USING MULTIPLEX[®] TO MANAGE NITROGEN VARIABILITY IN CHAMPAGNE VINEYARDS

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ABSTRACT

Nitrogen is a major fertilizer for grapevine and an important nutrient for yeast in the must. Identification of spatial variation patterns will be determinant for technicians to understand the effects of soil, climate and topography in order to establish decision rules to manage nitrogen inputs. Such decision rules would be useful to winegrowers in their effort to homogenize the nitrogen status within a plot. The determination of variation patterns can indeed lead to the implementation of inputs management such as differential winter pruning or variable fertilization rates. Recently, a vehicle-mounted optical sensor, Multiplex[®], was introduced to estimate the nitrogen status of grapevine leaves. The measuring principle of this proximal sensor is based on chlorophyll fluorescence screening. The aim of the present study was to correlate nitrogen contents at harvest to the Nitrogen Balance Index (NBI) measured with the Multiplex on leaves.

Multiplex measurements on leaves were recorded for each main stage during the growing seasons 2010 and 2011 on Plumecoq (Chouilly, France), the experimental vineyard of the Comité Champagne (CIVC). The plot was composed of three grape varieties (Chardonnay, Pinot noir and Meunier) planted in a Latin square pattern to minimize soil and microclimate effects. The Multiplex was mounted on a small caterpillar and connected to a DGPS. It provided NBI with high resolution. After two years of measurements, reliable correlations were obtained between the NBI in leaves, nitrogen in the must, wood pruning and leaves biomass.

Keywords: Grapevine, proximal sensing, nitrogen maps, viticulture.

INTRODUCTION

Nitrogen is a key element for plants and for grapevine in particular. Its availability and metabolism along the grapevine cycle is determinant for the vine development and its annual fruit production, as well as for a more long term balance of this perennial plant. Multi-year analyses of vine development are interesting and necessary for the winegrower to adapt its farming practices. The aim is to manage the vineyard to a good balance between vigour, yield and grape quality. Production targets should be adapted each year according to plant initial potential and how it can be realized depending on the annual climate conditions. Classical observations on vine and grape analysis give some elements, but it appears difficult to take into account the whole history of the vine to understand if it is correctly managed. Integrative indicators are needed to understand multi-year effects and vine development during the phenological stages. Precision viticulture and optical sensors indices such as the NDVI of the GreenSeeker (N-Tech, USA) and NBI from Multiplex (Force-A, France) seem to give good prospects to characterize, on the one hand, the canopy growth, and on the other hand, the leaf nitrogen content. A product index made from NDVI and NBI would be proportional to the global nitrogen uptake at the plot level. This new index should be better correlated to nitrogen content in the must, and should be a good indicator for multi-year management strategies concerning fertilizer inputs, pruning and yield control.

MATERIALS AND METHODS

Experimental field

Champagne is the wine region located in the North East of France which produces the famous sparkling wine called Champagne. The interests of Champagne are defended by the Comité Champagne, an organization funded by the growers and houses of Champagne under the supervision of the French Government. Plumecoq estate is an experimental vineyard of 10 hectares belonging to the Comité Champagne. All data presented in this article were obtained on a plot of this estate. This plot was planted in 1996 with the three specific cultivars of Champagne: Pinot Noir, Meunier and Chardonnay. Plantation was done respecting a Latin square pattern to minimize the effects of soil and microclimate. Its total area is 0.72 hectare. Farming methods were identical and homogeneous ever since plantation. This particular plot has been used for several years by Champagne technicians as an experimental field for vine physiology monitoring.

Classical viticultural measurements

Several descriptors related to the terroir were measured. The precise maps of soil, as well as usefull waterstock (RU), easily usable waterstock (RFU), elevation (Topo) and plot resistivity were obtained using RTK dGPS. The topographic wetness index (TWI) was then calculated and mapped to quantify topographic control on hydrological processes, and to describe wetness conditions at the catchment scale. This physical knowledge of the experimental field appears to be essential for a good understanding of vine development as it conditions water and thus nitrogen availability. Descriptors related to the vine agronomical behavior were also acquired regularly on an annual basis on the three cultivars. These descriptors were: phenological stages, predawn leaf water potential, main grape maturity descriptors, mean yield, and mean pruning weight. Main maturity parameters were measured on berry samples just before harvest. Specific attention was given to total nitrogen in the must. The latter is the result of complex mechanisms of nitrogen metabolism which are highly influenced by water availability and nutrition conditions throughout vine cycle. Nitrogen content in the must will have a great impact on the fermentation speed and on amino acids contents in wines, and therefore on their aromatic profile.

Precision viticulture measurements

The two optical sensors, GreenSeeker[®] and Multiplex[®], were mounted on a small caterpillar in order to collect optical data while driving through the vine rows. The technical challenges of the set-up and data collection were resolved on previous years (Debuisson et al. 2010). Data acquisition was performed about twice a month from early flowering until harvest.

NDVI measurements

GreenSeeker[®] (N-Tech, USA) is an integrated optical sensing system which measures the Normalized Difference Vegetative Index (NDVI). GreenSeeker sensor uses light emitting diodes (LED) to generate red and near infrared (NIR) light. The light generated is reflected off the crop and measured by a photodiode located at the front of the sensor head. NDVI is calculated according to the following equation: (NIR - RED)/ (NIR + RED) (Rouse et al., 1974). NDVI can vary between -1 and 1. A value of 1 represents a maximum coverage of surface by vegetation. The value 0 represents an absence of vegetation if the background color measured by the sensor is a perfect white. In the vineyards, a white board positioned behind the analyzed row was used. Thus, the GreenSeeker values vary between 0 and 1 depending on foliage porosity and leaves chlorophyll content. Healthy, vigorous biomass is characterized by a strong near-infrared reflectance and a very low red reflectance due to chlorophyll absorption.

Multiplex measurements

Multiplex[®] (Force-A, Orsay, France) is a hand-held multi-parametric fluorescence sensor dedicated to work in the field under daylight conditions on leaves and fruits. The measuring principle of the Multiplex is based on the chlorophyll fluorescence screening method (Agati et al., 2007, Cerovic et al., 2008). The method was validated on grapevine leaves (Kolb, 2005) and grapes (Kolb et al., 2003; Agati et al., 2007) using either UV light for flavonols (FLAV) or green light for anthocyanins (ANTH). A red beam, for which the epidermis is transparent, is used as a reference (Agati et al., 2007). The present version of the sensor has six 375-nm UV-A LED-matrices light sources and three Red-Blue-Green LED-matrices emitting light at 470 nm (blue, B), 516 nm (green, G) and 625 nm (red, R). There are three synchronized photodiodes for fluorescence recording: yellow (YF), red (RF) and far-red (FRF). The sensor illuminates an 8-cm diameter surface at a 10-cm distance from the

sources. The sensor provides hence twelve individual signals: 4 excitation x 3 emission. From these individual signals, several indices have been developed on leaves: FLAV for flavonol content, SFR_R for chlorophyll content and NBI_R for nitrogen content. In this paper, only the Nitrogen Balance Index (NBI) is reported. Indeed, NBI has been shown to respond best to nitrogen nutrition of the plant (Cartelat et al., 2005; Cerovic et al., 2009; Tremblay et al., 2012).

Data analysis

All measured parameters were averaged to have a mean value for each of the 27 sub-plots studied (Fig. 1), which means 9 spatial repetitions for each cultivar. Data were first analysed globally at the plot scale and from a kinetic point of view, and then by individual cultivar because they do not have the same agronomical behaviour. Same treatments were performed for 2010 and 2011 data.

Correlation coefficients were calculated between optical sensors data and agronomical parameters such as yield and total nitrogen content in the must.

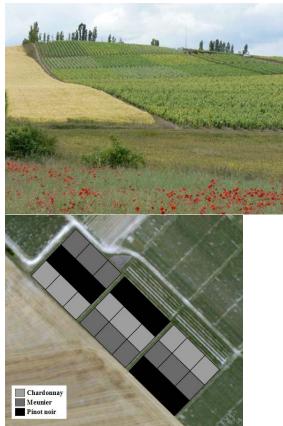
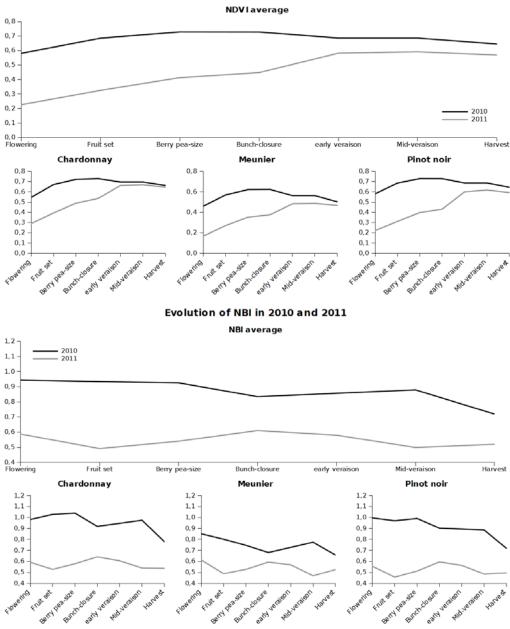


Fig. 1: The Plumecoq plot with the three cultivars.

RESULTS AND DISCUSSION

Multi-year analysis of NDVI and NBI

The NDVI average values are presented as a function of the vine development stage in Fig. 2. Measurements performed in 2010 and in 2011 are presented for a better understanding of annual variability of vine growth. The 2010 season is characterised by a rapid growth from mid-April (bud-burst) until mid-June (flowering) due to unusually high spring temperatures. It rained regularly avoiding any water stress for vine, hence at flowering, the canopy was already well furnished and first toppings were performed. NDVI reached an average value of 0.7 and then stabilizes between 0.6 and 0.7. Considering the three varieties, the growth of Chardonnay and Pinot noir was similar whereas Meunier showed a slightly lower total leaf area.



Evolution of NDVI in 2010 and 2011

Fig. 2: NDVI and NBI changes in 2010 and 2011

In 2011, NDVI data show a very different profile of vine development. The beginning of the season was dry and the temperatures high to a point where slow-down of growth rhythm until stop of growth was observed. Canopy porosity was still very high at flowering. The water deficit probably induced a weak mineralization of soil nitrogen and as consequence nitrogen deficiency in vines. At flowering, NDVI values were 0.2 and only reached 0.5 by the early ripening stages. NDVI has recently been shown to correlate with the canopy leaf area index (Johnson, 2003, Goutouly, 2006, Debuisson, 2009) and pruning weights (Dobrowski et al., 2003; Profitt and Malcom, 2005). More precisely, NDVI delivered by GreenSeeker sensor, used on the ground in a side view orientation, is mainly sensitive to the foliage porosity variations (Goutouly, 2006; Debuisson, 2009). So, it can be seen that the same vines had about 40% less leaves in 2011 at flowering compared to 2010, and about 30% less leaves at harvest, which obviously would have incidence on grape quality.

A similar reasoning can be performed on the NBI variations in 2010 and 2011 as a function of development stages (Fig. 2). The 2011 values are significantly lower than those measured in 2010. At flowering, the NBI values are 35 % lower in 2011 compared to NBI values in 2010. NBI did fluctuate during the season, but still, there was a constant decreasing trend with time (Fig. 2). It followed the known trend of the leaf nitrogen content decreasing during canopy development (Prieto et al., 2012). So, NBI behaved here as a proxy for the leaf mass-based nitrogen content (Meyer et al., 2006; Tremblay et al., 2012), which can be more or less diluted in leaves dependant on growth rates.

The correlation coefficients were investigated between NBI, NDVI, yields and total nitrogen contents in the must. They are positive, however irregular during the growth season.

Total nitrogen uptake

In viticulture, a main maturity parameter is the grape sugar content. Another way to analyze it is to consider the sugar uptake instead of the sugar content: the product of the sugar content by the yield. To manage nitrogen variability in vineyards, a similar approach can be applied, as already used in cereal crops. The nitrogen uptake throughout the season can be assessed thanks to the Combined index for Nitrogen Nutrition (CNN). CNN is defined here as:

$CNN = NDVI \times NBI$

Knowing the dependence of NDVI and NBI indices on the leaf area index (LAI) and the leaf nitrogen content (Nm), respectively, CNN can be seen as the product of the green biomass and nitrogen content of this biomass. Therefore, CNN should be proportional to the nitrogen uptake by the vine. Only the division of LAI by LMA (leaf mass per area) is missing to transform it into real mass-based units.

From a kinetic point of view, CNN shows a more regular evolution along vine development cycle (Fig. 3). Moreover, CNN still reflects correctly the contrast between 2010 and 2011 with very distinct evolution due to different climate conditions as mentioned previously.

Evolution of CNN in 2010 and 2011

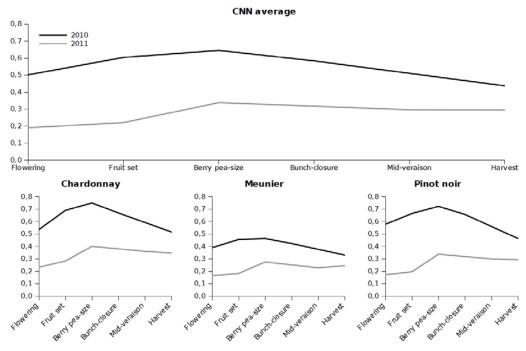


Fig. 3: CNN changes in 2010 and 2011

The CNN curve is characterized by two parts (Fig. 3). The first part from flowering to fruit set is defined by an increase that can be related to nitrogen intake by the plant dedicated to primary metabolism. The second part, from fruit set until harvest, reflects a decrease or a stabilization due to the leaf carbon accumulation. The nitrogen content decreased throughout the season (Prieto et al., 2012). Knowing that the NDVI values saturate at the end of the growing season (Debuisson et al., 2010) the CNN value might be underestimated at harvest.

Relationships between optical indices and agronomical parameters

The agronomical parameters for both years are presented in Table 1 and underline the typicity of each year. In 2011, due to the lower vegetative development, both yields and nitrogen contents in the must were strongly affected compared to 2010. Average yield for the plot decreased by almost 50%, and nitrogen content in the must dropped in average by 35% in 2011. As a result, nitrogen uptake in fruits decreased by almost 40% in 2011.

The 27 values of the data set, for each parameter, were standardized (mean=0, SD=1) to be able to correlate CNN with yield for both years. The yield correlated very well with CNN in 2010 and 2011 for the different dates of measurements. The linear regressions had a R² of 0.85 and 0.75 respectively in 2010 and 2011 (Fig. 4). Satisfactory correlations were also obtained between CNN and grape nitrogen uptake in the must (product of the yield and the total nitrogen in the must), regardless of the year or the date of measurement. Linear regression gave a R² of 0.7 (Fig. 4).

Table 1: Mean values of agronomical parameters per cultivar in 2010 and 2011

	2010			2011		
	СН	MN	PN	СН	MN	PN
CNN (1)	0,67	0,42	0,66	0,4	0,27	0,33
Potential alcool	10,5	10,2	8,6	9,9	9,6	9,3
% vol.						
Yield (kg/vine)	2,7	2,9	3,4	1,8	1,6	1,5
Total N must	275	400	241	221	246	157
(mg/L)						
N uptake in must	737	1163	831	401	411	239
(mg/vine)						

N uptake = yield x Total N must

(1) CNN 2010 at berry pea-size and CNN 2011 at bunch-closure

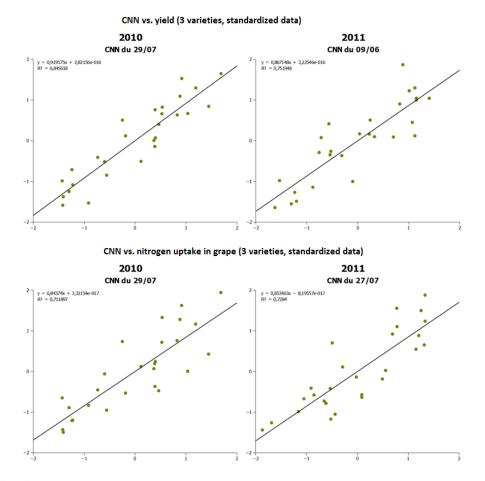


Fig. 4: Relationships between CNN and grape nitrogen uptake in grapes or yield on standardized values for both years.

CONCLUSION AND PERSPECTIVES

Classical agronomical parameters at harvest and physiological optical indices on leaves, obtained through high density non-destructive measurements, were assessed during the last two years. They bring new elements to understand grapevine development during the season on a multiyear perspective. Combined index such as CNN gives good information on physiological operating level of a grapevine as a result of water and nitrogen availability throughout the season. CNN is the product of the green biomass and the nitrogen content of this biomass. It gave good correlations with the yield and nitrogen uptake in grapes at harvest for both years. Provided a few more years of data for validation, this new indicator could be used, as early as flowering, to manage foliar applications of nitrogen, and to modulate yield or pruning levels. An important parameter from a multi-year point of view is the vine vigour, through quantity and quality of wood produced over a year. Lignification quality and sugar reserve in wood is a result of the past vegetative season, but also constitutes the starting point of the upcoming season. Development of a specific precision viticulture sensor to measure this wood parameter will soon be presented by the Comité Champagne (CIVC).

All these indicators should provide the necessary tools for the winegrower to improve the mastering of its vineyard. Better understanding of spatial variability across the vineyard could offer perspectives of classification of plots and differential farming practices, either to homogenize or to promote diversification of a vineyard's production.

REFERENCES

Agati, G., Meyer, S., Matteini, P., Cerovic, Z.G. (2007). Assessment of anthocyanins in grape (*Vitis vinifera L.*) berries using a non-invasive chlorophyll fluorescence method. J. Agric. Food Chem., 55: p. 1053-1061.

Debuisson, S., Grelier, M., Panigai, L. (2009). Mesure de la végétation avec un capteur NDVI embarqué. Le Vigneron Champenois décembre. p. 52-71.

Debuisson, S., Germain, C., Garcia, O., Panigai, L., Moncomble, D., Le Moigne, M., Fadaili, E.M., Evain, S. and Cerovic, Z.G. (2010). Using Multiplex[®] and greenseeker[™] to manage spatial variation of vine vigor in Champagne, in ICPA2010, Denver Co USA, pp 1-21.

Cartelat, A., Cerovic, Z.G., Goulas, Y., Meyer, S., Lelarge, C., Prioul, J.-L., Barbottin, A., Jeuffroy, M.-H., Gate, P., Agati, G., Moya, I., (2005). Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (*Triticum aestivum L*.). Field Crops Res. 91: p. 35-49.

Cerovic, Z. G., Moise, N., Agati, G., Latouche, G., Ben Ghozlen, N., Meyer, S. (2008). New portable optical sensors for the assessment of winegrape phenolic maturity based on berry fluorescence. J. Food Comp. Anal., 21: p. 650-654.

Cerovic, Z.G., Goutouly, J.P., Hilbert, G., Destrac-Irvine, A., Martinon, V., Moise, N. (2009). Mapping winegrape quality attributes using portable

fluorescence-based sensors. In FRUTIC 09. Conception, Chile. (Ed. S Best) (Progap INIA, Chillian, Chile), p. 301-310.

Dobrowski, S.Z., Ustin, S.L., Wolpert, J.A. (2003). Grapevine dormant pruning weight prediction using remotely-sensed data. Aust. J. Grape Wine Res. 9: p. 177-182.

Goutouly, J.P., Drissi, R., Forget, D., Gaudillère, J.P. (2006). Characterization of vine vigor by ground based NDVI measurements, VIth International Terroir Congress. p. 237-242.

Johnson, L.F. (2003). Temporal stability of an NDVI-LAI relationship in a Napa Valley vineyard. Aust. J. Grape Wine Res. 9: p. 96-101.

Kolb, C.A., Pfundel, E.E. (2005). Origins of non-linear and dissimilar relationships between epidermal UV absorbance and UV absorbance of extracted phenolics in leaves of grapevine and barley. Plant, Cell and Env. 25: p. 580–590.

Kolb, C.A., Kopecky, J., Riederer, M., Pfündel, E.E., (2003). UV screening by phenolics in berries of grapevine. Funct. Plant Biol. 30: p. 1177–1186.

Meyer, S., Cerovic, Z.G., Goulas, Y., Montpied, P., Desmotes-Mainard, S., Bidel, L., Moya, I. and Dreyer, E. (2006). Relationships between optically assessed polyphenols and chlorophyll content and the dry mass per leaf area ratio of woody plants: a signature of the carbon and nitrogen balance within leaf? Plant Cell Environ. 29: p.1338–1348.

Prieto, J.A., Louarn, G., Perez-Pena, J., Ojeda, H., Simoneau, T. & Lebon, E., 2012. A leaf gas exchange model that accounts for intra-canopy variability by considering leaf nitrogen content and local acclimation to radiation in grapevine (*Vitis vinifera L.*), Plant Cell and Environ.: in press.

Rouse, J.W., Haas, R.H., Schell, J.A., Deering, D.W., and Harlan, J.C. 1974. Monitoring the vernal advancement and retrogradation (greenwave effect) of natural vegetation. III Final Report, Greenbelt, Md. USA.

Tremblay, N., Wang, Z. and Cerovic, Z.G. (2012) Sensing crop nitrogen status with fluorescence indicators. A review. Agron Sust Develop 32: p.451-464.