FOLIAR SPRAYING OF NITROGEN AND SULFUR AT VERAISON: A VITICULTURAL TECHNIQUE TO IMPROVE AROMATIC COMPOSITION OF WHITE AND ROSÉS WINES.

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ABSTRACT

The aim of these studies is to evaluate the impact of nitrogen and sulfur foliar applications at the vineyard on the presence of varietal thiols in white and rosés wines.

Nitrogen pulverization at the veraison phenological stage improves significantly grape nitrogen status which is known to be a key factor of the varietal thiols release in wine. Also, these fruity thiols are linked to the plant sulfur metabolism by S-cysteine conjugates described as aroma precursors. Furthermore, cysteine is the main carrier of sulfur in plants.

Five French varieties - Sauvignon, Colombard, Gros Manseng and Négrette in the South-West of France vineyards, Melon and Sauvignon in the Loire Valley - were tested on experimental plots between 2005 and 2007. Nitrogen was sprayed under urea form in range from 10 to 20 kg per hectare in relation with the must nitrogen status of the non treated control. Sulfur was applied at the same time under its elemental soluble form in range from 5 to 10 kg per hectare. After fermentations at pilot scale in 30 liters tanks and under standardized protocols, varietal thiols in wines were analyzed as well as sensory analysis courses were performed by expert tasters in order to confirm the effect of the aromatic sulfur compounds.

Results show a spectacular impact of the practice on the quantity of the two molecules analyzed, 3-mercapto-hexanol (3MH) and 3-mercaptohexylacetate (3MHA). These results are confirmed in all the situations by wine tasting. No other secondary effects (yield, maturity, sanitary status) are noticed on vines.

Key words : foliar spraying, nitrogen, sulfur, varietal thiols, rosé wine, white wine

INTRODUCTION

Varietal thiols are aromatic compounds first identified in Sauvignon wines (Tominaga, 1998). As varietal aroma, they contribute to the typicity flavor of wines all around the world. They have been identified in several white wines from different varieties at high level (Tominaga et al., 2000) . They contribute also to the aroma of some Rosés and red wines (Murat and al., 2001). Among the thiols with pleasant flavors identified in wines, two molecules are always found, these are 3-mercaptohexanol (3MH) with smells of grapefruit and exotic fruit and 3-

mercaptohexylacetate (3MHA) with vegetal odor like box tree. We focus our work on them. These aromatic compounds are release in wine during alcoholic fermentation by the yeast enzymatic metabolism from odorless precursors identified in grapes as S-cysteine and S-glutathion conjugates (Peyros Des Gachons, 2000). Moreover, varietal thiols are present in less concentration (nanomole per liter-scale) and are very sensitive to oxidative reactions. The processes to extract precursors, to allow yeast to release them and finally to preserve them in the bottle demand full technology and knowledge for the winemaker and are the first primordial steps to control.

Recent works show that a high nitrogen (N) status of grape must is one of the factors that promote varietal thiols in wines (Choné et al., 2006). Thus, it is well known that high nitrogen status of the must could be obtained by high level of soil fertilization. But in this way, it increases vegetative growth, plant sensitivity to diseases, yield and decrease maturity. The management of the nitrogen is a long story in viticulture and the best approach developed is the control of genetic factors (cultivar, rootstock), cultural practices (soil management, canopy management, water availability) and environment factors (climate, soil). In the summer period, around veraison a large part of the nitrogen uptake by the grapevine is found in the bunches (Conradie, 1986). This moment could be the worth management time for viticulturist who would optimized the grape nitrogen composition by technique without unbalancing the plant physiology. Even if roots are the organs for assimilation of nitrogen, as many plants, grapevine is able to uptake nitrogen under urea form by the leaves. Experiment using stable nitrogen isotope (^{15}N) showed on maize, wheat and tomato the fastness assimilation of urea by leaves with few losses on the soil (Genter and al., 1998). Thus, foliar spraying appears to be a technique that involves some benefits like less amount of N to bring, soil composition and water status independency, assimilation fastness (Gooding and *al.*, 1992). Combination of nitrogen and sulphur (S) foliar spraying on wheat crop at anthesis phenological stage induce modification of the nitrogen composition in the grain by increasing in particular its concentration in glutathione and sulphur amino-acids (Téa, 2004).

We try to reproduce this kind of result on grapevine in the aim to modify and improve the nitrogen composition of the grape must and in order to increase *in fine* the varietal thiols concentration in wines. First results have already been carried out and have shown the efficiency of the technique (Charrier and Dufourcq, 2007) (Lacroux and al., 2008). This paper summarises results observed between 2005 and 2007 in different vineyards and on different varieties.

MATERIALS ET METHODS

Vineyard and cultivar

Five different varieties were tested in different regions: Colombard, Gros Manseng, Négrette and Sauvignon in the South-West of France vineyards (Gascony, Fronton, Gaillac) ; Melon and Sauvignon in the Loire Valley (Muscadet, Touraine) (table 1).

Foliar spraying

Foliar spraying consists in two applications around veraison of urea (FOLUR™ - Tradecorp) at 10, 15 or 20 kilograms of N per hectare solubilized in 400 liters water solution. When sulfur is associated, it is in its elementary form (Microthiol™ - CerexAgri) from 5 to 10 kilograms of S per hectare mixed with urea.

We worked on experimental plot (100-vines scale) divided in 3 blocs per modality with non sprayed control to compare.

Wine making

Grapes were processed in our experimental cellars in each region and under a strict non-oxidative protocol, using carbonic gas and cold temperature in pre-fermentation, selected yeast strains and controlled temperature for alcoholic fermentation. Around 30 to 50 liters of wine were made for each modality. Colombard, Sauvignon, Gros Manseng and Melon were processed in dry white wine ; Négrette in Rosés wine and one Gros Manseng 2007 in sweet wine (40g/l sugar).

Analysis

Nitrogen of the must was characterized by ammonia and amino-acid nitrogen concentrations on KonéLab Arena20 analyzer – Thermo Electron Corporation. Ammonia nitrogen (NH_4^+) was analyzed with enzymatic kit "Trace ammonia – Thermo Electron Corporation – Ref: TR60101". Amount of nitrogen from amino acid sources was held by colorimetric OPA (Orthophtaldéhyde) method.

Varietal thiols were analyzed at the UMR SPO INRA laboratory in Montpellier (France) with the method described by Schneider et al. (2003). Wine tasting ran in each region with local experts.

RESULTS AND DISCUSSION

Nitrogen spraying at veraison and must nitrogen composition

Most of the time nitrogen under urea form sprayed at veraison on grapevine involved an increase of the nitrogen concentration of the must (figure 1). In our experiment we managed almost one hundred of samples from 10 to 20 kg of N per hectare. On average, the increase of the nitrogen in must was linear. For 10 kilograms per hectare you can attempt around 50% of increasing, and for 20 kg upper than twice. The variability in response has to be noticed. We observed also in some cases no response in the must. Many factors occur in spraying at vineyard: crop management, period and time of application, weather conditions, urea formulation…No simple explanation could be proposed.

Another result was to compare the effects on must nitrogen concentration of urea and sulfur mix compared with urea spraying. Some results showed a modification in the final composition (wheat grain – Téa, 2004) and suggested sulfur synergic effect in assimilation of nitrogen. In our conditions, no differences appeared within the both treatments (figure 2). We can consider that nitrogen and sulfur mix sprayed on vines did not increase the level of nitrogen status of a must in comparison with the same dose of nitrogen applied in the same conditions.

Foliar spraying of nitrogen and sulfur at veraison: relation with varietal thiols composition in wines.

All the wine making processes were optimized in order to produce varietal thiols in wine. The grapes were protected again oxidation and specific yeast strains were used for alcoholic fermentation. The table 2 shows the main results. Some varieties like Colombard and Gros Manseng were high producer of varietal thiols according to the particular conditions of production (soil, climate and cultural practices). In Gascony, these varieties are conducted to produce fresh and fruity young white wines with vigorous and up-yield vines. In this case, varietal thiols are the main aromatic flavor that contributes to the expected quality. The others white varieties of Sauvignon and Melon in Touraine, Gaillac and Muscadet were under-controlled to produce more structured wines. Grapes presented low to moderate nitrogen concentrations (table 2). The concentration in varietal thiols measured in the controls ranged

within 0,4 and 2,4 nanomoles per liter for the molar sum of the 3MH and 3MHA. At this level, the thiols contribute more or less to the wine bouquet but are not over expressed. The varietal thiols analyzed in the Rosés from Négrette were present in interesting quantity (1,8 to 5,9 nanomoles per liter). It suggests that they contributed surely to the flavor of the wine and were in relation with the process of wine making.

The spraying of the mix of nitrogen and sulfur was conducted in two times, beginning around 20% of berries veraison. No secondary effects were noticed on vines within application and harvest: sanitary status stayed at the same level, maturity was slightly delayed (more acidity) but never significantly, no differences occurred in yield. The grapes were harvested at the same time on each plot. The varietal thiols obtained in wine were compared to the non sprayed control. As we searched effects on the quantities of molecules, we used the molar sum of the two compounds as indicator, according that these molecules come from the same precursor and also that 3MHA is only a transformation (acetyltransfer) of part of the 3MH under the alcoholic fermentation conditions (Sweigers and Pretorius, 2007).

There always was a gain in varietal thiols measured in wines from the sprayed plots in comparison with controls (figure 3). On average, we observed three times more aromas in wines and up to twelve times more. Even if the control wine was high concentrated in thiols (10 to 40 nanomoles per liter) we obtained from the sprayed plot highest level of aromas in wines. This suggests that this technique is fully influential for the production of such compounds. When a low rate of thiols was present in controls (0,4 to 6 nanomoles per liter), the increasing of concentrations was less scattered and in the same range (figure 3).

The must nitrogen status is a factor that contributes to the aroma potential expression (Bell and Henschke, 2005) (Choné and *al.*, 2006). We observed the distribution of the gain in aromas (nitrogen-sulfur spraying/control ratio) in relation with the nitrogen concentration of the control must. The trend was: the less nitrogen is concentrated in must, the more, after spraying of nitrogen and sulfur at vineyard, gain in varietal thiols is observed in wines (figure 4).

All the wines were tasted in the year following harvest by experts. These tasting courses shown up differences, most of the time significantly, within wines coming from sprayed plots and controls. These courses checked the robustness of the technique in the way to transfer it in industrial conditions. They confirmed also that no undesirable sulfur perception occurred in wines.

Spraying of nitrogen and sulfur on vines appears to be a powerful technique. On deficient vine with poor aromatic expression, it could allow to maintain grass covered vineyard which are known to induce low level of nitrogen in must and in consequence some off-flavors in wine (Spring and Lorenzini, 2006). On some varieties it may promote over-expression of the varietal thiols flavor, for the best, or instead of aromatic complexity.

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Table 1 : Main characteristics of the experimental plots

Variety	Origin	Vintage	Density vines/ha	Product tons/ha	Water stress at vineyard	Nitrogen in control must
Colombard	Gascony	2005	3333	15-20	Moderate	High
		2006	3333	15-20	Weak	High
		2006	3571	10-12	Moderate	Medium
		2007	3571	10-12	No	Medium
G. Manseng		2007	4000	15-20	No	Medium
		2007	4000	12-15	No	Medium
Sauvignon	Gaillac	2006	4000	10-12	Moderate	Medium
		2007	4000	12-15	No	Low
	Touraine	2007	6000	6-7	No	Medium
Melon	Muscadet	2007	6500	7-9	No	Medium
		2007	6500	7-9	No	Medium
		2007	6500	7-9	No	Medium
Négrette	Fronton	2006	5000	6-8	Weak	Low
		2006	5000	8-10	Weak	Medium
		2007	4329	7-9	No	Medium

Table 2: varietal thiols concentrations in wines and nitrogen contents in must

			molar sum [3MH+3MHA] ($nmol.l^{-1}$)		Nitrogen in must ($mg.l^{-1}$)	
vintage	variety	origin	N+S spraying	Control	N+S spraying	Control
2007	Colombard	Gascony	26,8	14,4	218	153
2007	Gros Manseng	Gascony	59,5	11,5	141	84
2007	Gros Manseng	Gascony	80,9	12,7	153	94
2007	Melon	Muscadet	4,2	1,0	255	130
2007	Melon	Muscadet	5,6	2,3	241	112
2007	Melon	Muscadet	6,8	2,4	231	116
2007	Négrette	Fronton	19,6	5,8	197	108
2007	Sauvignon	Gaillac	5,4	0,4	n.a	n.a
2007	Sauvignon	Touraine	3,2	1,4	114	81
2006	Colombard	Gascony	31,0	19,8	363	141
2006	Colombard	Gascony	57,6	37,9	237	197
2006	Négrette	Fronton	7,4	1,8	228	59
2006	Négrette	Fronton	11,2	4,5	183	130
2006	Sauvignon	Gaillac	13,4	1,9	144	65
2005	Colombard	Gascony	36,4	26,9	212	187

(n.a : not available)

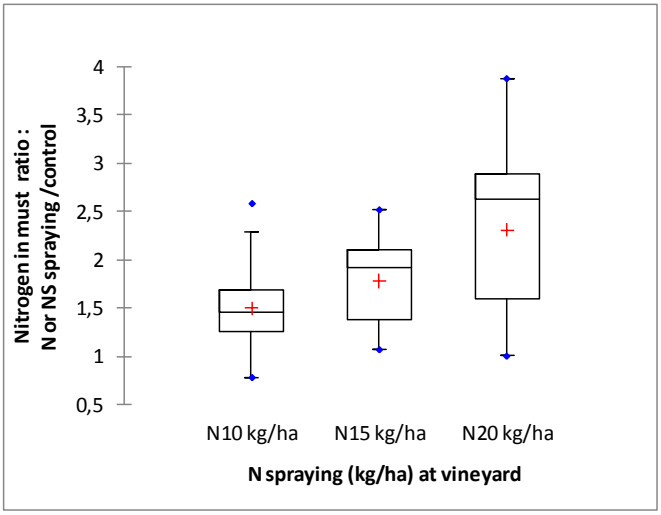

Figure 1: nitrogen gain in must in relation with the quantity of urea sprayed at vineyard (101 observations)

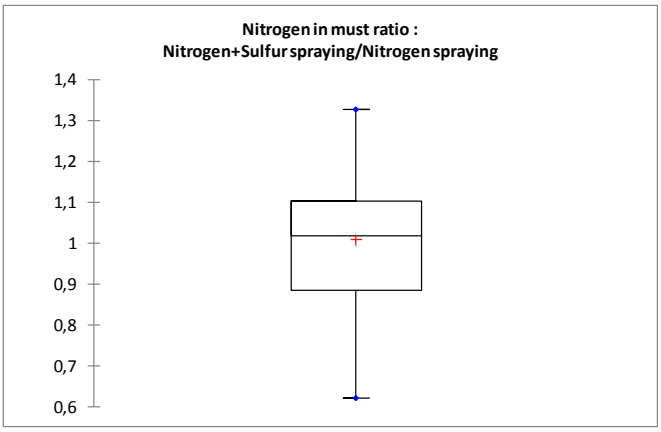

Figure 2: Effect of nitrogen and sulphur spraying versus nitrogen spraying on the must nitrogen status (42 observations).

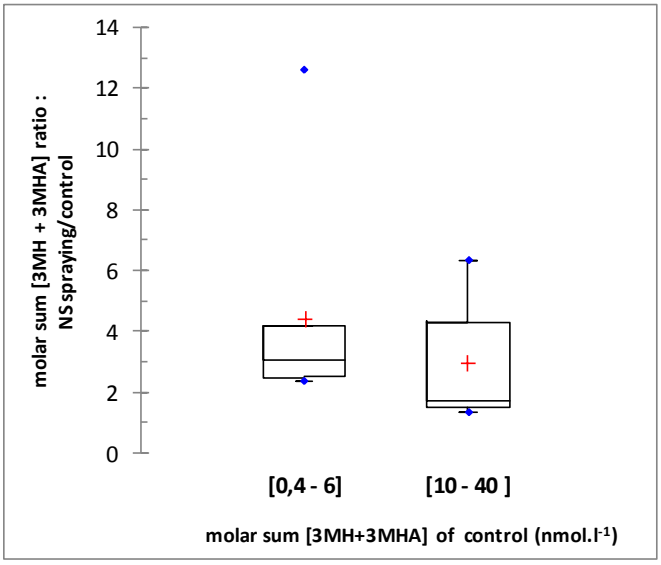

Figure 3 : varietal thiols gain in wines after nitrogen and sulphur spraying at vineyard (15 observations)

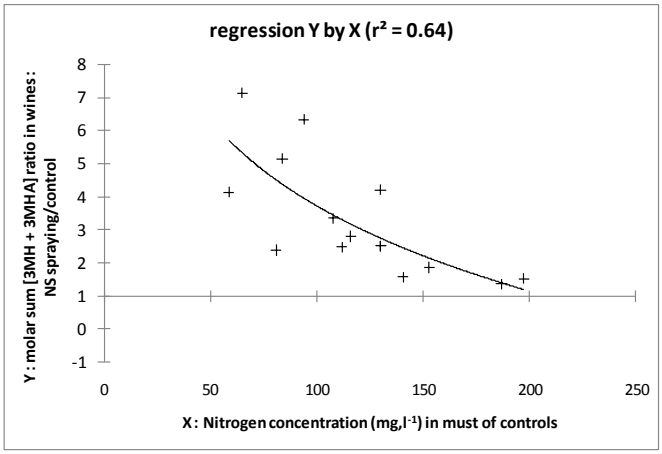

Figure 4 : relation within nitrogen concentration in control must and varietal thiols gain in wines after nitrogen and sulphur spraying at vineyard.