L of YAN would also guarantee optimal organoleptic results in terms of wine quality (Spring and Lorenzini 2006).

In case of YAN deficiency, the direct addition of diammonium phosphate (DAP) in the must improves the fermentation kinetics, although its positive effect on the wine aromas has not yet been established (Lorenzini and Vuichard 2012). Indeed, only the amino acids – the main part of YAN – act as precursors in the synthesis of wine aromas (Rapp and Versini 1991).

YAN concentration can be significantly increased in the must through the addition of nitrogen in the form of urea directly on the vine canopy (Lacroux et al. 2008, Dufourcq et al. 2009). Urea application is usually recommended at veraison for increasing the must YAN concentration without increasing the vigour of the vine (Lasa et al. 2012; Verdenal et al. 2015).

The present study follows on the project of Reynard et al. (2011) which highlighted the large role of the soil parameters (i.e. structure, depth, water holding capacity) in the grape N content and their impact on the final wine characteristics and quality. The relatively wide variation of N use reported for grapevines suggest that nutrient management recommendations should be developed on a regional basis (Conradie 2005). On the initiative of the Fédération Vaudoise des Vignerons, Agroscope set an experiment throughout the vineyard of the Lemman region, Switzerland, to observe the YAN fluctuation in function of vintage and soil type and to evaluate the impact of urea fertilisation in the different situations on the YAN concentration in the must and further on the wine quality.

MATERIALS AND METHODS

Vineyard sites and experimental setup

Five plots of Doral and five plots of Gamaret were chosen in the Lemman wine region. The vines were all grafted on rootstocks 3309 C, planted in 2003 (except Gamaret in Changins planted in 2007) and trellised in a single Guyot pruning system. Plant density varied from 5000 vines/ha in Pully up to 7800 in Villeneuve and Cully. The **Table 1** describes the soils profiles of each site. Villeneuve was the only site with fallen stones (hyperskeletal leptosol) and a very small water holding capacity (50 to 75 mm). Cully and Pully soils were composed of gravely moraine (eutric cambisol). Pully soil had particularly big water holding capacity of 230 mm. Whereas Vufflens and Changins soils were made of moraine over molasses and basal compact moraine respectively (both calcaric cambisol with

endostagnic conditions). Each plot was divided in two treatments of 60 vines each: a treatment which received foliar urea at veraison (20 kg/ha N applied in four times) and a control treatment with no fertilisation.

Table 1: Soil description of the ten sites of the study. The soil water holding capacity corresponds to the water accessible for vine root uptake.

	Site	Altitude (m)	Soil class	Rock type	Hydromorphy	Depth	Water holding capacity (mm)	Stones (%)
Doral	Villeneuve	462	Hyperskeletal leptosol	Fallen stones	-	140	50	75
	Cully	490	Eutric cambisol	Gravelly moraine	-	150	150	20
	Pully	469	Eutric cambisol	Gravelly moraine	-	180	230	15
	Vufflens	487	Calcaric cambisol	Moraine over molasse	Endostagnic	150	200	5
	Changins	442	Calcaric cambisol	Basal moraine	Endostagnic	150	185	5
Gamaret	Villeneuve	433	Hyperskeletal leptosol	Fallen stones	-	150	75	70
	Blonay	518	Eutric cambisol	Moraine over molasse	-	160	205	20
	Pully	469	Eutric cambisol	Gravelly moraine	-	180	230	15
	Begnins	567	Calcaric cambisol	Fluvio-glacial deposits	-	160	195	25
	Changins	442	Calcaric cambisol	Basal moraine	Endostagnic	150	185	5

Field measurements, sampling and analysis

The phenological stage flowering was dated. The fertility was estimated on 20 vines and was expressed as the average number of clusters per shoot. The total leaf area was estimated in August on 10 vines twice per treatment. The chlorophyll index (N-tester, Yara, France) which permitted the monitoring of the chlorophyll concentration in the leaves throughout the season, was measured every 3 weeks for each treatment on 30 primary leaves of the medial part of the canopy. The light-exposed leaf area (m²/m² of soil) was determined using the Carbonneau's method (1995). The vigour of the vines was assessed during winter by weighing 50 one-meter long pruned canes per plot and was expressed in grams per meter (g/m). The carbon isotope discrimination ($\delta^{13}\text{C}$), showing the global water constraint from veraison to harvest, was determined at the Stable Isotopes Laboratory of the University of Lausanne. Water restriction is considered high when $\delta^{13}\text{C}$ is above -23 ‰, moderate between -23 and -24.5 ‰ and weak to null under -24.5 ‰ (van Leeuwen et al. 2009). A leaf diagnosis was carried out after the four urea applications on 25 leaves (limb + petiole) per treatment from the medial part of the canopy and analysed at the registered laboratory Sol-Conseil (Gland, CH) in order to assess the N, P, K, Ca and Mg contents. The grape maturation was controlled weekly (200 berry sample) at the Agroscope laboratory using the following parameters: berry weight (g), titratable acidity (TA, g/L as tartaric acid), tartaric (g/L) and malic acids (g/L), total soluble solids (TSS, °Brix), pH and yeast available nitrogen (YAN, mg/L). Finally, 50 kg of grapes from each treatment were harvested and vinified separately at the Agroscope winery. Wine sensory analysis was realised by the Agroscope tasting panel.

Statistics

The data description and the significance of the differences between treatments, sites and vintages were statistically evaluated using analysis of variance (ANOVA, p values < 0.05), multiple comparison Newman-Keuls test and principle component analysis (PCA) realised with ©XLSTAT 2015.1.02. Results are presented as average ± 1 SD.

RESULTS AND DISCUSSION

Climate and phenology

Total precipitations from April to October (vegetative period) were 645 mm in 2012, 820 mm in 2013 and 825 mm in 2014. Average temperatures over the same period were 17.2 °C in 2012, 16.7 °C in 2013 and 16.1 in 2014 (Pully weather station). 2012 was a relatively early vintage with Doral flowering occurring on the 15th of June. On the other hand, 2013 was a late vintage with flowering on the 1st of July. Changins vineyard production was lost in 2013 due to hailstorm. Leaf diagnosis did not show any deficiency in terms of mineral nutrients N, P, K, Mg and Ca (results not shown). Average N concentration in the leaves was 2.1 ± 0.2 % of dry weight.

Physiology, yield and grape maturity

The **Table 2** shows the main results of physiology and must composition. Fertility was slightly higher in 2013. Yield was maintained at approximately 1.2 kg/m². Doral at Villeneuve had a lower yield because of important symptoms of millerandage for the three vintages. As a consequence, leaf-fruit ratio varied from 0.9 to 2.6 m²/kg. Consistent differences between sites in terms of grape maturity were observed over the years, but remained smaller than the differences due to the overwhelming impact of the vintage in terms of climate. As an example, average TTS for Doral was 22.0 ± 0.7 °Brix in 2012, 19.2 ± 0.7 °Brix in 2013 and 21.1 ± 0.6 °Brix in 2014. No differences could be observed between treatments within a site, except for YAN as described further.

Table 2: Variability of vine parameters, yield and must composition at harvest in function of site. There was no difference between control and fertilised treatments, thus only averages per site are presented. (cv. Doral and Gamaret, average 3 years)

	Site	Bud fertility (Clusters/shoot)	Yield (kg/m ²)	Leaf-fruit ratio (m ² /kg)	Pruning weight (g/m)	TSS (°Brix)	pH	TA (mg/L)
Doral	Villeneuve	1.7 b	0.5 b	2.5 a	53 c	20.8 bc	3.03 d	9.0 b
	Cully	1.6 b	1.0 a	1.4 bc	57 b	20.2 c	3.06 c	9.1 b
	Pully	2.0 a	1.1 a	1.1 c	75 a	21.2 b	3.18 a	9.2 ab
	Vufflens	1.7 b	1.1 a	1.1 c	60 b	20.4 bc	2.99 e	9.7 a
	Changins	1.7 b	0.6 b	1.7 b	48 c	22.0 a	3.09 b	8.9 b
Gamaret	Villeneuve	1.7 c	0.9 abc	1.5 a	37 c	22.4 a	3.17 c	6.5 b
	Blonay	1.9 bc	1.0 ab	1.3 a	35 c	21.6 bc	3.25 b	6.5 b
	Pully	2.3 a	1.1 a	1.4 a	54 a	22.0 ab	3.40 a	5.9 b
	Begnins	1.9 bc	0.8 bc	1.5 a	49 ab	21.0 c	3.22 b	7.5 a
	Changins	2.1 ab	0.7 c	1.7 a	47 b	21.8 ab	3.24 b	6.3 b

Principle component analysis

To better understand the links between the different parameters measured in this study, a global PCA – Including the physiological parameters, the yield components and the must analysis – was realised for each cultivar and is presented in **Figure 1**. A major distinction was observed due to the different grape maturities between the vintages, mainly in terms of TSS and TA concentrations in the musts. Despite the vintage effect, we can see the site effect with identical site differentiation within the three vintages. As an example for Doral, on the extreme right hand side, Pully consistently presented more vigour (higher berry, cluster and pruning weights) over the years. Water holding capacity and soil depth seemed to be discriminant between sites and correlated with vigour and YAN level. On the other side, Villeneuve had the lowest vigour, the highest leaf-fruit ratio, which seemed to be correlated with its high soil stone percentage and its low water holding capacity. The plant density did not play a key role in the final YAN concentration: the correlation which was observed was mainly due to the particularities of Pully (soil structure, high vigour). Site altitude and water constraint were not discriminating parameters, since altitude variation between sites was negligible (478 ± 41 m) and water supply from rain was not restrictive ($\delta^{13}\text{C} = -26.9 \pm 0.6$ ‰). N leaf content and chlorophyll index were not discriminant either since N level in the vines were high and non-restrictive everywhere.

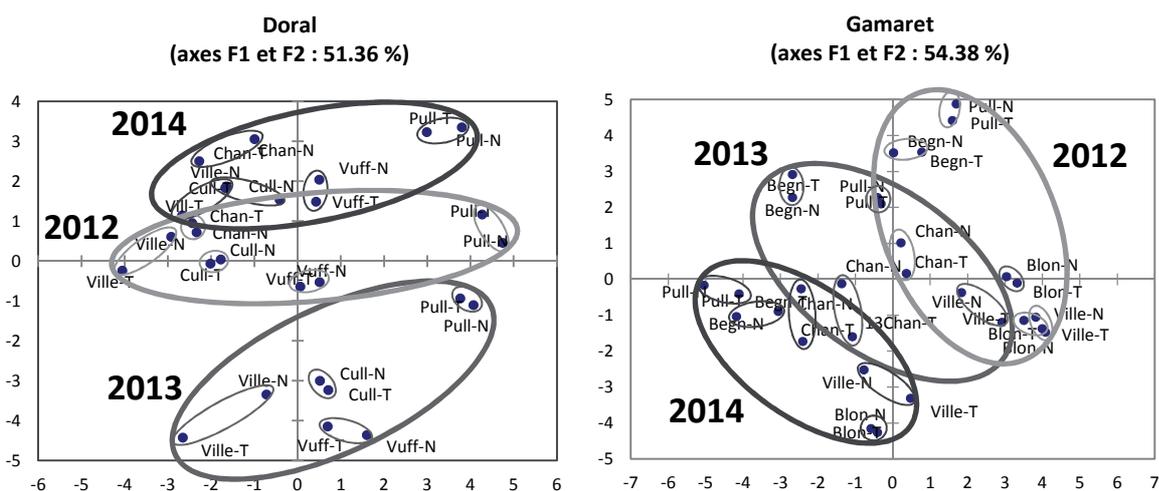


Figure 1: The principle component analysis (cv. Doral et Gamaret, 3 years) shows the disparities in terms of physiological behaviour between vintages, between sites (Cull=Cully, Pull=Pully, Vill=Villeneuve, Chan=Changins, Vuff=Vufflens, Begn=Begnins, Blon=Blonay) and between treatments (T=control treatment, N=treatment with urea). Closer are the points, higher are the similarities. The vines in Changins were hit by hail in 2013. The vintage effect represents the main explanation of the plot differentiation, followed by the site effect, and then by the treatment effect.

YAN concentration in the must

Figure 2 shows the YAN concentrations per site and per treatment for both cultivars. The 3 year average YAN concentrations in musts from control treatments were 139 ± 60 mg/L for Doral and 118 ± 53 mg/L for Gamaret. The 3 year average gain in YAN due to foliar-N application were 69 ± 32 mg/L for Doral and 52 ± 27 mg/L for Gamaret.

For all vintages, Pully had the highest YAN concentration for both cultivars (232 ± 44 mg/L for Doral and 165 ± 50 mg/L for Gamaret). Doral at Villeneuve had the largest YAN gain (+ 106 mg/L in average). On the other hand, the Gamaret in Changins had the smallest YAN gain (+ 37 mg/L).

YAN concentration in the must from control treatments was positively correlated with the vine vigour represented by pruning weight ($R^2_{Doral} = 0.27$, $R^2_{Gamaret} = 0.39$). Vigour was itself positively correlated with soil depth ($R^2_{Doral} = 0.67$, $R^2_{Gamaret} = 0.30$) and water holding capacity ($R^2_{Doral} = 0.38$, $R^2_{Gamaret} = 0.30$). YAN concentration was also negatively correlated with Phosphor ($R^2_{Doral} = 0.65$, $R^2_{Gamaret} = 0.47$) and Magnesium ($R^2_{Doral} = 0.53$, $R^2_{Gamaret} = 0.39$) in the leaves. No correlation could be pointed out neither between YAN concentration and leaf N content nor between initial YAN in control treatment and YAN gain after treatment. A correlation between YAN and pH in the must could be pointed out ($R^2_{Doral} = 0.56$, $R^2_{Gamaret} = 0.12$). The YAN gain between control and fertilised treatments was correlated with leaf-fruit ratio for Doral only ($R^2_{Doral} = 0.57$, $R^2_{Gamaret} = 0.08$), but this last result needs to be confirmed because of important millerandage symptoms in Villeneuve site. The YAN gain was not correlated to the plantation density nor to the leaf area, but it was negatively correlated to the yield ($R^2_{Doral} = 0.50$, $R^2_{Gamaret} = 0.19$).

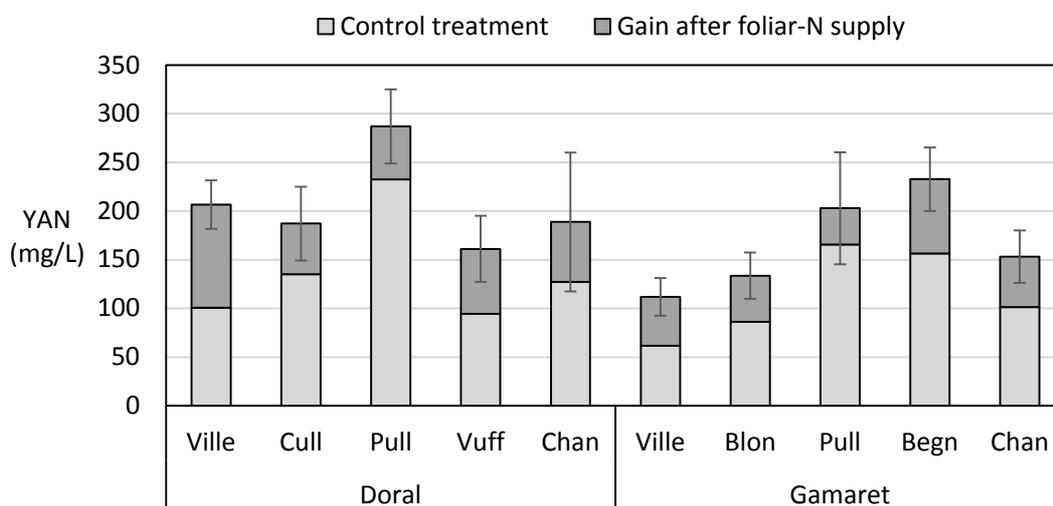


Figure 2: YAN content in the must at harvest in control treatment (mg/L) and gain after foliar-N supply per site for both cv. Doral and Gamaret (average 3 years \pm standard deviation)

Wine quality

Because of vinification issues in 2012, the wines from Vufflens and Changins could not be tasted by the Agroscope panel, and in 2013, no grape could be harvested from Changins due to hail. The wines from Pully of both Doral and Gamaret were significantly preferred for all vintages in comparison with the other sites. Doral 2012 wines from Villeneuve were presented to 67 winemakers on the occasion of a hedonistic blind tasting: they significantly preferred the wine from the treatment with urea. The impact of foliar-N supply on the wine quality was not regular and affected various criteria (colour, bouquet and/or structure), but it was globally either positive or non-significant. Urea supply was more efficient on Doral: the impact of urea has never been negative on the wine quality and 7 wines into 12 were significantly preferred to the associated control treatment wine. Urea supply was less efficient on Gamaret wines, since the YAN concentration remained insufficient in most of the musts (< 140 mg /L). Indeed, the gain in wine quality becomes obvious when the YAN in the must rises from a restrictive to a non-restrictive concentration, above 200 mg/L (Spring and Lorenzini

2006). A strong correlation could be pointed out between the YAN in the must and the overall appreciation of the Doral wines (Fig. 3, $R^2 = 0.70$), while the same correlation was not significant for Gamaret ($R^2 = 0.07$)

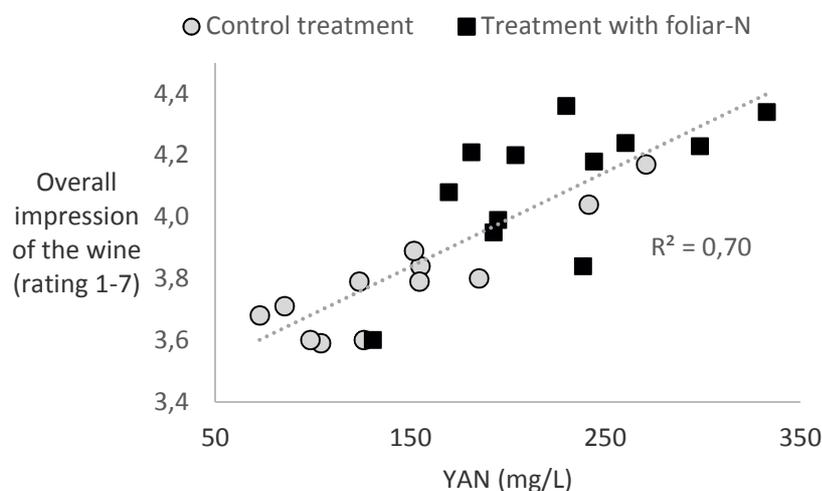


Figure 3: Correlation between the YAN content in the must and the overall appreciation of the wine (cv. Doral, 3 years)

Conclusion

The effect of foliar urea application at veraison significantly enhances the YAN concentration in the must in all the sites of the study without influencing the vine vigour and maturity parameters such as soluble sugars and acids concentrations in the must. This result confirms earlier publications (Lasa et al. 2012, Hannam et al. 2013; Verdenal et al. 2015). For Doral, the sites Villeveuve and Pully benefited of a gain in terms of wine quality (colour, bouquet) whereas Cully and Vufflens did not present any difference for the three vintages. The YAN concentration in the must was the parameter that best explained the enhancement in wine quality since it was the only parameter which significantly changed in the must between control and fertilised treatments. The absence of wine quality enhancement in some sites can be justified by the YAN concentration which remained under the threshold of non-restrictive YAN concentration (200 mg/L) despite the urea application, as mentioned by Spring and Lorenzini for the cv. Chasselas (2006). The effect of urea fertilisation was clearly negligible in comparison with the vintage effect. Despite the vintage effect and the relatively constant leaf N content between site, some sites regularly presented low YAN concentration in grape must (Villeneuve, Blonay, Cully, Vufflens), whereas other sites benefited of high YAN concentrations (Begnins, Pully). This site effect was stronger than the urea treatment effect and seemed to be correlated with the soil, more precisely the root zone size and structure. Further investigation should be realised on the influence of root activity (growth, uptake, reserves) on the YAN concentration in the must.

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Abstract

Aims: Agroscope investigated the efficiency of nitrogen fertilisation via foliar urea application at veraison with the aim of raising the yeast assimilable nitrogen (YAN) concentration in the musts. The observations were conducted over three vintages on two grapevine cultivars in several pedoclimatic conditions of the Lemman wine region, Switzerland. Knowing that the YAN in the must plays a key role in wine quality, the aim of this study was finding the main parameters affecting the final YAN level in order to better control them.

Methods and results: Five plots of Doral (white grape, Chasselas x Chardonnay) and five plots of Gamaret (red grape, Gamay x Reichensteiner) were chosen over 80 km of vineyards. Pedologic profiles were realised. Vegetal materials, date of plantation and cultivation practices were kept constant for comparison purposes. Each plot was divided in two treatments of 60 vines each: a control treatment and a nitrogen fertilized treatment (20 kg N/ha as foliar urea applied at veraison). Phenological development, nitrogen status and grape maturation of vines were monitored. 50 kg of grapes from each treatment were harvested and then vinified separately using a standard protocol. YAN levels in musts were significantly enhanced by foliar-nitrogen fertilisation, but strong vintage, site and cultivar effects were pointed out: average YAN gain over 3 years was 69 ± 32 mg N/L in Doral must and 52 ± 27 mg N/L in Gamaret must. Some sites consistently presented higher gains (e.g. Doral at Villeneuve, +106 mg N/L). The bigger water holding capacity and the deeper effective root zone seemed to mainly enhance vine nitrogen status. No correlation could be established between initial leaf N content and the variation of YAN gain. YAN in must was the parameter that best explained the positive variations in wine sensory characteristics and, in the case of Doral only, was highly correlated to the overall appreciation of the wines ($R^2 = 0.70$).

Significance and impact of the study: This work confirms that YAN level in must, in relation to climate and soil characteristics, contributes to the terroir effect on the wine quality. YAN concentration is clearly influenced by pedoclimatic conditions and cultivar. The impact of foliar-N supply is not always sufficient for a significant improvement of wine overall appreciation particularly for the cv. Gamaret. This observations may assist the development of sustainable practices to increase the YAN concentration in musts.

Keywords: terroir, yeast assimilable nitrogen YAN, leaf urea fertilisation, wine quality, terroir