Investigating the impact of grape exposure and UV radiations on rotundone in *Vitis vinifera* L. cv. Tardif grapes under field trial conditions

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Abstract

Rotundone is the main aroma compound responsible for peppery notes in wines whose biosynthesis is negatively affected by heat and drought. Through the alteration of precipitation regime and the increase in temperature during maturation, climate change is expected to affect wine peppery typicality. In this context there is a demand for developing sustainable viticultural strategies to enhance rotundone accumulation or limit its degradation. It was recently proposed that ultraviolet (UV) radiations could stimulate rotundone production. The aim of this study was to investigate under field trial conditions the impact of grape exposure and UV treatments on rotundone in *Vitis vinifera* L. cv. Tardif, an almost extinct grape variety from south-west France that can express particularly high rotundone levels. Four different treatments were compared in 2021 to a control treatment using a randomised complete block design with three replications per treatment. Grape exposure was manipulated through early or late defoliation. Leaf and laterals shoots were removed at Eichorn Lorenz growth stages 32 or 34 on the morning-sun side of the canopy. During grape maturation, UV radiations were either reduced by 99% by installing UV radiation-shielding sheets, or applied four times with the aim of activating plant signalling pathway. The results of this study are preliminary as the samples still have to be analysed for their rotundone content. However, the fact that the studied techniques had a limited impact on bunch zone air temperature determined by loggers displayed in solar radiation shields shall enable to assess the single impact of UV radiations on rotundone biosynthesis. Defoliation induced a significant increase in grape anthocyanin content in accordance with previous results, while the application of UV-C provoked a significant increase in wine bitterness at tasting for a similar grape total phenolic index, suggesting a modification in phenolic composition. The experiment will be repeated in 2022, 2023, and complementary measurements notably penetration testing will be conducted to better understand the changes induced by the UV-C treatment.

Introduction

Rotundone, a sesquiterpene first identified in Syrah wine, is the main contributor to peppery notes in grapes and wines (Wood et al., 2008). The biosynthesis of rotundone is negatively affected by heat and drought (Geffroy et al., 2014; Zhang et al., 2015). Through the alteration of precipitation regime and the increase in temperature during maturation, climate change is expected to affect wine peppery typicality. In this context there is a demand for developing sustainable viticultural strategies to enhance rotundone accumulation or limit its degradation.
It was recently proposed that ultraviolet (UV) radiations could stimulate rotundone production (Geffroy, Kleiber, & Jacques, 2020). The mechanisms are currently unknown but could involve i) a direct stimulation of the rotundone synthesis pathway or ii) cause an overproduction of reactive oxygen species (Ros) which would oxidize α-guaiene, the precursor of rotundone, to rotundone.

The objectives of this work conducted in ecophysiological conditions are to better understand the impact of leaf removal and UV exposure, and to develop a new strategy for maximizing rotundone based on UV applications using LED lights.

Materials and methods

Experimental design and treatments

A field trial was conducted in 2021 using a complete randomized block design on a plot of *Vitis vinifera* L. Tardif (PDO Saint Mont), a genotype known for its high potential for rotundone production. The 0.4 ha-vineyard (lat. 43°, 38', 11.0''N; long. 0°, 7', 25.6'' W), typical of the area with 2.20 m × 1 m vine spacing, was planted in 2018. Orientation of the rows was north-west to south-east, and vines were trained with vertical shoot positioning on a single Guyot pruning system. The soil was managed by chemical weed control under the vines and by grass cover or mechanical weeding in every second inter-row. Each treatment was replicated 3 times on elementary plots made up of 12 adjacent vines.
4 different treatments were investigated and compared to a control treatment (CTRL):

- Early defoliation (ED): elimination of leaves in the fruit-bearing zone up to the 3rd node on the side of least exposure, on June 30, i.e. 18 days after flowering (Eichhorn and Lorenz 32)
- Late defoliation (LD): elimination of leaves in the fruit-bearing zone up to the 3rd node on the side of least exposure, on August 6, i.e. 12 days before veraison (Eichhorn and Lorenz 34)
- Late defoliation combined to UV-screens (LD - UV): after late defoliation performed as for the LD treatment, a UV radiation-shielding sheets filter blocking 99% of UV radiation (Altuglas, La Garenne-Colombes) was placed in the bunch area (Figure 1A).
- Late defoliation combined to UV applications (LD + UV): after late defoliation performed as for the LD treatment, a UV-C treatment was applied on August 18 (veraison), August 30, September 10 and September 23 using the Boxilumix™ device (Asclepios Tech, Tournefeuille) (Figure 1B).

Figure 1. Illustration of the late defoliation combined to (A) UV-screens or to (B) UV applications treatments

Analytical parameters and microclimate measurements

On October 12 at harvest time, 200 berries were sampled for each elementary plot from the side of the row exposed to the treatments and weighed for analysis of classic oenological parameters (°Brix, titratable acidity, pH, tartaric and malic acids, anthocyanins, Total Polyphenols or TPI, yeast assimilable nitrogen), as well as for the analysis of $\delta^{13}C$. This latter parameter reflects the level of water stress experienced by the plant during maturation (van Leeuwen et al., 2009). For each harvested vine, the yield and the number of clusters were also measured. 100g-berry samples were collected and then frozen at -18°C for rotundone analysis according to a previously published protocol (Siebert, Wood, Elsey, & Pollnitz, 2008).
As radiation-shielding sheets are like to impact bunch microclimate, EBI 20-T1 temperature loggers (Ebro, Ingolstadt, Germany) displayed in solar radiation shields were positioned in the bunch area to monitor air temperature during ripening on the control, LD, and LD - UV treatments. The percentage of hours when the

temperature exceeds 25°C (Dh25), an indicator well correlated to the level of rotundone in grapes (Zhang et al., 2015), was calculated.

Mini-vinification and sensory analysis

After harvest, grapes were transported to our experimental cellar to be vinified. After destemming, crushing, sulphite addition at 40 mg/L and yeast addition at 200 mg/L with 522 Davis strain (Lamothe Abiet, Canéjan), maceration was carried out at 25°C for 8 days with an unique daily punching down. Given the small amount produced for each treatment, the wines were not filtered before bottling. The sensory analysis was carried out by a trained panel composed of 9 panelists. Wines were rated on a discontinuous scale from 0 to 9 using 15 sensory descriptors including peppery/spicy notes. Two consecutive 3-alternative forced choice (3-AFC) tests (100 µL of a 10% vol. ethanol solution vs 100 µL of a 1 mg/L-rotundone solution in 10% ethanol using 2.5mL-flasks) were conducted at the end of the session to detect anosmic panelists to rotundone.

Statistical processing

Statistical processing was performed using Xlstat software (Addinsoft, Paris). Data were treated through a two-factor analysis of variance (ANOVA) (block x treatment or panelist x treatment) followed by a Fisher's test.

Results and discussion

Impact on bunch microclimate

Differences in bunch zone air temperature between the treatments were not very marked (Figure 2). For the LD treatment, temperature appears to be slightly cooler in the afternoon, which could be explained by a greater wind penetration in the canopy and consequently a better ventilation. On the opposite, the LD - UV treatment exhibited higher temperature in the morning, when solar radiations were incident, probably in connection with the greenhouse effect generated. However with regard to Dh25, no significant difference could be demonstrated at $P<0.05$ between the control (Dh25=10.47% ± 0.63), LD (Dh25=10.63% ± 0.85) and LD - UV (Dh25=10.65% ± 0.90) treatments.

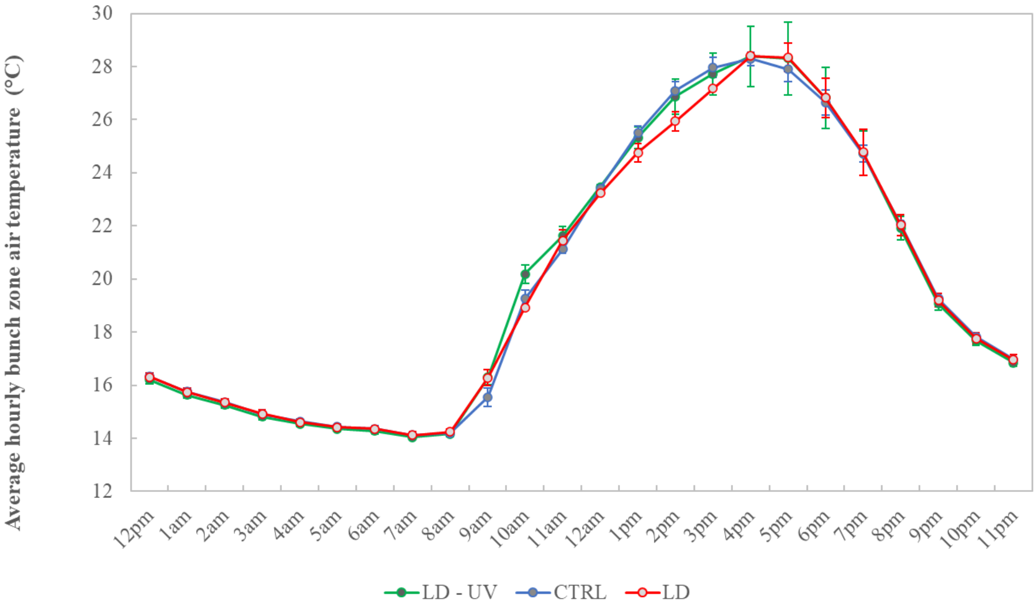

Figure 2. Impact of the late defoliation (LD) and late defoliation combined to UV-screens (LD - UV) treatments on the average hourly bunch zone air temperature between veraison and harvest.
Error bars refer to variability between vineyard blocks.

Impact on yield, grapevine water status and basic fruit parameters

Statistical data processing did not reveal any significant differences for number of bunches and yield (Table I) which tends to demonstrate the homogeneity of our experimental design. Indeed, no impact of the studied techniques on yield parameters was expected. Although Tardif is known for the production of small and light bunches, the extremely low crop level is the consequence of the spring frost which damaged the buds/primary shoots enhancing the development of lesser fertile lateral shoots. This exceptional climatic event that occurred in April 2021 was also accompanied by a large heterogeneity in vegetative and fruit developments which can be clearly visible on the raw data by a marked variability between the blocks. Among the measured parameters, the content in anthocyanins was the only one significantly impacted by the treatments. In comparison with the *control*, a greater concentration was observed with the ED and LD treatments which is relevant with previous findings (Hunter, De Villiers, & Watts, 1991). It also worth mentioning that a lesser level of water constraint as reflected by lower $\delta^{13}C$ values would have been expected for the defoliated treatment as a consequence of a lesser plant transpiration.

Table 1. Impact of the studied treatments on yield, grapevine water status and basic fruit parameters. Different letters indicate means significantly different at $P<0.05$ by Fisher's test. ED stands for Early Defoliation, LD Late Defoliation, '+ UV' UV application and '- UV' UV blocking screens.

Parameter	CTRL	ED	LD	LD - UV	LD + UV	*P*
Weight of 200 berries (g)	266 a	281 a	261 a	263 a	271 a	0.610
Sugar concentration (°Brix)	23.3 a	23.2 a	23.5 a	23.4 a	22.8 a	0.489
Titratable acidity (g/L tartaric acid)	10.23 a	10.01 a	9.20 a	9.67 a	10.01 a	0.726
pH	3.09 a	3.00 a	3.06 a	3.11 a	3.04 a	0.257
Tartaric acid (g/L)	5.71 a	6.12 a	5.85 a	5.92 a	5.93 a	0.485
Malic acid (g/L)	5.31 a	4.82 a	4.47 a	4.53 a	4.97 a	0.182
Amino acids (mg/L)	97.0 a	61.3 a	58.9 a	80.1 a	67.6 a	0.208
NH_4^+ (mg/L)	9.33 a	0.00 a	0.03 a	8.33 a	8.73 a	0.616
K^+ (g/l)	1.42 a	1.38 a	1.34 a	1.54 a	1.38 a	0.074
Total Phenolic Index (TPI)	190 a	218 a	252 a	272 a	217 a	0.092
Anthocyanins (mg/L)	1653 b	2489 a	2442 a	1886 ab	1978 ab	0.027
$\delta^{13}C$	-26.29 a	-26.53 a	-26.98 a	-26.41 a	-26.77 a	0.554
Number of bunches per vine	15.8 a	13.9 a	14.4 a	14.7 a	15.8 a	0.669
Crop load per vine (kg)	0.61 a	0.61 a	0.61 a	0.87 a	0.73 a	0.250

Impact on rotundone and wine sensory characteristics

At the time of writing, the analytical method for the determination of rotundone was not fully operational and the results will be available by the beginning of June 2022. Among the 9 trained panelists, two were not able to succeed in both 3-AFC tests. These panelists were considered as anosmic respondents and their related data were removed before performing statistical treatment. Besides *bitterness*, no sensory attributes enabled to discriminate the five experimental wines. The wine made from the LD + UV treatment was characterized by a higher *bitterness* score. Despite that no analytical differences were noticed for TPI, it can be hypothesized that UV-C treatments provoked a modification in phenolic composition and notably an increase in low molecular weight phenols whose contribution to bitterness in wine has been previously demonstrated (Gonzalo-Diago, Dizy, & Fernández-Zurbano, 2014). As wines were made from all the grapes found on the experimental site and not only from berries collected on the side exposed to the treatments, it can be assumed that the observed effect is systemic or particularly high in intensity. In an analogous manner, it can be pointed out that the fact that the intensity of peppery notes at tasting was not impacted doesn't necessarily mean that no difference in rotundone will be observed for the grape samples.

Conclusion

The results of our study are still preliminary as the samples still have to be analysed for their rotundone content. However, the fact that the studied techniques had a limited impact on bunch zone air temperature shall enable to assess the single impact of UV radiations on rotundone biosynthesis. Defoliation induced a significant increase in anthocyanins in accordance with previous results, while the application of UV-C provoked a significant increase in bitterness at tasting which suggests modification in phenolic composition. The experiment will be repeated in 2022 and in 2023. Complementary measurements and notably penetration testing will be conducted in 2022 to better understand the changes induced by the UV-C treatment. Additionally, the conditions for applying UV-C will be refined on the basis of experiments carried out on simpler grape models (calluses and fruiting cuttings).

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