OENO One
vine & wine

DOI: https://doi.org/10.20870/oeno-one.2026.60.3.9626

OPEN ACCESS




SPECIAL ISSUE

# Assessment of grape exposure and UV radiation effects on rotundone in *Vitis vinifera* L. cv. Tardif under warm and dry vintage conditions

Olivier Geffroy[1,*], Thomas Baerenzung dit Baron[1,2], Christian Chervin[3], Chloé Cuif[1], Marie Denat[1], Carole Feilhès[4], Élodie Gassiolle[5], Jeanne Laforge[1], Laura Lescot[2], Ricardo López[6], Ignacio Ontañón Alonso[6], Jean-Pierre Petit[7], Christine Roynette[8], Anthony Santacreu[1], Valérie Simon[2], and Alban Jacques[1]

[1] Physiologie Pathologie et Génétique Végétales (PPGV), Université de Toulouse, École d'Ingénieurs de Purpan, 31076 Toulouse Cedex 3, France
[2] Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, Toulouse INP, INRAE, 31030 Toulouse, France
[3] LRSV, Université de Toulouse, INP-ENSAT, 31326 Auzeville-Tolosane, France
[4] Institut Français de la Vigne et du Vin Pôle Sud-Ouest, V'innopôle, 81310 Peyrole, France
[5] Plaimont Producteurs, 32400 Saint-Mont, France
[6] Laboratory for Aroma Analysis and Enology, Instituto Agroalimentario de Aragón-IA2 (Universidad de Zaragoza-CITA), Department of Analytical Chemistry, Faculty of Sciences, Zaragoza, E-50009 Spain
[7] Écophysiologie et Génomique Fonctionnelle de la Vigne (EGFV), Université de Bordeaux, Bordeaux Sciences Agro, INRAE, ISVV, 33882 Villenave-d'Ornon, France
[8] Asclepios Tech, 31170 Tournefeuille, France

**Article number: 9626**

*correspondence:
olivier.geffroy@purpan.fr

*Associate editor:*
Simone Castellarin

*Received:*
20 September 2025

*Accepted:*
10 February 2026

*Published:*
1 July 2026

This article is published under the **Creative Commons licence** (CC BY 4.0).

*Use of all or part of the content of this article must mention the authors, the year of publication, the title, the name of the journal, the volume, the pages and the DOI in compliance with the information given above.*

This article is a short communication article published in cooperation with the 16th International Terroir Congress and the 3rd ClimWine Symposium (July 5–9, 2026), hosted by the École Supérieure des Agricultures in Angers, France.

Guest editors: Cécile Coulon-Leroy and Etienne Neethling.

## ABSTRACT

Rotundone is the main aroma compound responsible for peppery notes in wines. Its biosynthesis is negatively affected by heat and drought, whereas the impact of light, particularly ultraviolet (UV) radiation, remains unestablished. This study aimed to investigate, under field conditions, the effects of grape exposure and UV treatments on rotundone in *Vitis vinifera* L. cv. Tardif. During the warm 2022 season, four treatments were compared to a control using a randomised complete block design with three replicates per treatment: early defoliation at Eichhorn & Lorenz stage 32 (ED), late defoliation at stage 34 (LD), exclusion of UV-A and UV-B radiations on late-defoliated vines using radiation screens (LD-UV), and four UV-C modulated light applications during daytime between mid-veraison and harvest on late-defoliated vines (LD+UV). No differences were observed between the control and ED, likely due to leaf regrowth limiting the initial increase in cluster exposure. In contrast, the LD treatment resulted in a significant 33 % increase in rotundone. LD-UV did not affect bunch zone air temperature but caused a significant reduction in rotundone, highlighting the key role of UV in its biosynthesis. UV-C treatments applied during daytime had no effect, whereas preliminary results on a limited number of fruiting cuttings suggest a substantial increase when treatments were applied at night. Overall, these results indicate that winegrowers cultivating Tardif can use late defoliation to enhance rotundone biosynthesis even during warm vintages. The effects of UV-C treatments warrant further validation on a larger number of vines under field conditions.

**KEYWORDS:** Terclim 2026, peppery aroma, defoliation, timing, UV screens, UV-C treatments

## INTRODUCTION

Rotundone is the main contributor to peppery notes in grapes and wines (Wood *et al.*, 2008). The biological function of this sesquiterpene has not yet been elucidated, but it could be involved in plant natural response, notably regarding powdery mildew *(Erysiphe necator)* (Geffroy *et al.*, 2015). As any other compound from the same class, its biosynthesis and emission by the plant could be affected by environmental factors such as temperature, plant water constraints, and light (Duhl *et al.*, 2008). Indeed, previous studies have shown that temperatures particularly above 25 °C, were detrimental (Zhang *et al.*, 2015) whereas water supply through irrigation effectively enhances rotundone levels in grapes and wines (Geffroy *et al.*, 2016).

The influence of light and ultraviolet (UV) radiation is not yet clearly established, with studies reporting contrasting outcomes. Indeed, investigations were generally focused on defoliation under field conditions, where it was challenging to separate the respective effects of light and temperature. Defoliation applied on both sides of the row markedly reduced rotundone concentration in Duras wines from south-west France (Geffroy *et al.*, 2014). Contradictory outcomes were reported in the north-east USA on Noiret, where fruit exposure either had no effect or even enhanced rotundone levels (Homich *et al.*, 2017). Similar results were observed in Fer cultivated under temperate conditions with very cool nights (Geffroy *et al.*, 2019a), highlighting the complexity of environmental interactions.

Other research works suggest a stimulatory role of light and UV radiation, as correlations between rotundone concentration and indices of solar radiation, including mean irradiation and sunshine duration during ripening, have been described (Geffroy *et al.*, 2019b). Although no controlled studies have specifically examined rotundone, evidence suggests that UV exposure can influence its biosynthesis. For example, UV treatment of grapevine leaves has been shown to enhance the production of the sesquiterpene *(E)*-nerolidol (Gil *et al.*, 2012). From a mechanistical point of view, UV is perceived by the UVR8 photoreceptor, which activates signalling cascades involving reactive oxygen species (ROS) and defence-related phytohormones such as jasmonic acid, salicylic acid, and ethylene (Liu *et al.*, 2015). In the case of rotundone, an oxygenated derivative of its precursor α-guaiene, it remains possible that ROS contribute directly by enhancing the oxidation reaction (Huang *et al.*, 2014).

This field research aimed to investigate in greater depth the effects of different timings of defoliation, as well as the influence of UV exposure, either reduced by UV screens following a previously published approach (Šuklje *et al.*, 2014), or enhanced through external UV-C treatments. Although the trial was initially planned over three consecutive seasons (2021–2023), only data from the 2022 vintage were retained for analysis due to severe climatic and phytosanitary constraints in 2021 and 2023, respectively.

## MATERIAL AND METHODS

### 1. Experimental site and design

The trial was conducted in 2022 using a complete randomised block design on a plot of *Vitis vinifera* L. Tardif (PDO Saint-Mont), an almost extinct genotype that is regaining popularity and is known for its high rotundone production (Baerenzung dit Baron *et al.*, 2025; Geffroy *et al.*, 2025). 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 three times on experimental units made up of 12 adjacent vines. It should be emphasised that this study was conducted as part of a three-year collaborative project with a cooperative cellar, which began in early 2021 and concluded in late 2023. For the 2021 and 2023 seasons, reliable data collection was not possible. This was due to an extreme spring frost in April 2021 and severe downy mildew *(Plasmopara viticola)* damage in 2023, both of which reduced the available plant material and introduced heterogeneity, preventing reliable data collection. During these two seasons, relocating the trial to an undamaged vineyard was not feasible, as other Tardif vineyards planted after 2020 were still too young for experimentation.

### 2. Treatments

Four different treatments were investigated and compared to a control treatment without intervention *(Control)* (Figure 1):

- *Early defoliation* (ED): elimination of leaves in the fruit-bearing zone up to the 3rd node on the side of least exposure (*i.e.*, north-east), on June 22, which corresponds to 22 days after mid-flowering (Eichhorn & Lorenz 32).
- *Late defoliation* (LD): elimination of leaves in the fruit-bearing zone up to the 3rd node on the side of least exposure (*i.e.*, north-east), on July 26, which corresponds to 13 days before mid-veraison (Eichhorn & Lorenz 34).
- *Late defoliation combined with UV-screens* (LD-UV): after late defoliation performed as per the LD treatment, a UV radiation-shielding sheet filter blocking 99 % of UV-A and UV-B radiation (Altuglas, La Garenne-Colombes, France), hereafter referred to as UV screens, was installed in the bunch area on the north-east side of the row from August 8, which corresponds to mid-veraison, until harvest.
- *Late defoliation combined to UV treatments* (LD+UV): after late defoliation performed as for the LD treatment, four UV-C modulated light treatments were applied for 1 min on the north-east side of the row at a distance of 10 cm from the fruits on August 8 (mid-veraison),

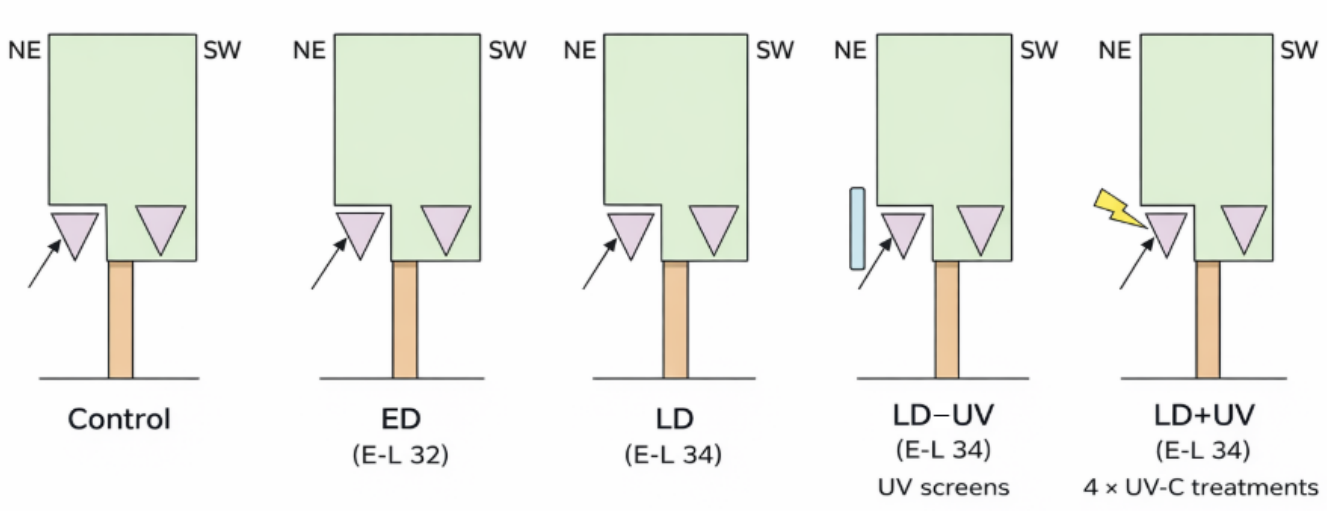



**FIGURE 1.** Schematic representation of the treatments applied to experimental Tardif grapevines. The arrows indicate the bunches from which the berries were collected to perform basic fruit and rotundone analyses. ED stands for Early Defoliation; LD, Late Defoliation; LD-UV, Late Defoliation combined with UV screens; LD+UV, Late Defoliation combined with UV-C treatments. NE, northeast; SW, southwest.

August 19, August 30, and September 12 using the Boxilumix™ patented device (Asclepios Tech, Tournefeuille, France) (Roynette & Roynette, 2024). The treatments were conducted at 11 am for practical reasons, as the experimental site was located a two-hour drive from our laboratory. With a wavelength ranging from 200 to 280 nm, UV-C is more energetic than UV-A and UV-B and is usually blocked from reaching the lower atmosphere by the ozone layer (Paulo *et al.*, 2019). The detailed settings (*i.e.*, wavelength, frequency, and modulation time), which cannot be divulged for confidentiality reasons, were obtained based on previous experiments conducted with grapevine cell cultures, highlighting a 10-fold increase in several sesquiterpenes (*i.e.*, bergamotene, β-caryophyllene, and α-humulene) using the protocol proposed by Diot *et al.* (2023). A key advantage of the system lies in its use of modulated light-emitting diodes (LEDs) in place of conventional mercury vapour lamps. This approach significantly reduces thermal load and enables precise, real-time control of irradiance and energy delivery at ultralow intensities (*e.g.*, modulated <100 $W \cdot m^{-2}$ *vs* continuous ~10,000 $W \cdot m^{-2}$ for mercury lamps). Such modulation in a sustainable way minimises the risk of photothermal and phytotoxic effects, thereby allowing for safer and more controlled exposure conditions in plant physiological experiments (Roynette & Roynette, 2024).

## 3. Phenological data and weather measurements

Phenological data were collected across the entire experimental site and from the reproductive organs on both sides of the row. The percentage of flowering was determined by estimating the percentage of opened flowers per inflorescence for 100 inflorescences. The percentage of veraison was determined by randomly collecting 100 berries from several parts of the bunch and by counting the number of soft berries. Linear regression tests were performed using Excel software (Microsoft Corp., Redmond, WA, USA) to calculate mid-flowering and mid-veraison dates.

To characterise the season accurately from a climatic point of view, daily high-resolution data provided by Météo France (Toulouse, France), the French weather service, were obtained for the experimental site using ANTILOPE (Champeaux *et al.*, 2011) for rainfall and AROME (Brousseau *et al.*, 2011) for air temperature (minima, maxima, and mean values) for the 2011–2025 period. The data were used to calculate the Huglin index, and cumulative rainfall between April 1 and September 30, and between January 1 and December 31 (Tonietto & Carbonneau, 2002).

As radiation-shielding sheets are likely to impact bunch microclimate, EBI 20-T1 temperature loggers (Ebro, Ingolstadt, Germany) displayed in solar radiation shields were positioned in the bunch area, on removable stakes at a height of 1 m above the ground, on the north-east side of the row, to monitor air temperature during ripening on the control, LD, and LD-UV treatments. For this latter treatment, loggers were positioned at 20 cm from UV shields. Such loggers were not used for the two other treatments, as the temperature is expected to be similar to that of LD for LD+UV, and intermediate between the control and LD for ED, as a consequence of foliage renewal for this early stage defoliation (Homich *et al.*, 2017). The percentage of hours when the temperature exceeds 25 °C (Dh25), an indicator anti-correlated to the level of rotundone in grapes (Zhang *et al.*, 2015), was calculated.

## 4. Berry sampling, basic fruit analysis, and yield components

On October 3, at harvest time, 400 berries were sampled for each experimental unit from the side of the row exposed to the treatments. A cooler with ice packs was used to transport the samples from the vineyard to the laboratory.

Half of the berries were weighed, gently crushed, and the resulting juice was centrifuged for basic fruit analyses. Sugar concentration (°Brix) was determined with a digital hand-held Pocket refractometer PAL (Atago, Japan). Titratable acidity was measured according to the OIV method (OIV, 2009) and pH measured with a HI 3221 pH meter (Hanna Instruments, France). A Konelab Arena 20 sequential analyser (Thermo Electron Corporation, USA) associated with enzyme kits provided by several suppliers was used to determine amino acids, ammonium (Megazyme, Ireland), and malic acid (Thermo Fisher Scientific, USA). Potassium determination was carried out by flame photometry (Bio Arrow, France) according to the OIV method (OIV, 2009) and tartaric acid determination by colorimetric titration (Hill & Caputi, 1970). Anthocyanins and Total Phenolic Index (TPI) were quantified in grapes according to Cayla *et al.* (2002) using an Evolution 100 spectrophotometer (Thermo Electron Corporation, USA). This analytical method for phenolic determination in grapes is based on a 1 h micromaceration in a hydroalcoholic and acidic medium. $\delta^{13}C$, a parameter reflecting the level of water deficit experienced by the plant during maturation, was determined according to a previously published protocol (Coplen, 2011). All determinations were carried out in duplicate. The remaining berries were frozen at –18 °C for rotundone analysis, which was performed without technical replication.

The number of clusters per vine was determined, and crop load was measured individually with a Precia Molen C20 K balance (Precia SA, Privas, France).

## 5. Rotundone analysis

Rotundone analysis was conducted using the protocol proposed by Baerenzung dit Baron *et al.* (2025) on an UltraTRACE gas chromatograph equipped with a split/splitless injector and coupled to an ITQ 900 ion trap mass spectrometer (Thermo Scientific, Courtaboeuf, France). A TriPlus autosampler (Thermo Scientific, Courtabœuf, France) was used for sample introduction. Compound separation was achieved using an HP-5ms column (60 m × 0.25 mm ID × 0.25 µm film thickness; Agilent, Santa Clara, CA, USA). This protocol can be summarised here briefly.

The still-frozen 200-berry samples were resampled into 120 g batches, thawed, and homogenised using a GRINDOMIX GM 200 knife mill (Retsch, Eragny, France). After centrifugation, juice was collected, and solids were extracted with ethanol/water (1:1) spiked with $d_5$-rotundone with purity of 95 % provided by Eptes (Vevey, Switzerland) as an internal standard. The extract was shaken for 24 h, sonicated, centrifuged, combined with the juice, filtered (0.45 µm), and diluted to 200 mL with MilliQ water before solid phase extraction (SPE). SPE cartridges (SDB-L, 500 mg/6 mL) provided by Phenomenex (Torrance, CA, USA) were conditioned and loaded with the extract, washed, and eluted with *n*-pentane/ethyl acetate (9:1). The eluate was evaporated under nitrogen, reconstituted in ethanol, and diluted with Milli-Q water. Analysis was performed by direct immersion solid-phase microextraction coupled with gas chromatography–mass spectrometry (DI-SPME-GC-MS) using a PDMS/DVB fiber (Sigma-Aldrich, Darmstadt, Germany), with incubation at 40 °C, 60 min immersion, and desorption at 250 °C. Mass spectrometry was conducted in EI+ mode (70 eV) with full scan acquisition (120–230 amu). Rotundone and *d5*-rotundone were identified by comparison of retention time (respectively 65.60 ± 0.10 min and 65.42 ± 0.10 min) and mass spectra with standard data. Identification was confirmed by ion ratios (*m/z* 218/203 and 223/208). Calibration curves (20–980 ng/kg) were linear (y = 0.7591x + 0.0105, $R^2$ = 0.9995) with an LOQ of 20 ng/kg (S/N = 10) and LOD of 7 ng/kg (S/N = 3). Repeatability tests at 5×, 25×, and 50× LOQ yielded RSDs of 9 %, 4 %, and 3 %, while recovery ranged from 98–107 %.

## 6. Additional experiment in growth chamber

To obtain preliminary insights on how the time of the day (daytime *vs* nighttime) could affect the efficiency of the UV treatments, an additional experiment was conducted in growth chamber conditions. Twenty-five fruiting cuttings of *Vitis vinifera* L. Syrah, another rotundone-producing genotype were used to run this complementary experiment. Such cuttings produced using a previously published protocol (Ollat *et al.*, 1998) were maintained from mid-veraison at 20 °C and 40 % relative humidity, supplied with four irrigations of 25 mL of water every 24 h, and subjected to a night/day cycle of 8 h/16 h with a day light intensity of 250 ± 50 µmol/m²/s. Thirteen plants were used as controls and received no treatment, while twelve plants received three UV-C treatments at the beginning of the night (mid-veraison + 2 days, mid-veraison + 12 days, mid-veraison + 22 days) using the same equipment and conditions as for the field trial. Fifty days after mid-veraison, the bunches were harvested, and the berries destemmed. For each treatment, the berries were pooled, divided into three batches of 110 g, and frozen for subsequent rotundone extraction and quantification, while the remaining fruit was used for basic fruit analyses. Due to the small quantity of material available, basic fruit analyses were limited to sugar concentration using the method previously described. However, rotundone was quantified in extracts obtained following the previously described protocol (Baerenzung dit Baron *et al.*, 2025) by stirbar sorptive extraction (SBSE) combined with multidimensional gas chromatography-mass spectrometry (GC-GC-MS) to get a higher sensitivity (Wen *et al.*, 2018) than the previous approach, which had a LOQ of 20 ng/kg. Indeed, lower sesquiterpene (SQT) levels and thus rotundone concentrations were expected in controlled conditions, for which grapes are less exposed to abiotic and biotic stressors that can enhance SQT biosynthesis (Schwab & Wüst, 2015). For the SBSE-GC-GC-MS analysis, a stirbar coated with 126 µL polydimethylsiloxane (20 mm length × 1.0 mm thickness; Gerstel, Mülheim an der Ruhr, Germany) was added to a 25 mL Erlenmeyer flask containing 5 mL of berry extract

and 50 µL of internal standard solution. The mixture was stirred at 750 rpm for 30 min at room temperature. Following extraction, the stirbar was removed from the flask, rinsed briefly with Milli-Q water and dried with a lint-free tissue. Each stirbar was then transferred into a thermal desorption tube and placed in the autosampler tray. Thermal desorption was conducted in splitless mode at 270 °C for 7 min. The multidimensional chromatographic system comprised a first-dimension DB-5MS column (J&W Scientific, Folsom, CA, USA) coupled to a second-dimension SAPIENS-WAX MS column (Teknokroma, Barcelona, Spain) via a Deans switch (Agilent Technologies, Santa Clara, CA, USA). During the elution of rotundone and its corresponding deuterated standard from the first column, the Deans switch was programmed to divert the flow to the second column to enhance separation. A quadrupole mass detector was connected to the second column and operated using the parameters previously published.

### 7. Statistical processing

Statistical processing was performed using XLSTATS software (Addinsoft, Paris). Data were treated through a two-factor analysis of variance (ANOVA) (treatment × block) followed by a Fisher's test. The interaction factor was included in the model only for variables with replicate measurements for each experimental unit (*i.e.*, number of bunches per vine and crop load per vine). Data obtained from the additional experiment conducted under growth chamber conditions were subjected to a Student's *t*-test.

## RESULTS AND DISCUSSION

At the experimental site, the average bioclimatic data for the 2011–2025 period (Figure 2) were 2,108 ± 161 for the Huglin index, 399 ± 104 mm for cumulative rainfall from April 1 to September 30, and 899 ± 256 mm for annual cumulative rainfall (January 1 to December 31). According to the Huglin index, 2022 was the warmest season recorded since 2011. It was also the third driest year for both the April–September and the full-year periods.

Data loggers were not able to detect modifications in mean hourly bunch zone air temperature among the three monitored treatments (Figure 3). Similarly, for Dh25, no statistically significant differences were observed at $P < 0.05$ between the control (33.08 % ± 0.83), LD (32.81 % ± 0.56), and LD-UV (33.08 % ± 0.49) treatments. This finding is particularly important, as it demonstrates that UV suppression can be assessed without a concomitant increase in bunch zone air temperature. The mean hourly temperature data also illustrate the particularly warm conditions experienced during maturation on the experimental site, with mean temperature exceeding 30 °C between 1 pm and 5 pm. Although, to our knowledge, no studies have been conducted in the southwest of France to determine Dh25 using data loggers, research from Australia did not report values for Syrah above 15 % (Zhang *et al.*, 2015), indicating that low rotundone concentrations are expected in grapes collected at our experimental site.

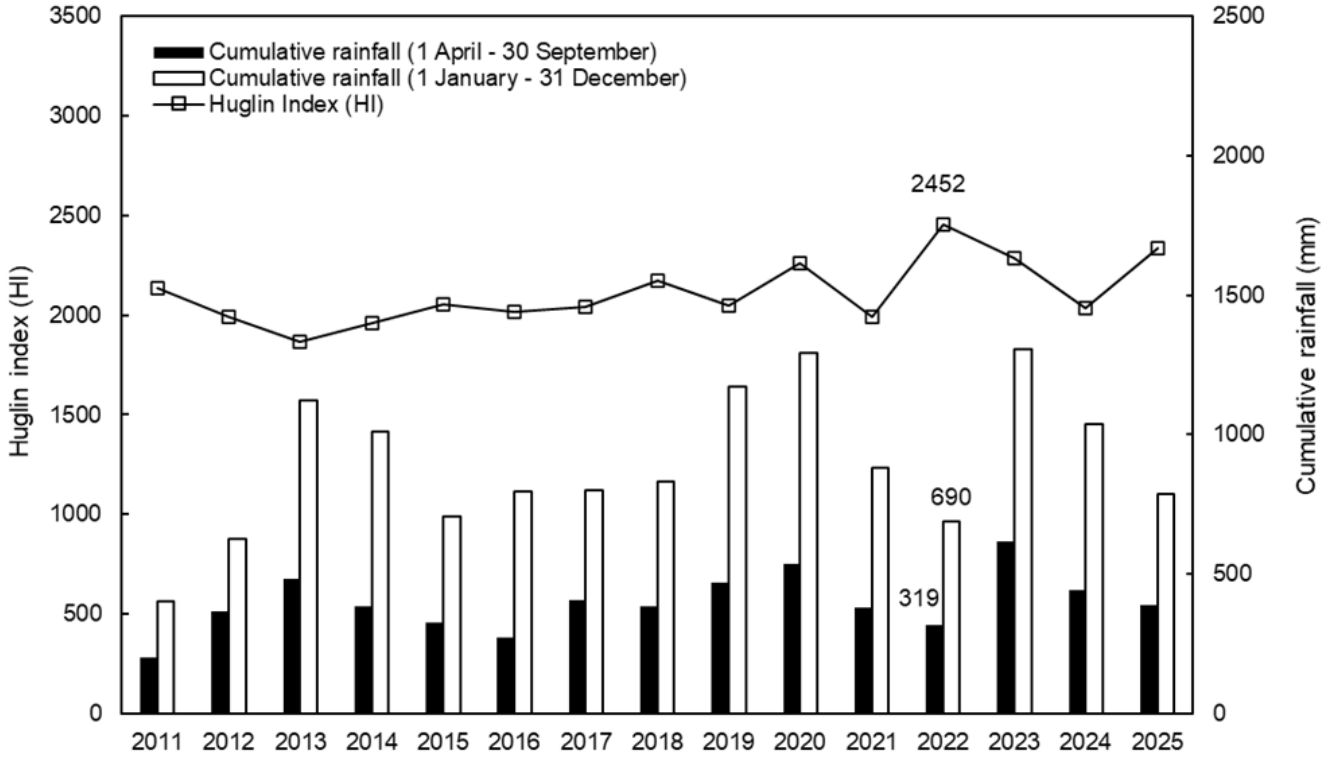



**FIGURE 2.** Huglin index, cumulative rainfall between April 1 and September 30, and between January 1 and December 31 from 2011 to 2025 at the experimental site (data source: Météo France).

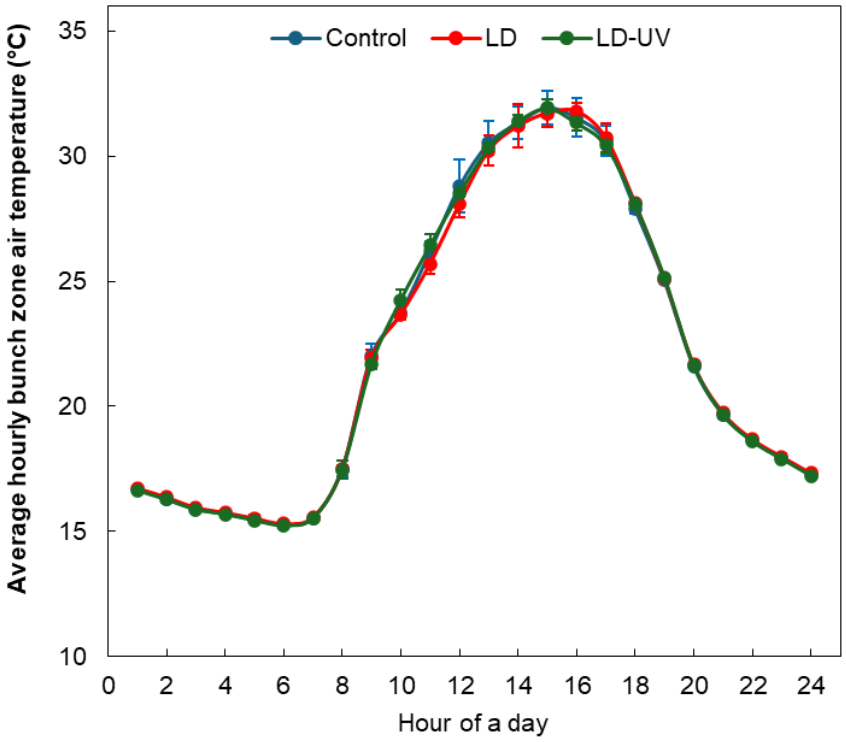



**FIGURE 3.** Impact of the late defoliation (LD) and late defoliation combined with UV screens (LD-UV) treatments on the average hourly bunch zone air temperature on the north-east side of the row between veraison and harvest. Error bars refer to variability between vineyard blocks.

Sunburn damage was not observed on the side of the row subjected to the treatments. The number of bunches and crop load per vine did not differ among treatments (Table 1), which illustrates the homogeneity of our experimental vineyard. Indeed, although early defoliation at flowering or before flowering has been shown to impact berry set and yield through source limitation, no impact of the studied techniques, which were applied much later, was expected on yield parameters (Verdenal *et al.*, 2019).

Among the measured parameters, pH and the content of potassium were the only variables significantly impacted by the treatments. For ED, LD, and LD+UV, lower berry potassium content was observed, which could result from the reduced leaf area limiting the translocation of assimilates and minerals from vegetative organs to the fruits (Mpelasoka *et al.*, 2003). Logically, this resulted in a decrease in pH for ED and LD+UV treatments, as potassium is known to influence the acid balance in the must (Mpelasoka *et al.*, 2003). For unknown reasons, this decrease was not observed for LD. It must be outlined that the potassium content was not affected for LD-UV in comparison to the control, which suggests that UV exclusion mitigated stress and stabilised berry metabolism (Mpelasoka *et al.*, 2003), allowing potassium levels to be maintained despite reduced leaf area. No effect of leaf removal on berry sugar concentration was observed, likely because the removed leaves were older and photosynthetically less active, or due to compensatory mechanisms, as reported in previous studies (Geffroy *et al.*, 2019a; Verdenal *et al.*, 2019). It is worth noting that a lower level of water constraint, as reflected by lower $\delta^{13}C$ values, could have been expected for the defoliated treatments due to reduced plant transpiration, but $\delta^{13}C$ values showed no significant variation (Table 1).

Surprisingly, despite previous studies reporting in most cases higher phenolic content, notably anthocyanins in grapes or wines from defoliated vines (Verdenal *et al.*, 2019), the treatments had no significant effect on anthocyanins or TPI, although a trend toward an increase was observed with greater UV exposure (Table 1). It cannot be discarded that the very high temperature experienced on the experimental site may have disrupted anthocyanin accumulation, whose biosynthesis is negatively affected by temperature above 30 °C in berry skin (Yamane *et al.*, 2006). It should also be noted that berry weight was not affected by the treatments, indicating that variations in skin-to-juice ratio are not likely to influence rotundone concentration in berries, considering that this compound is mainly found in the grape exocarp skin (Caputi *et al.*, 2011). This allows for a direct comparison of the effects of the treatments on rotundone biosynthesis.

As expected under such extreme climatic conditions, rotundone concentrations were low (Figure 4) compared to previous studies conducted on high rotundone producers for which levels up to 3,450 ng/kg on Noiret and 6,130 ng/kg on Vespolina have been observed (Geffroy *et al.*, 2020). Data are scarcer for Tardif, with a maximal reported level of about 600 ng/kg in grapes (Baerenzung dit Baron *et al.*, 2025).

**TABLE 1.** Impact of the studied treatments on basic fruit parameters, $\delta^{13}C$ determined on berries harvested on the north-east side of the row and yield components (mean values ± standard deviations). Different letters indicate means significantly different at $P < 0.05$ by Fisher's test. ED stands for Early Defoliation; LD, Late Defoliation; LD-UV, Late Defoliation combined with UV screens; LD+UV, Late Defoliation combined with UV-C treatments.

| | Weight of 200 berries (g) | Sugar concentration (°Brix) | Titratable acidity (g/L as tartaric acid) | pH | Tartaric acid (g/L) | Malic acid (g/L) | Amino acids (mg/L) | $NH_4^+$ (mg/L) | $K^+$ (g/L) | Anthocyanins (mg/kg) | TPI | $\delta^{13}C$ | Number of bunches per vine | Crop load per vine (kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 236 ± 4 a | 24.7 ± 0.3 a | 5.46 ± 0.12 a | 3.51 ± 0.01 a | 6.12 ± 0.08 a | 1.76 ± 0.21 a | 89.4 ± 8.4 a | 9.90 ± 4.15 a | 1.72 ± 0.05 a | 1,709 ± 269 a | 237 ± 25 a | –24.14 ± 0.17 a | 18.3 ± 4.8 a | 1.16 ± 0.33 a |
| ED | 232 ± 8 a | 24.8 ± 0.2 a | 5.58 ± 0.07 a | 3.44 ± 0.04 b | 5.99 ± 0.15 a | 1.71 ± 0.04 a | 77.2 ± 6.6 a | 6.04 ± 3.07 a | 1.55 ± 0.04 b | 2,035 ± 353 a | 281 ± 56 a | –24.34 ± 0.26 a | 18.3 ± 5.8 a | 1.02 ± 0.36 a |
| LD | 231 ± 12 a | 25.4 ± 0.1 a | 5.42 ± 0.28 a | 3.50 ± 0.04 a | 6.03 ± 0.28 a | 1.71 ± 0.07 a | 73.6 ± 9.7 a | 5.15 ± 2.53 a | 1.58 ± 0.06 b | 1,981 ± 270 a | 268 ± 40 a | –24.48 ± 0.34 a | 18.8 ± 4.8 a | 1.08 ± 0.37 a |
| LD-UV | 235 ± 15 a | 25.1 ± 0.8 a | 5.12 ± 0.27 a | 3.48 ± 0.03 a | 6.02 ± 0.18 a | 1.66 ± 0.01 a | 69.2 ± 16.8 a | 5.51 ± 2.75 a | 1.66 ± 0.05 a | 2,115 ± 251 a | 310 ± 336 a | –24.43 ± 0.49 a | 16.6 ± 5.4 a | 1.04 ± 0.37 a |
| LD+UV | 245 ± 4 a | 25.5 ± 0.8 a | 5.37 ± 0.35 a | 3.41 ± 0.03 b | 6.01 ± 0.29 a | 1.76 ± 0.15 a | 65.5 ± 4.6 a | 2.59 ± 1.12 a | 1.58 ± 0.05 b | 2,207 ± 156 a | 323 ± 28 a | –24.47 ± 0.44 a | 17.1 ± 7.0 a | 1.01 ± 0.45 a |
| *P* (treatment) | 0.500 | 0.318 | 0.366 | 0.019 | 0.971 | 0.855 | 0.141 | 0.115 | 0.026 | 0.349 | 0.166 | 0.776 | 0.509 | 0.394 |
| *P* (block) | 0.673 | 0.380 | 0.884 | 0.524 | 0.975 | 0.582 | 0.509 | 0.326 | 0.661 | 0.711 | 0.683 | 0.467 | 0.987 | 0.100 |
| *P* (treatment × block) | — | — | — | — | — | — | — | — | — | — | — | — | 0.520 | 0.420 |

— Not applicable.

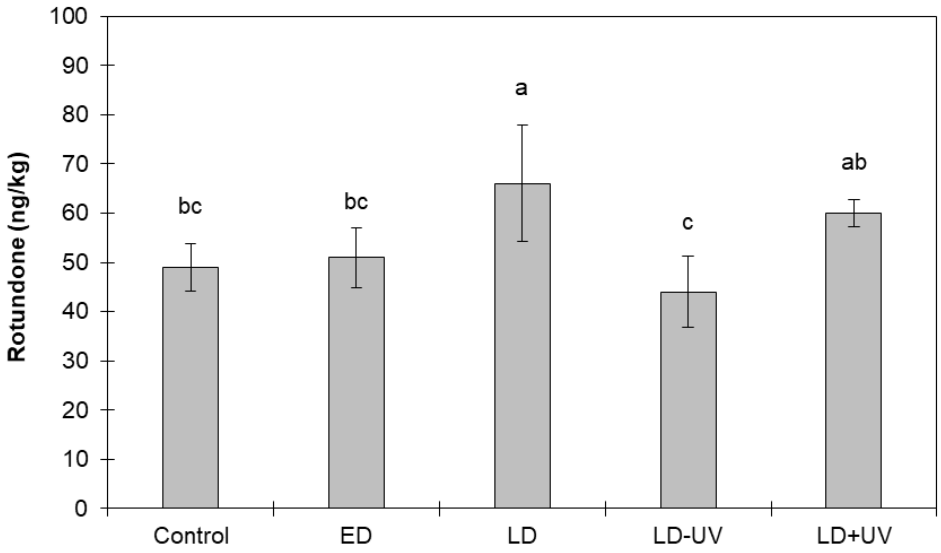



**FIGURE 4.** Impact of the studied treatments on rotundone concentration (mean values ± standard deviations) in berries harvested on the north-east side of the row. Different letters indicate means significantly different at $P < 0.05$ by Fisher's test. ED stands for Early Defoliation; LD, Late Defoliation; LD-UV, Late Defoliation combined with UV screens; LD+UV, Late Defoliation combined with UV-C treatments.

Nevertheless, the treatments had a significant effect on rotundone concentration ($P = 0.027$). Its content was similar to that of the control for ED (Figure 3), whereas for LD, it was significantly greater (+33 %). These findings align with previous studies highlighting the advantages of late defoliation at veraison or treatments that sustain fruit-zone sunlight exposure, compared to early defoliation, where regrowth of leaves may negate the initial increase in cluster exposure (Homich *et al.*, 2017).

In comparison with LD, the rotundone concentration observed for LD-UV was significantly lower (–33 %) (Figure 4), indicating that, given the absence of previously described temperature differences between the two treatments, UV-A and UV-B exclusion negatively impacts rotundone biosynthesis. In contrast, the UV-C treatments (LD+UV) did not lead to an increase in rotundone.

As previously discussed, the mechanisms underlying the effects of UV on rotundone remain unclear but may involve either a direct stimulation of its biosynthetic pathway or an overproduction of reactive oxygen species (ROS), which could oxidise α-guaiene, the precursor of rotundone, into rotundone (Huang *et al.*, 2014). The UV-C treatments were applied on our experimental site during daytime, while it has been proven that at night, UV-C induces higher ROS accumulation mainly because antioxidant defences are reduced, photorepair is suppressed, and metabolism is slower, allowing ROS to persist longer (Urban *et al.*, 2016). It cannot be excluded that a stimulating effect would have been observed if the treatments were conducted at night.

In the same way, during this warm and dry vintage, it can be hypothesised that the excessive amount of UV received by the vines, particularly for the LD+UV treatment, may have had detrimental effects such as extreme oxidative stress and potential damage to plant tissue (Matsuura *et al.*, 2013).

Indeed, under controlled growth chamber conditions, the effect induced by the UV-C treatment differed from that observed under field conditions. Although the UV-C treatments induced some light stress symptoms on leaves surrounding the bunches, there was no significant difference in sugar concentration between the two treatments (16.6 °Brix ± 1.9 for the control *vs* 15.7 °Brix ± 0.8 for the UV treatments, $P = 0.477$). However, berries treated with the UV-C device exhibited a significantly higher rotundone content (176.2 ng/kg ± 105.4) compared with the control (7.1 ng/kg ± 3.5) at $P < 0.05$.

Although derived from a limited number of fruiting cuttings and pending validation under field conditions, these preliminary results suggest a positive effect of UV-C modulated light treatments on rotundone biosynthesis when applied nocturnally.

## CONCLUSION

This study enabled the investigation of the impact of two timings of defoliation performed on the side of least exposure on rotundone in *Vitis vinifera* L. Tardif. Although the early defoliation treatment had no impact on rotundone, the late treatment appears to be a suitable practice for winegrowers to enhance rotundone biosynthesis. It should be noted that different results might have been obtained if regrowth had been removed and the fruiting area had remained exposed during the whole season. In the same way, it can be acknowledged that analyses were conducted only on grapes collected from the treated row. The observed effect was moderate and would likely be even smaller under industrial conditions, where grapes are harvested from both sides of the row.

Excluding UV-A and UV-B exposure led to a reduction in rotundone concentration, confirming the role of this abiotic factor in rotundone biosynthesis. The UV-C treatments during daytime, under field trial conditions, did not increase rotundone levels, whereas a similar treatment applied at night on a limited number of fruiting cuttings produced in the growth chamber resulted in a substantial rotundone increase. This discrepancy, which may be linked to decreased photorepair when UV-C treatments are applied at night, highlights the need for further investigation to elucidate the underlying mechanisms, to validate the observed effects across a broader range of grapevines and environmental conditions before UV-C can be considered a viable strategy to enhance rotundone in grapes.

## ACKNOWLEDGEMENTS

The authors would like to thank the Occitanie Region for funding the Pepper your Wine project through the Recherche et Société(s) program and for the doctoral thesis grant of Thomas Baerenzung dit Baron. We are also grateful to Maria Angélica Usta and Marie Homs from École d'Ingénieurs de Purpan, to Laure Gontier from IFV, as well as to Alain Deloire, formerly of Institut Agro Montpellier, and Nathalie Ollat from EGFV, for their technical support and practical advice.

## REFERENCES

Baerenzung dit Baron, T., Lescot, L., Asieieva, D., Cuif, C., Yobrégat, O., Jacques, A., Simon, V., & Geffroy, O. (2025). A survey on the rotundone content of eighteen grape varieties sourced from a germplasm collection during a cool season. *Journal of Food Composition and Analysis*, *138*, 107024. https://doi.org/10.1016/j.jfca.2024.107024

Brousseau, P., Berre, L., Bouttier, F., & Desroziers, G., (2011). Background-error covariances for a convective-scale data-assimilation system: AROME–France 3D-Var. *Quarterly Journal of the Royal Meteorological Society*, *137*, 409–422. https://doi.org/10.1002/qj.750

Caputi, L., Carlin, S., Ghiglieno, I., Stefanini, M., Valenti, L., Vrhovsek, U., & Mattivi, F. (2011). Relationship of changes in rotundone content during grape ripening and winemaking to manipulation of the 'peppery' character of wine. *Journal of Agricultural and Food chemistry*, *59*(10), 5565-5571. https://doi.org/10.1021/jf200786u

Cayla, L., Cottereau, P., & Renard, R. (2002). Estimation de la maturité phénolique des raisins rouges par la méthode ITV standard. *Revue Française d'Œnologie*, *193*, 10-16.

Champeaux, J. L., Laurantin, O., Mercier, B., Mounier, F., Lassegues, P., & Tabary, P. (2011). Quantitative precipitation estimations using rain gauges and radar networks: inventory and prospects at Meteo-France. *WMO Joint Meeting of CGS Expert Team on Surface-based Remotely-sensed Observations and CIMO Expert Team on Operational Remote Sensing*, 5–9 December 2011, Geneva, Switzerland.

Coplen, T. B. (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid communications in mass spectrometry*, *25*(17), 2538-2560. https://doi.org/10.1002/rcm.5129

Diot, A., Denat, M., Laforge, J., Baerenzung dit Baron, T., Djari, A., Maza, E., Geffroy, O., & Chervin, C. (2023). Ethylene effects on grapevine cells (cv. Gamay), links with sesquiterpene synthesis. In *XII Symposium of Ethylene*, Toulouse, France.

Duhl, T. R., Helmig, D., & Guenther, A. (2008). Sesquiterpene emissions from vegetation: a review. *Biogeosciences*, *5*(3), 761–777. https://doi.org/10.5194/bg-5-761-2008

Geffroy, O., Buisson, C., Nogues, G., Desport, G., Bessac, F., Hoyau, S., Lacaze-Dufaure, C., Gassiolle, E., & Simon, V. (2025). Impact of protein fining agents and bentonite on rotundone concentration in red wine made from *Vitis vinifera* L. cv. Tardif. *OENO One*, *59*(3). https://doi.org/10.20870/oeno-one.2025.59.3.9431

Geffroy, O., Descôtes, J., Levasseur-Garcia, C., Debord, C., Denux, J.-P., & Dufourcq, T. (2019b). A 2-year multisite study of viticultural and environmental factors affecting rotundone concentration in Duras red wine. *OENO One*, *53*(3). https://doi.org/10.20870/oeno-one.2019.53.3.2341

Geffroy, O., Dufourcq, T., Carcenac, D., Siebert, T., Herderich, M., & Serrano, E. (2014). Effect of ripeness and viticultural techniques on the rotundone concentration in red wine made from *Vitis vinifera* L. cv. Duras. *Australian Journal of Grape and Wine Research*, *20*(3), 401-408. https://doi.org/10.1111/ajgw.12084

Geffroy, O., Kleiber, D., & Jacques, A. (2020). May peppery wines be the spice of life? A review of research on the 'pepper' aroma and the sesquiterpenoid rotundone. *OENO One*, *54*(2), 245–262. https://doi.org/10.20870/oeno-one.2020.54.2.2947

Geffroy, O., Li Calzi, M., Ibpfelt, K., Yobregat, O., Feilhes, C., & Dufourcq, T. (2019a). Using common viticultural practices to modulate the rotundone and 3-isobutyl-2-methoxypyrazine composition of *Vitis vinifera* L. cv. Fer red wines from a temperate climate wine region with very cool nights. *OENO One*, *53*(4). https://doi.org/10.20870/oeno-one.2019.53.4.2459

Geffroy, O., Siebert, T., Herderich, M., Mille, B., & Serrano, E. (2016). On-vine grape drying combined with irrigation allows to produce red wines with enhanced phenolic and rotundone concentrations. *Scientia horticulturae*, *207*, 208-217. https://doi.org/10.1016/j.scienta.2016.05.031

Geffroy, O., Yobrégat, O., Dufourcq, T., Siebert, T., & Serrano, E. (2015). Certified clone and powdery mildew impact rotundone in red wine from *Vitis vinifera* L. cv. Duras N. *Journal International des Sciences de la Vigne et du Vin*, *49*(4), 231-240. https://doi.org/10.20870/oeno-one.2015.49.4.46

Gil, M., Pontin, M., Berli, F., Bottini, R., & Piccoli, P. (2012). Metabolism of terpenes in the response of grape (*Vitis vinifera* L.) leaf tissues to UV-B radiation. *Phytochemistry*, *77*, 89-98. https://doi.org/10.1016/j.phytochem.2011.12.011

Hill, G., & Caputi, A. (1970). Colorimetric determination of tartaric acid in wine. *American Journal of Enology and Viticulture*, *21*(3), 153-161. https://doi.org/10.5344/ajev.1970.21.3.153

Homich, L. J., Elias, R. J., Vanden Heuvel, J. E., & Centinari, M. (2017). Impact of fruit-zone leaf removal on rotundone concentration in Noiret. *American Journal of Enology and Viticulture*, *68*(4), 447-557. https://doi.org/10.5344/ajev.2017.16106

Huang, A. C., Burrett, S., Sefton, M. A., & Taylor, D. K. (2014). Production of the pepper aroma compound, (–)-rotundone, by aerial oxidation of α-guaiene. *Journal of Agricultural and Food Chemistry*, *62*(44), 10809-10815. https://doi.org/10.1021/jf504693e

Liu, L., Gregan, S., Winefield, C., & Jordan, B. (2015). From UVR8 to flavonol synthase: UV-B-induced gene expression in Sauvignon blanc grape berry. *Plant, Cell & Environment*, *38*(5), 905-919. https://doi.org/10.1111/pce.12349

Matsuura, H. N., de Costa, F., Yendo, A. C. A., & Fett-Neto, A. G. (2013). Photoelicitation of bioactive secondary metabolites by ultraviolet radiation: mechanisms, strategies, and applications. In S. Chandra, H. Lata, & A. Varma (Eds.), *Biotechnology for Medicinal Plants: Micropropagation and Improvement* (pp. 171-190). Berlin, Heidelberg: Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-29974-2_7

Mpelasoka, B. S., Schachtman, D. P., Treeby, M. T., & Thomas, M. R. (2003). A review of potassium nutrition in grapevines with special emphasis on berry accumulation. *Australian Journal of Grape and Wine Research*, *9*(3), 154-168. https://doi.org/10.1111/j.1755-0238.2003.tb00265.x

OIV. (2009). *Recueil des méthodes internationales d'analyse des vins et des moûts*. Organisation Internationale de la Vigne et du Vin.

Ollat, N., Geny, L., & Soyer, J. (1998). Grapevine fruiting cuttings: validation of an experimental system to study grapevine physiology. I. Main vegetative characteristics. *OENO One*, *32*(1). https://doi.org/10.20870/oeno-one.1998.32.1.1061

Paulo, M. S., Adam, B., Akagwu, C., Akparibo, I., Al-Rifai, R. H., Bazrafshan, S., Gobba, F., Green, A. C., Ivanov, I., Kezic, S., Leppink, N., Loney, T., Modenese, A., Pega, F., Peters, C. E., Prüss-Üstün, A. M., Tenkate, T., Ujita, Y., Wittlich, M., & John, S. M. (2019). WHO/ILO work-related burden of disease and injury: Protocol for systematic reviews of occupational exposure to solar ultraviolet radiation and of the effect of occupational exposure to solar ultraviolet radiation on melanoma and non-melanoma skin cancer. *Environment International*, *126*, 804-815. https://doi.org/10.1016/j.envint.2018.09.039

Roynette, C., & Roynette, P. (2024). *Improved photobiological treatment device* [Patent Nº EP4408193]. European Patent Office. https://register.epo.org/application?&lng=fr&number=EP22789974

Schwab, W., & Wüst, M. (2015). Understanding the constitutive and induced biosynthesis of mono-and sesquiterpenes in grapes (*Vitis vinifera*): a key to unlocking the biochemical secrets of unique grape aroma profiles. *Journal of Agricultural and Food Chemistry*, *63*(49), 10591-10603. https://doi.org/10.1021/acs.jafc.5b04398

Šuklje, K., Antalick, G., Coetzee, Z., Schmidtke, L. M., Baša Česnik, H., Brandt, J., du Toit, W., Lisjak, K., & Deloire, A. (2014). Effect of leaf removal and ultraviolet radiation on the composition and sensory perception of *Vitis vinifera* L. cv. Sauvignon Blanc wine. *Australian Journal of Grape and Wine Research*, *20*(2), 223-233. https://doi.org/10.1111/ajgw.12083

Tonietto, J., & Carbonneau, A. (2002). Régime thermique en période de maturation du raisin dans le géoclimat viticole : indice de fraîcheur des nuits – IF et amplitude thermique. In *Proceedings of the Symposium International sur le Zonage Vitivinicole*, 17–20 June 2002, Avignon, France (Organisation Internationale de la Vigne et du Vin, Paris, France), pp. 279–289.

Urban, L., Charles, F., de Miranda, M. R. A., & Aarrouf, J. (2016). Understanding the physiological effects of UV-C light and exploiting its agronomic potential before and after harvest. *Plant Physiology and Biochemistry*, *105*, 1-11. https://doi.org/10.1016/j.plaphy.2016.04.004

Verdenal, T., Zufferey, V., Dienes-Nagy, A., Bourdin, G., Gindro, K., Viret, O., & Spring, J.-L. (2019). Timing and intensity of grapevine defoliation: an extensive overview on five cultivars in Switzerland. *American Journal of Enology and Viticulture*, *70*(4), 427-434. https://doi.org/10.5344/ajev.2019.19002

Wen, Y., Ontañon, I., Ferreira, V., & Lopez, R. (2018). Determination of ppq-levels of alkylmethoxypyrazines in wine by stirbar sorptive extraction combined with multidimensional gas chromatography-mass spectrometry. *Food Chemistry*, *255*, 235-241. https://doi.org/10.1016/j.foodchem.2018.02.089

Wood, C., Siebert, T. E., Parker, M., Capone, D. L., Elsey, G. M., Pollnitz, A. P., Eggers, M., Meier, M., Vossing, T., Widder, S., Krammer, G., Sefton, M. A., & Herderich, M. J. (2008). From wine to pepper: rotundone, an obscure sesquiterpene, is a potent spicy aroma compound. *Journal of Agricultural and Food Chemistry*, *56*(10), 3738-3744. https://doi.org/10.1021/jf800183k

Yamane, T., Jeong, S. T., Goto-Yamamoto, N., Koshita, Y., & Kobayashi, S. (2006). Effects of temperature on anthocyanin biosynthesis in grape berry skins. *American Journal of Enology and Viticulture*, *57*(1), 54-59. https://doi.org/10.5344/ajev.2006.57.1.54

Zhang, P., Barlow, S., Krstic, M., Herderich, M., Fuentes, S., & Howell, K. (2015). Within-vineyard, within-vine, and within-bunch variability of the rotundone concentration in berries of *Vitis vinifera* L. cv. Shiraz. *Journal of Agricultural and Food Chemistry*, *63*(17), 4276-4283. https://doi.org/10.1021/acs.jafc.5b00590