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ORIGINAL RESEARCH ARTICLE

Sensory and aroma impact of mitigation strategies against sunburn in Riesling

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ABSTRACT

Climate change is an environmental challenge that impacts the wine industry due to frequent sunburn damage triggered by heat waves, sun radiation and severe water deficits. This leads to severe yield losses and sensory changes in the resulting wines, presumably caused by climate-induced off-flavours. This study aims to develop viticultural and oenological strategies to mitigate sunburn damage in the highly sensitive variety Riesling and its detrimental sensory properties. In 2020, we combined the timing and intensity of defoliation measures with the application of kaolin and calcium hydroxide suspensions, reflecting a portion of the solar radiation. Seven treatments were replicated in three field experiments. Grapes from each field replication were maintained as a fermentation replication and, therefore, separately vinified following a standardised protocol. Replicates, including field and fermentation replicates, were further used as sensory replications. Descriptive analysis (DA) and temporal dominance of sensations (TDS) revealed a significant sensory impact of experimental trials in eight of twelve attributes. Additionally, wines were analysed by gas chromatography-mass spectrometry. The early sun exposition treatment with partial defoliation of the grape zone after flowering, followed by a second defoliation at berry closure, reduced fruity aroma but increased smoky notes due to enhanced 4-vinylguajacol formation as well as the intensity of the atypical ageing note (ATA) reminiscent of acacia blossom, naphthalene and fusel alcohols. Applying kaolin or calcium hydroxide particles on the berry skin slightly mitigated these unpleasant effects and even increased fruitiness and sweetness. Late defoliation at berry closure diminished the green note, which was most prominent in the non-defoliated control. Further smokiness and ATA intensities were lowered, in contrast to their rise due to early defoliation. However, late defoliation increased 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), causing the petrol off-flavour. TDS analysis revealed a higher fruity and sweet dominance during the first 10 seconds due to early defoliation, while late defoliation fostered dominant and lingering sourness. In conclusion, early defoliation, which lowers sunburn incidence due to an early adaptation towards sun exposure, increases smoky and ATA flavours and diminishes fruitiness. These detrimental sensory effects could be significantly mitigated by applying reflecting particles of kaolin and calcium hydroxide on berry skins.

KEYWORDS: climate change, sunburn damage, kaolin, off-flavour, sensory, wine, aroma analysis

INTRODUCTION

Climate change is having a huge influence on humanity, especially in the field of agriculture, including grape growing and, subsequently, on wine quality. According to Droulia *et al*. and the IPCC Climate Change Reports of 2007 and 2014 (Droulia and Charalampopoulos, 2021; IPCC., 2014) climate change is one of the greatest environmental concerns facing humankind in the 21st Century. More frequent and severe extreme weather events such as heat waves and drought periods increase UV-B radiation harming leaves and grapes and intensifying evaporation which limits water availability of the vines. The sharp CO_2 rise enhances temperature, leading to growing evaporation rates not only in vines but also diminished soil moisture by 30 to 50 % in Western Europe (Schultz, 2000). At the same time, rising CO_2 concentration stimulates net assimilation rates in grape vines, more efficient water use and higher single berry weights but no differences in the number of bunches per vine as was found for the cultivars Riesling and Cabernet-Sauvignon (Wohlfahrt *et al.*, 2018). According to the studies by Wohlfahrt *et al.,* these effects of the changing CO_2 level could lead to an alteration of grape and wine composition. The effect of climate change on the aromatic potential of grapes and wine quality is described to lower herbaceous and vegetal notes due to rising average temperatures and increased formation of 3-isobutyl-2-methoxypyrazines due to water stress, both in Cabernet-Sauvignon and Sauvignon blanc (Pons *et al*., 2017). Nevertheless, concentrations of carotenoid derivates, namely C_{13}-norisoprenoids, rise when grapes are more exposed to light and higher temperatures, which can be both positive and negative for the aroma profile of a wine (Pons *et al*., 2017). An indirect consequence of rising temperature is the resulting water stress for plants, which has an influence on the physiology of the vines and, consequently, on the biosynthesis of aromatic compounds and their precursors as well. For the typicity of a German Riesling wine, climatic conditions play a huge role and are more important than other environmental factors in particular soil (van Leeuwen and Darriet, 2016). It is common practice to protect grapes against excessive sunlight to prevent the formation of off-flavours such as 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) or the atypical ageing note (ATA) or those masking varietal character such as 4-vinylguajacol (Fischer, 2007). A rise in temperature is also relevant as the grapevine requires a minimal temperature sum during the vegetation period for proper vegetative growth and grape ripening, which are defined by the Huglin Index (HI) or Growing Degree Days (GDD) (Huglin, 1978). In contrast, high temperatures hinder sugar accumulation and favour malate degradation, leading to wines of low acidity (Coombe, 1987, Droulia and Charalampopoulos, 2021). Overall, a higher temperature can benefit the fruity and floral aroma of wines as long as it is not excessively high (Pons *et al.*, 2017). Moreover, the aroma and, therefore, the occurring climate-related off-flavours of wine are not only dependent on a singular aroma compound but on the profile and interactions of the multiple odour-active compounds found in the wine and the release of aroma compounds from their odourless precursors during wine maturation (Robinson *et al.*, 2014).

Global warming actuates warm spring temperatures, which leads to early flowering, faster ripening and, consequently, higher grape maturity with increased sugar accumulation (Fischer, 2007). Secondary metabolites such as anthocyanins and carotenoids are also increased to protect berries against detrimental effects, which results in wines with modified varietal aroma and deeper colour (Droulia and Charalampopoulos, 2021; González-Barreiro *et al*., 2015; Mori *et al*., 2007). Temperatures above 35 °C negatively affect the plant's photosynthetic system and trigger severe skin damage, which leads to the oxidation of polyphenols and shrivelling, as well as the higher incidence of fungal infections in grapes (Berry and Björkman, 1980; Steel and Greer, 2008). The phenomenon of sunburn can be differentiated between sunburn necrosis and sunburn browning. The latter is a result of both high sun exposure and high temperature, especially occurring after véraison. Sunburn necrosis can be led back to much higher temperatures than necessary for sunburn browning and is likely to appear yet at pre-véraison (Gambetta *et al.*, 2021). Gambetta *et al*. mentions different strategies to mitigate sunburn, such as moderate or no defoliation, coverage by shading nets, and application of reflecting particles such as kaolin. Early defoliation after flowering seems to lower the vulnerability of grapes by promoting a higher accumulation of photoprotectants such as catechins, cinnamic acid derivates, anthocyanins and carotenoids. In contrast, removing leaves at a later stage at véraison had no mitigation effect, which was also true for not defoliated grapes (Gambetta *et al.*, 2021, Pastore *et al.*, 2013). Application of kaolin particles on the leaves of cv. Merlot did not affect the volatile composition of the resulting wine, while in the same trial, a water deficit enhanced terpene alcohols and C_{13}-norisoprenoids (Ou *et al*., 2010). In previous studies, defoliation measures, especially early leaf removal at flowering, induced compositional changes of the flavonoids in white wine where the content and concentration of phenolics such as skin quercetin glycosides and hydroxycinnamic acids significantly increased due to more intense illumination (Friedel *et al.*, 2015). So far, the sensory implication of sunburn has not been studied yet. Data on the combination of defoliation with the application of reflective particle films are still very limited, especially regarding the aroma composition and sensory properties of the resulting wines.

Thus, it is the objective of the present study to investigate the main effects of timing and extent of defoliation and the application of a reflecting particle film, as well as their interactions, examining the composition of volatiles and sensory modification in Riesling wines.

MATERIALS AND METHODS

1. Viticultural treatments and weather conditions

Experiments were conducted in a vineyard of the Staatsweingut Neustadt in the Pfalz region in Germany

(49°N, 8°E, 145 m above sea level), which was planted in a west–east direction between 1996 (5 C Geisenheim) and 1999 with the rather compact Riesling clone N90 on the moderate-vigorous SO4 rootstock using a vertical shoot positioning trellising. This clone shows a lower potential for free and bound TDN formation (Ziegler *et al.*, 2020). We chose Riesling for its high susceptibility to sunburn. Rustioni *et al*. (2015) classified white cultivars on a scale ranging from highly susceptible against sunburn (e.g., Cornichon blanc, Riesling, Muscat of Alexandria) to tolerant (e.g., Moscato Giallo, Chardonnay, Sauvignon Blanc), based on their ability to protect Chlorophyll from photodegradation (Gambetta *et al*., 2021). The vegetation period 2020 was characterised by a HI of 1683 and 2178 GDD. Sunburn-inducing weather conditions occurred around the 27th of June 2020 with a maximum temperature of 30.7 °C, on the 9th of July 2020 with 31.8 °C, around the 19th of July 2020 with 29.5 °C, around the 31st of July 2020 with 36.2 °C, around the 9th of August 2020 with 37 °C and around the 21st of August 2020 with 34.3 °C. All temperatures were measured at 2 m height at the experimental station located at a 1 km distance from the field trial, but presumably, the temperature on the berry surface has been much higher (Supplementary Figure 1).

The control consisted of no grape zone defoliation and no application of reflecting particles. Early defoliation was done shortly after flowering (69 BBCH scale according to Lorenz *et al*., 1995) on the 8th of June 2020, removing 75 % of the leaves in the grape zone from both sides. Late defoliation with 100 % leave removal was executed at grape closure before véraison (77–79 BBCH scale) on 16th July 2020. Due to cluster shading by re-growth, a second defoliation was executed in the early defoliated variants.

The reflecting particle suspension was applied using a portable diffusor at pea size (75 BBCH scale) right before the first possible sunburn event occurred on 22nd June 2020. Due to wash-offs caused by a rain event, a reapplication on 7th July 2020 was necessary for the early defoliation treatments. Clusters of late defoliation treatments were treated on 17th July 2020 for the first time, with reapplications on 29th July 2020 and 20th August 2020, respectively. At these times, early defoliation treatments were reapplied as well.

TABLE 1. Composition of experimental wines measured via FTIR WineScan.

Name	Treatments	Rep	EtOH [Vol-%]	Residual sugar content [g/L]	Tartaric acid [g/L]	Malic acid [g/L]	Lactic acid [g/L]	Total acidity [g/L]	pH value
No defoliation	No treatment	1	12.27	0.2	3.4	3.9	0.2	8.0	3.23
		2	11.48	0.2	3.8	3.6	0.5	8.0	3.19
		3	11.85	0.4	2.9	4.3	0.5	8.1	3.28
Early defoliation	Partial defoliation (75 %) after flowering and at grape closure	1	12.43	0.3	3.7	3.6	0.3	7.9	3.23
		2	12.35	0.4	4.0	3.0	0.4	8.0	3.13
		3	12.40	**6.1**[1]	3.8	2.8	0.4	7.8	3.20
Early defoliation + kaolin	Partial defoliation (75 %) after flowering and at grape closure + application of 5 % kaolin	1	12.14	0.7	3.3	3.7	0.5	7.8	3.21
		2	12.43	0.9	3.5	3.0	0.4	7.6	3.16
		3	11.58	0.0	**4.4**	3.4	0.4	**8.4**	3.10
Early defoliation + $Ca(OH)_2$	Partial defoliation (75 %) after flowering and at grape closure + application of 2 % $Ca(OH)_2$	1	11.80	0.1	3.4	3.5	0.2	7.6	3.21
		2	12.42	0.4	2.9	3.6	0.3	7.4	3.29
		3	12.24	**0.7**	3.5	3.4	0.5	7.4	3.30
Late defoliation	Complete defoliation (100 %) at grape closure	1	11.37	0.2	3.6	3.8	0.5	8.0	3.18
		2	11.32	0.3	4.3	3.2	0.5	8.0	3.13
		3	11.66	1.0	4.1	**2.7**	**1.4**	7.5	3.14
Late defoliation + kaolin	Complete defoliation (100 %) at grape closure + application of 5 % kaolin	1	11.72	0.1	3.8	3.3	0.4	7.8	3.21
		2	11.06	0.0	3.7	3.1	0.5	7.7	3.15
		3	11.93	0.1	3.8	**0.4**	**1.8**	**6.6**	3.24
Late defoliation + $Ca(OH)_2$	Complete defoliation (100 %) at grape closure + application of 2 % $Ca(OH)_2$	1	11.65	0.2	2.9	3.7	0.4	7.3	3.29
		2	11.41	0.3	3.4	3.1	0.4	7.1	3.22
		3	11.56	0.0	3.4	3.1	0.3	7.2	3.19

[1]bold and underlined values indicate the analytical clues, why the respective field/fermentation replication was not included in the sensory analysis.

2. Wines

Grapes of three field replication were distributed across the whole experimental vineyard. Field repetitions were separately harvested and analysed (Supplementary Figure 2). Thus the evaluated wines comprise the variance due to spatial soil differences, which will be described further in a separate publication, and individual fermentation. For experimental winemaking, field repeats were not pooled but kept separately as three replications for the impact of grape processing and fermentation. A total of 60 kg of grapes from each field repetition underwent whole cluster pressing. The juice was treated with a pectinase (Lallzyme C-max, Lallemand, Montreal, Canada) and 0.2 g/L pea protein (LittoFresh Origin®, Erblösh, Geisenheim, Germany) was added to support flotation with nitrogen gas for juice clarification to 50 NTU. The clear juice of each field repetition was racked after 2 h into a 25 L carboy, inoculated with 0.2 g/L of the *Saccharomyces cerevisiae* strain Anaferm Riesling (Zefüg, Langenlonsheim, Germany), which was rehydrated in a water/juice 50:50 mixture supplemented by 0.4 g/L GoFerm (Lallemand, Montreal, Canada). Fermentation was done at the constant temperature of 18 °C. Two weeks after fermentation was completed, wines were racked off the lees into a 20 L carboy, sulfited with 60 mg/L SO_2 and moved to cold storage at 7 °C. Two months later, wines were adjusted to 25 mg/L free SO_2, bottled after membrane filtration (0.45 µm) and closed using screw caps.

To prevent fatigue by sensory judges, the number of sensory replicates were limited to two. After analytical screening using FTIR WineScan (FOSS, Hillerød, Denmark) (Table 1) and informal sensory evaluation, we removed those fermentation replicate of each triplicate, which did not finish fermentation to dryness or performed unintended malolactic fermentation (MLF). Clearly, these analytical outliers were not linked to viticultural aspects but to unknown fermentation conditions during microvinification. Residual sugar, as well as lowered acidity due to MLF, may have masked the perception of the phenolic composition, which varied due to the experimental trials. The MLF product diacetyl, with its strong buttery odour, may have masked varietal aroma or changes induced by the viticultural treatments. For all analytical parameters, all three replicates were measured.

3. Sensory evaluation

Sensory analysis was conducted with a trained panel of seven females and 10 males (18–58 years old; average of 40 years), all of whom had previously participated in sensory studies of wine. The study was performed in an odour-free, well-lit room with an ambient temperature of 21 °C that was equipped according to ISO13301:2018., 2018.

The sensory attributes (Supplementary Table 1) were developed in free napping of all experimental wines, in which judges individually discriminated the sample in a plane and described those sensory properties that best distinguished the wines. After the panel discussion, a set of standards for the sensory attributes for each training and tasting session were freshly prepared in a dry Riesling (Staatsweingut mit Johannitergut, Neustadt/Weinstraße, Germany) of the vintage 2018. During three training sessions, judges were familiarised with the standard solutions in varying concentrations (Supplementary Table 1). For descriptive analysis, seven wines were presented in randomised order at room temperature in clear tulip-shaped glasses (Sensus, DIN 10960, Zwiesel Kristallglas, Zwiesel, Germany). Replicates of the experimental treatments were served in different sessions. Attribute intensities were rated on a 10 cm line scale, anchored by low and high intensity. Odour descriptors were assessed in a comparative set-up, rating the intensity of one attribute in all wines before progressing to the next attribute. Orally perceived attributes were evaluated monadically, assessing all descriptors in one wine before continuing to the next one after neutralizing the palate with tap water at room temperature.

4. Temporal dominance of sensations

Temporal Dominance of sensations (TDS) is a method developed in 1999 at the "Centre Européen des Sciences du Goût" in the LIRIS lab. It consists in presenting to the judges the complete list of attributes on a computer screen. Thereafter, the judges are asked to assess which of the attributes is perceived as dominant (i.e., the most striking perception at a given time) and to give a score to this dominant sensation (Pineau *et al*., 2009). For the temporal dominance of sensations of this study, eight in-mouth attributes (sweet, sour, fruity, mineral, green/vegetative, dull/ATA, bitter, astringent) were chosen, similar to the attributes of the descriptive analysis generated in consensus with the judges. Three training sessions were performed to familiarise the judges with the method. Each descriptor of the TDS was displayed as one button on the screen. To balance order effects, the sequence of buttons differed among judges, but for each judge, the order of the attributes stayed the same to ease the search for the right button. The evaluation started when clicking the start button simultaneously to sipping the entire wine sample of 10 mL. Judges were asked to activate the button with the attribute, which is currently the dominant one. Each attribute can be activated several times. After 10 sec, the judges were requested to expectorate the wine but continue the evaluation. When no attribute is perceived any longer, they shall stop the recording. The data were collected every 0.5 s. Fourteen wines were presented twice in randomised order in overall four sessions. Dominance rates were calculated to get TDS curves for each treatment and field/fermentation replicate. Analysis of variance was applied to those dominance curves, which were above the chance level (p = 1/number of attributes, here: 12.5 %). Significance was determined for the parameters "maximum dominance rate" (D_{max}), "duration of dominance" and "area under the curve", which were extracted from the curves for each attribute of the experimental treatments and replicates, separately.

5. Chemicals

The standards for the aroma analysis were purchased by Carl Roth GmbH & Co. KG (Karlsruhe, Germany), Merck KGaA (Darmstadt, Germany) and VWR International

GmbH (Darmstadt, Germany). As reflecting particles for the application in the grape zone, two different preparations were used, both with 600 L/ha. The first is a 5 % aqueous suspension of 99 % kaolin (CutiSan) which was supplemented by 3.5 L/ha of the surfactant CropCover CC-1000 (both, Biofa GmbH, Münsing, Germany). The second preparation is a 2 % aqueous suspension of calcium hydroxide (fiMUM®-Fruchtkalk®) composed of 74 % calcium oxide equivalents, 24 % crystalline water and 2 % magnesium oxide, plus 0.03 % of the surfactant PIMP® (both, Schneider Verblasetechnik e. K., Kleines Wiesental-Wies, Germany). The surfactants are added to increase the even distribution of the reflecting particles on the grape surface in a thin liquid layer and avoid the formation of larger drops. The surfactants do not have any reflecting properties and do not contribute to the whiteness of the particles. These two properties, however, are essential factors to mitigate sunburn.

6. Gas chromatographic analysis of the aroma compounds by HS-SPME-GC-MS

Aroma analysis of the experimental wines was carried out by a headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) method for volatiles in Riesling wine (Schwinn *et al*., 2019, Schmarr *et al.*, 2016). Quantification was performed per stable isotope dilution assay (SIDA) by using deuterated standards of the odourants and by linear calibration in models for all compounds in a model wine consisting of 1.5 g/L L(+)-tartaric acid, 1.5 g/L malic acid, 1.7 g/L lactic acid, 15 g/L D(+)-glucose, 15 g/L D(-)-fructose, 6 g/L glycerin, 1 g/L 2,3-butandiol, 12 Vol-% ethanol and pH 3.5 in deionised water. Supplementary Table 2 lists the internal deuterated Standards, their concentrations, quantifiers, retention times (t_R), LRI on the used column and CAS-Number. Supplementary Table 3 lists the standards for the external calibration, their calibrated range, quantifiers, retention times (t_R), LRI on the used column and CAS-Number. For HS-SPME analysis, 8 mL of saturated aqueous sodium chloride solution was mixed with 2 mL of the sample and 10 µL of the ethanolic deuterated standard solution in a 20 mL Headspace-Vial (CZT, Kriftel, Germany). HS-SPME was performed by a Combi PAL Autosampler (CTC Analytics AG, Zwingen, Switzerland), using the agitator module for incubation and extraction. The samples were incubated for 5 min at 40 °C followed by an extraction time of 20 min with a pre-heated SPME fibre (1 cm PDMS, 100 µm df, 40 °C, CTC Analytics AG). Samples were desorbed in the GC injector at 240 °C for 2 min, applying splitless mode and purging after 2 min. Before analysing each sample, the fibre was conditioned at 250 °C for 2 min. GC analysis was performed by a Trace GC Ultra (Thermo Fisher Scientific, Dreieich, Germany) coupled to a Trace DSQ quadrupole mass spectrometer (Thermo Fisher Scientific, Dreieich, Germany). The analytical column was a 30 m × 0.25 mm i.d. fused silica capillary, coated with a 5 % phenyl containing polydimethylsiloxane stationary phase of 0.25 µm film thickness (Rxi-5SilMS from Restek Corporation, Bellefonte, USA). The oven gradient started at 30 °C for 3 min, increased temperature to 150 °C at 4 °C/min, followed by a second ramp to 300 °C at 50 °C/min and maintained the temperature for 5 min. Electron impact ionisation (EI+) was conducted at 70 eV. The chromatogram was generated in full scan mode within the range of m/z = 29 – 300.

7. Statistical analysis

Data from the descriptive analysis and GC aroma analysis were compared using a three-way-mixed model analysis of variance (ANOVA), where judges were treated as a random effect while wine and replications were fixed. For the TDS curves, which express the percentage of judges selecting the particular attribute as the dominating one, the factor "judge" is no longer available. Therefore a two-way ANOVA was performed with wine and repetition as the sources of variation (Sokolowsky and Fischer, 2012). Post-hoc mean comparison test was done by the least significant difference test (LSD) (significance level $\alpha = 0.1$). PCA was done based on mean centring (correlation) and no rotation was applied. Sensory results were correlated to chemical data using a multifactor analysis with PCA-type co-variance and mixed data type. All statistical analyses were performed using XLSTAT (XLSTAT version 2021.5, Addinsoft, Paris, France).

RESULTS

1. Viticultural impact of mitigation strategies

Sunburn damages on clusters were assessed right before véraison. Late defoliation resulted in significantly higher sunburn frequency and severity than observed for early defoliation or for the non-treated control. The largest but not significant effect in lowering sunburn damage was due to the combination of early defoliation and application of kaolin. However, this putative protecting effect of kaolin was not observed when the application was made in the late defoliation treatment. Calcium hydroxide application did not affect sunburn frequency and severity at the early defoliation stage. However, applied in the late defoliation treatment, calcium hydroxide lowered the frequency of sunburn events but not their severity. These results only display the effects of viticultural treatments conducted in the vegetation period of 2020.

2. Sensory impact of sunburn mitigation treatments

According to the ANOVA (Table 2), eight out of twelve attributes, including colour, varied significantly among treatments. In contrast, replicates combining the field and fermentation variation were insignificant in all attributes except for colour.

First, the data with significant attributes are presented in the form of a spiderweb diagram (Figure 1) to illustrate the differences between the variations with different defoliation and application treatments.

Wines were best discriminated by their yellow colour. The control without defoliation displayed the weakest yellow colour. Early defoliation yielded the deepest colour and the application of reflecting particles led to significantly

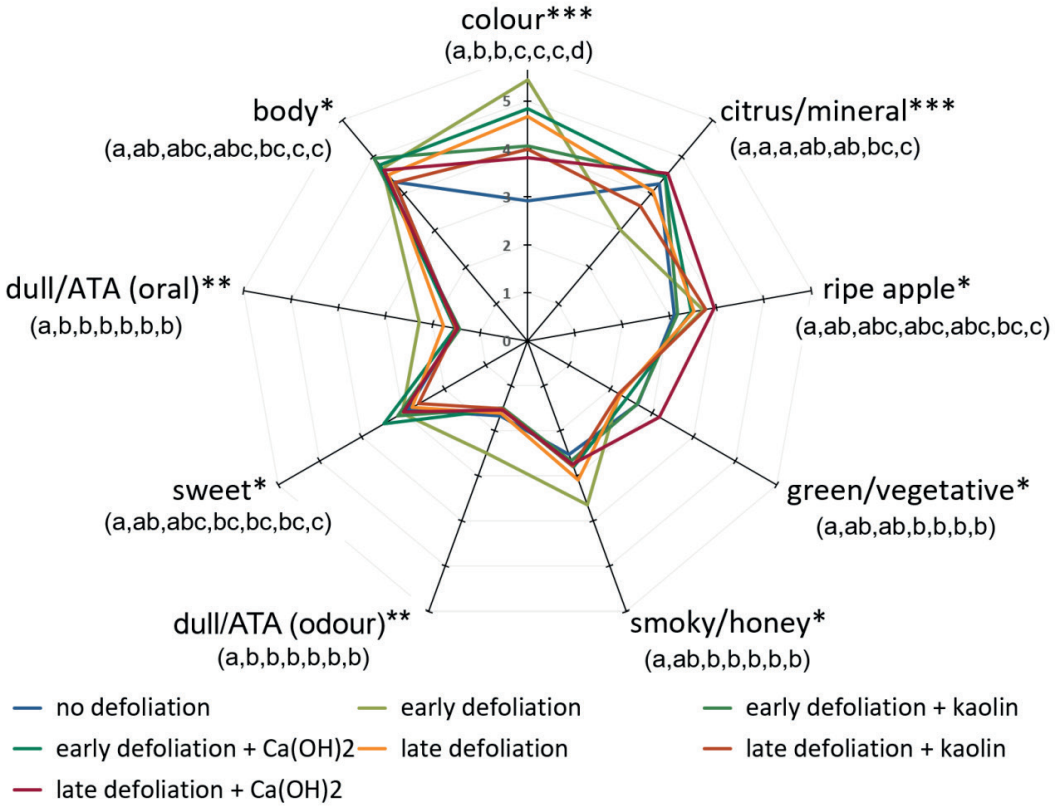


FIGURE 1. Impact of viticultural treatments on odour and in-mouth intensities obtained from descriptive analysis (17 J × 2 Rep.; * $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$, letters indicate significant differences among treatments according to Fishers Least Significant Difference (LSD) test, ATA = atypical ageing note).

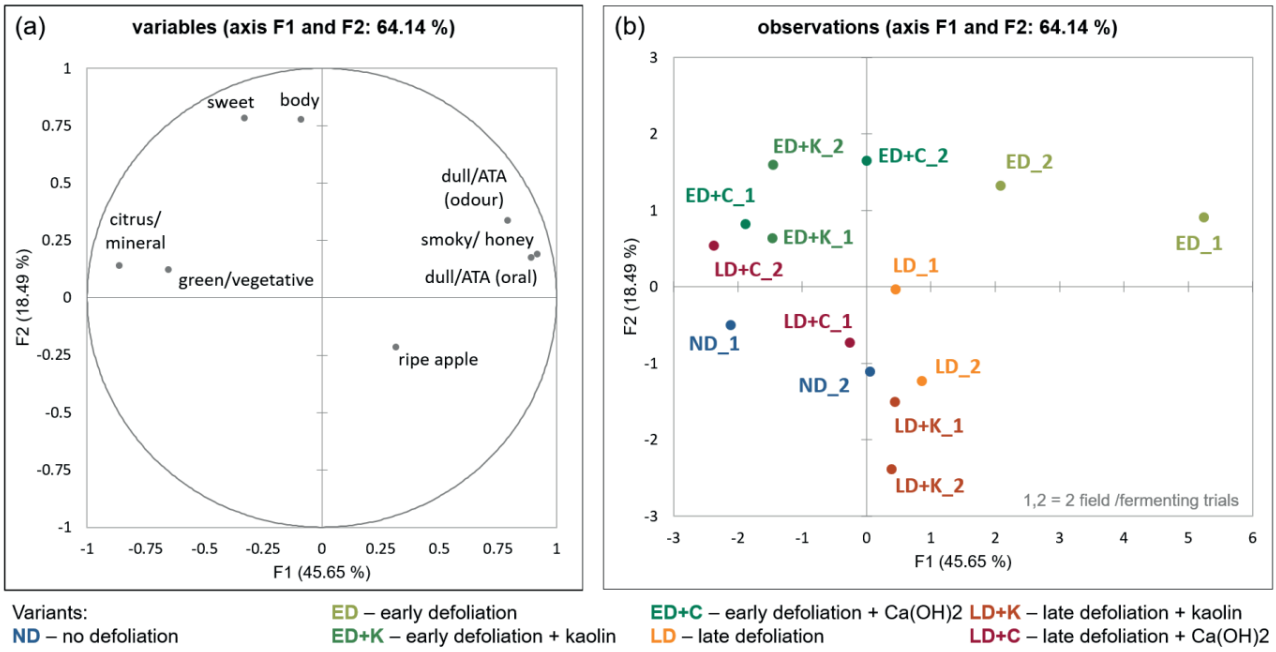


FIGURE 2. Principal component analysis displaying the loadings of sensory attributes on the left (a) and scores of experimental wines on the right (b). Replicates combining field, fermentation and sensory variability are denoted with 1 and 2.

weaker colours. The same effect of colour attenuation by the reflecting particles was observed for late defoliation, albeit at a lower level. Early defoliation showed the least intensity of citrus/mineral, while the application of reflecting particles on early defoliated grapes yielded significantly higher intensities. Parallel to the colour enhancement, the early defoliation showed the strongest smoky/honey notes and atypical ageing off-flavour. Early defoliation treatment alone and with particle application produced the sweetest wines, which is presumably not an effect of residual sugar.

The holistic presentation in a PCA in Figure 2 reveals in the loadings plot (a) a horizontal separation explaining 45.6 % of total variation by the off-notes dull/ATA (ortho- and retronasal) and smoky/honey on the right side versus the citrus/mineral and green notes on the left side. The vertical separation explaining 18.5 % of the total variance is governed by sweet and full-bodied wines in the upper half and, to a smaller extent, by ripe apple in the lower half.

In the score plot (b), replicated tasting of the two field/fermentation replicates of each treatment are mapped in proximity to each other. Early defoliation treatment plotted in the direction of dull/ATA and smoky/honey while showing low intensities of citrus/mineral and green/vegetative. Applying the reflecting particles kaolin (K) and calcium hydroxide (C) shifted the scores of the wines to the left, fruitier side. Overall late defoliation treatments were in the centre and lower part of the score plot indicating less dull/ATA and smoky/honey intensities but more pronounced citrus/mineral, green/vegetative and ripe apple notes. Reflecting particles modified the sensory properties of the wines to a smaller extent than during early defoliation, except for the calcium hydroxide application. The control without defoliation yielded wines with citrus/mineral and green flavours and the least sweetness and body.

3. Modification of aroma composition by sunburn mitigation treatments

For aroma analysis, all three replicates of the treatments were measured. According to ANOVA, 13 of the 17 analysed odourants varied significantly due to the treatments in Table 3. Variance due to field and fermentation replications was significant only for the esters ethyl-butanoate, ethyl hexanoate, ethyl octanoate and 2/3-methylbutyl acetate. Among the grape-derived aroma compounds, linalool varied the most between 26 and 63 µg/L and TDN between 0.9 and 3.2 µg/L. Both aroma compounds increased with defoliation at both time points (Figure 3). The application of reflecting particles lowered the TDN concentrations, but only calcium hydroxide in the early defoliation yielded a significant decrease in TDN. For linalool, a significant difference occurred only between the undefoliated control and all the other defoliation treatments. 4-Vinyl-guaiacol and β-damascenone, however, were not significantly modified by the treatments. Among the esters, variations of ethyl butanoate and ethyl hexanoate were most prominent, as they exceeded their published odour threshold by factors up to 20 and 70, respectively. However, no clear impact of mitigation strategies was observed. The impact of the treatments on 2-aminoacetophenone was the most significant one; however, the concentration in all experimental wines remained below

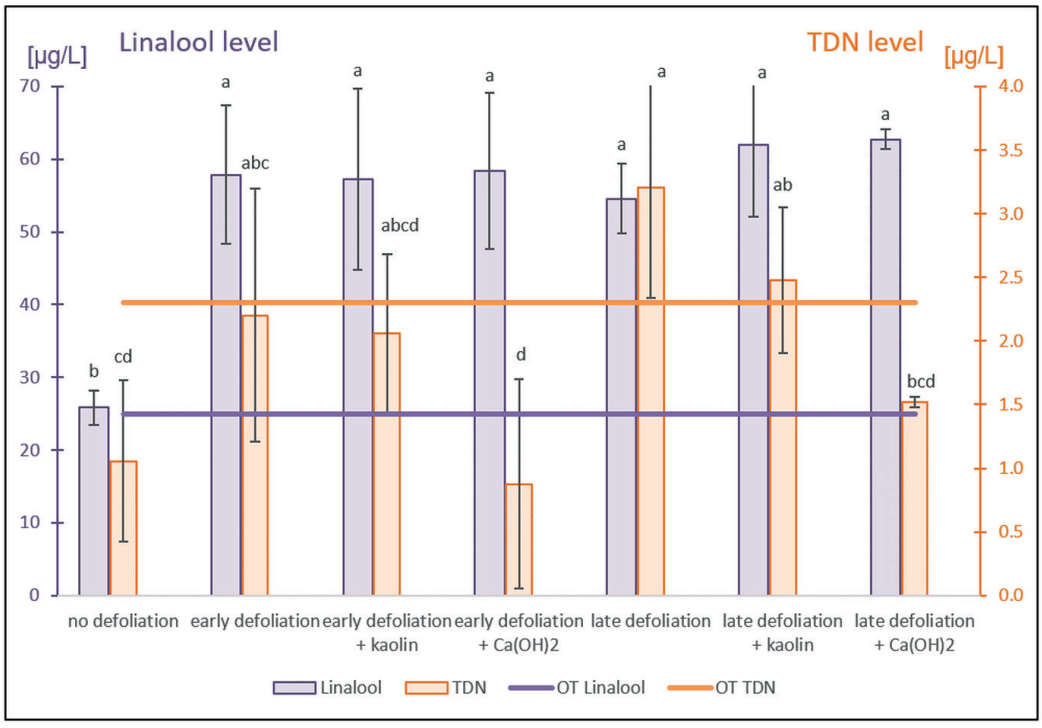


FIGURE 3. Concentrations of linalool and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) in the experimental wines and their general odour thresholds in the wine of 2.3 µg/L for TDN according to Ziegler *et al.* (2019) and 25 µg/L for Linalool according to Ferreira *et al.* (2000) (letters indicate significant differences among treatments according to Fishers Least Significant Difference (LSD) test).

TABLE 2. Sensory data of the different treatments and three-way analysis of variance of the data of the descriptive analysis (letters indicate significant differences among treatments according to Fisher's Least Significant Difference (LSD) test).

treatment		colour	citrus/ mineral	ripe apple	yellow fruits	green/ vegetative	smoky/ honey	dull/ATA (odour)	sweet	sour	fruity	dull/ATA (oral)	body	mouthfeel	astringent	bitter
no defoliation		2.9 d	4.3 ab	3.1 c	3.1	2.7 ab	2.5 b	1.7 b	2.9 bc	5.5	3.9	1.5 b	4.3 c	4.2	3.4	2.4
early defoliation		5.4 a	3.0 c	3.7 abc	3.3	2.2 b	3.6 a	2.5 a	3.0 abc	5.4	3.8	2.3 a	4.7 abc	4.5	3.5	2.9
early defoliation + kaolin		4.1 c	4.5 a	3.2 bc	3.4	2.6 ab	2.7 b	1.5 b	3.1 ab	5.2	4.1	1.4 b	5.0 a	4.5	3.4	2.5
early defoliation + $Ca(OH)_2$		4.8 b	4.5 a	3.5 abc	3.6	2.4 b	2.8 b	1.5 b	3.5 a	5.3	4.4	1.5 b	4.8 ab	4.2	3.0	2.6
late defoliation		4.7 b	4.1 ab	3.5 abc	3.3	2.2 b	3.1 ab	1.6 b	2.8 bc	5.8	3.7	1.8 b	4.5 bc	4.6	3.1	2.6
late defoliation + kaolin		4.0 c	3.7 bc	3.8 ab	3.4	2.2 b	2.8 b	1.5 b	2.6 c	5.4	3.9	1.5 b	4.3 c	4.4	3.3	2.2
late defoliation + $Ca(OH)_2$		3.8 c	4.6 a	4.0 a	3.4	3.2 a	2.7 b	1.5 b	3.0 bc	5.2	4.1	1.5 b	4.7 abc	4.2	3.1	2.7
F value	treatment	23.09	5.07	1.99	0.53	2.58	2.67	3.36	2.84	1.06	1.59	3.03	2.30	0.50	1.26	1.70
p		***	***	*	ns	*	*	**	*	ns	ns	**	*	ns	ns	ns
F value	judge	27.22	12.96	17.68	12.35	12.08	14.96	12.42	27.41	32.19	18.73	10.70	39.61	24.61	53.31	38.94
p		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
F value	replication	8.07	1.53	1.32	0.22	0.61	0.14	0.08	0.74	0.22	0.02	1.01	0.03	0.26	2.33	1.03
p		**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Data represents mean values with n = 2, level of significance: * $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$; ns = not significant.

TABLE 3. Aroma analysis data of the different treatments and two-way analysis of variance of the data of the aroma analysis (letters indicate significant differences among treatments according to Fisher's Least Significant Difference (LSD) test).

treatment	TDN free	vitispirane free	β-damas cenone	4-vinyl-guaiacol	linalool	2-phenyl-ethanol	2/3-methyl-butanol	hexanol	ethyl butanoate	ethyl hexanoate	ethyl octanoate	ethyl decanoate acetate	ethyl acetate	hexyl acetate	2/3-methyl-butyl acetate	2-phenyl-ethyl acetate	2-amino-acetophenone
	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[mg/L]	[mg/L]	[mg/L]	[µg/L]	[µg/L]	[mg/L]	[mg/L]	[mg/L]	[µg/L]	[mg/L]	[µg/L]	[µg/L]	[µg/L]
no defoliation	1.06 cd	7.35 cd	0.63	9.31	25.84 b	13.66	232.45	3.17 ab	335.98 b	806.82 bc	3.22 c	1.22 abc	63.83 ab	168.86 b	2.19 a	250.59 ab	0.23 b
early defoliation	2.20 abc	12.87 abc	1.32	9.54	57.85 a	19.75	253.06	2.64 bc	311.13 bc	750.54 c	3.00 cd	1.06 bcd	68.04 a	88.81 d	1.29 d	200.20 c	0.23 b
early defoliation + kaolin	2.06 abcd	11.30 abcd	0.91	10.34	57.23 a	16.97	245.37	3.09 ab	328.58 b	937.09 a	3.66 ab	1.26 ab	61.51 ab	147.48 bc	1.64 cd	192.44 c	0.37 a
early defoliation + $Ca(OH)_2$	0.88 d	5.15 d	1.08	8.12	58.40 a	16.44	258.89	2.40 c	391.83 a	994.84 a	4.05 a	1.40 a	68.32 a	131.58 c	2.15 a	272.60 a	< 0.01 e
late defoliation	3.21 a	17.87 a	0.81	10.94	54.53 a	18.11	249.84	2.38 c	314.37 bc	891.85 ab	3.10 cd	0.96 cd	58.73 bc	119.19 cd	1.72 bc	213.55 bc	0.13 cd
late defoliation + kaolin	2.48 ab	16.74 ab	1.34	12.60	61.98 a	17.14	241.81	3.16 ab	284.86 c	713.39 c	2.80 d	0.85 d	53.78 cd	123.24 cd	1.64 cd	205.83 bc	0.17 bc
late defoliation + $Ca(OH)_2$	1.52 bcd	9.78 bcd	0.56	8.43	62.73 a	20.38	265.38	3.55 a	324.70 b	973.57 a	3.41 bc	1.14 abc	50.32 d	214.46 a	2.08 ab	272.60 a	0.05 de
odour threshold	2.3 [1]	101.3 [1]	0.05 [2]	40 [2]	25 [3]	14 [2]	30 [2]	8.0 [2]	20 [2]	14 [2]	0.005 [2]	0.2 [2]	12.3 [2]	1800 [2]	0.16 [2]	2400 [2]	1.0 [4]
F – treatment	2.94	2.38	1.3	1.23	4.15	1.99	0.58	4.08	7.65	6.10	6.45	3.16	5.10	7.59	4.30	3.74	11.24
p – treatment	*	*	ns	ns	*	ns	ns	*	**	**	**	*	**	**	*	*	***
F – replication	1.69	1.15	1.59	0.32	0.22	2.82	1.16	0.68	8.68	6.05	6.78	0.63	1.24	7.17	3.52	1.25	0.55
p – replication	ns	ns	ns	ns	ns	ns	ns	ns	**	*	*	ns	ns	**	*	ns	ns

Data represents mean values with n = 3, level of significance: * p < 0.1, ** p < 0.01, *** p < 0.001; ns = not significant). [1]Ziegler *et al.* (2019). [2]Waterhouse *et al.* (2016), [3]Ferreira *et al.* (2000), [4]Fischer and Sponholz (2000).

the published odour threshold of 1.29 µg/L (Fischer and Sponholz, 2000). Nevertheless, the lowest concentration was found in the two treatments with the application of the calcium hydroxide suspension.

4. Temporal dominance of sensations (TDS)

Assessing the sensory modulation over time, the TDS graphs revealed the greatest differences among the defoliation treatments. The wines of early defoliated grapes all showed a higher sweetness dominance in the first 10 seconds. The dominating sourness lasted longer for the late defoliation treatments and non-defoliated control. Astringency dominance remained below the significance threshold, except for the application of reflecting particles on the late defoliated grapes, where astringency dominated after 30 and 40 seconds at a significant level (Figure 4). Analysis of variance (ANOVA) was applied to those dominance curves, which were above the chance level (p = 1/number of attributes, here: 12.5 %). Significance was determined for the parameters "maximum dominance rate" (D_{max}), "duration of dominance" and "area under the curve", which were extracted from the curves for each attribute of the experimental treatments and replicates separately. Unfortunately, none of these parameters proved significant and are, therefore, not considered further.

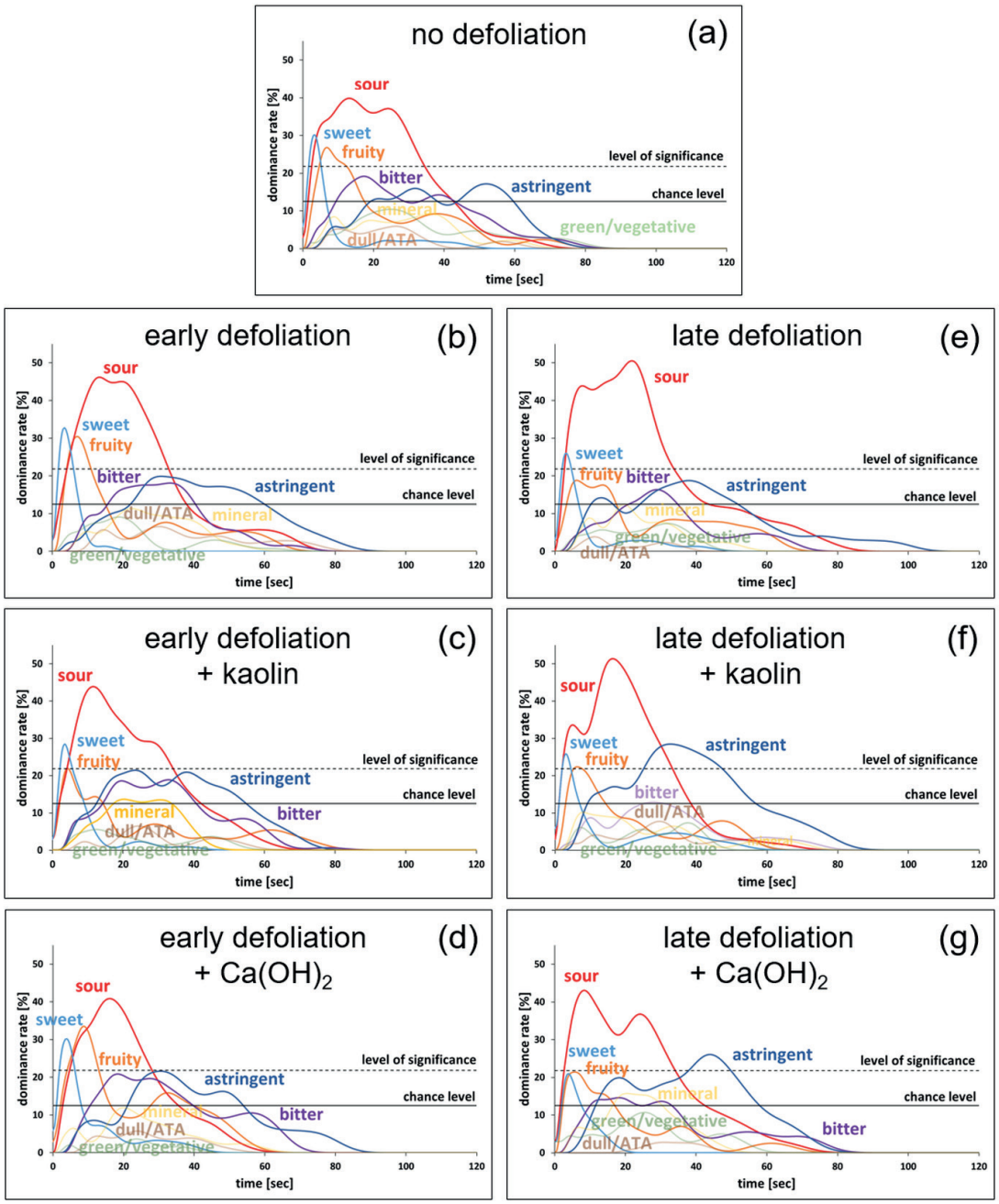


FIGURE 4. Smoothed TDS curves of the different treatments. (a) no defoliation, (b)–(d) treatments with early defoliation, (e)–(g) treatments with late defoliation. Level of significance with α = 0.05, chance level 1/p with p = number of attributes (12.5 %) (17 J × 2 Rep.). Dominance curves not reaching the chance level are displayed transparently.

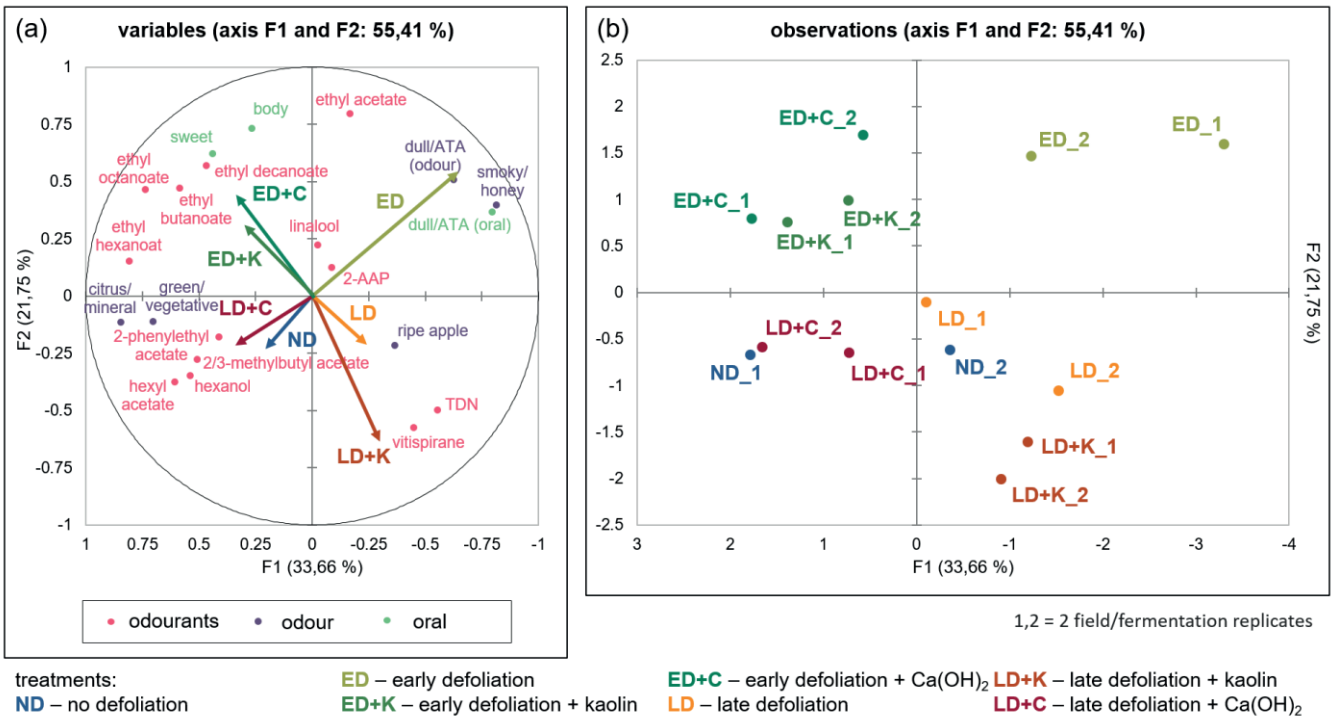
DISCUSSION

According to our study, an early defoliation results in the plant adaptation to increased sun exposure by producing protecting secondary plant compounds, such as carotenoids and polyphenols, which may act as precursors for TDN, vitispirane and 4-vinylguaiacol as well (Ziegler *et al.*, 2019). The increased content of these phytochemicals results in a more intense yellow colour of the wines. In contrast, late defoliation at berry closure fosters sunburn events because the grapes had insufficient time to adapt to sun exposure and high temperature. Application of both reflecting particle suspensions seems to contribute to mitigating sunburn events. At the same time, lower colour and less ATA perception in the wines of these grapes suggest a diminished formation of protective carotenoids and polyphenols. This could be rationalised by the protective effect of the reflecting particles themselves. For the petrol off-flavour in TDN, this effect occurred when particles were applied after early defoliation, but not in the case of kaolin after late defoliation. Ongoing sun exposition after véraison decreases carotenoid and polyphenol levels. This catabolism also leads to a higher content of the degradation products, such as the odour-active TDN, vitispirane and 4-vinylguaiacol (Marais *et al.*, 1992, Yuan and Qian, 2016).

The multiple factor analysis (MFA) with the significant sensory and aroma analysis data (Supplementary Table 4) of each replicate in Figure 5 allows the holistic interpretation of the treatment effects in regard to the sensory properties of the wines in combination with their aroma composition.

A positive correlation between hexyl acetate and a citrus/mineral and green/vegetative odour impression seems to be obvious. The odour of hexyl acetate, in general, enhances the fruity aroma of a wine, while one of its precursors, hexanol, is described as herbaceous (Forde *et al.*, 2011, Dennis *et al.*, 2012). Wine made from the non-defoliated control and the treatment with late defoliation combined with $Ca(OH)_2$ application yielded the highest concentration of hexyl acetate and hexanol and accordingly were evaluated with more intense green/vegetative and citrus/mineral odour. According to Dennis *et al.* (2012), multiple C_6 alcohols and aldehydes are grape-derived precursors for hexyl acetate. Thus, the post-fermentative concentration and fruity impression depend on the sum of the precursors present in the initial grape juices. At the same time, precursors which have not been used for ester formation by the yeast may influence the aroma properties of the wine as well.

Ethyl acetate is shown on the right side in the upper half of the MFA. Ethyl acetate has a typical odour of nail polish remover and is a result of the esterase activity of yeasts (Waterhouse *et al.*, 2016). According to Table 3, ethyl acetate varied between 50 and 68 mg/L, surpassing its published odour threshold of 12.3 mg/L in 10 % water/ethanol mixture (Escudero *et al.*, 2004). Lower concentrations of ethyl acetate may contribute to a fruity impression, while higher concentrations mask fruity and other attributes. According to the MFA in Figure 5, ethyl acetate fostered the dull/ATA and



treatments:
ND – no defoliation
ED – early defoliation
ED+K – early defoliation + kaolin
ED+C – early defoliation + $Ca(OH)_2$
LD – late defoliation
LD+K – late defoliation + kaolin
LD+C – late defoliation + $Ca(OH)_2$

FIGURE 5. Multi-factor analysis (MFA) of data from sensory and aroma analysis: On the left (a), the loadings of significant sensory attributes, the concentration of odourants and scores averaged over both replications (vectors). On the right (b), the scores of each treatment replicate. Individual scores 1 and 2 reflect variability due to field, fermentation and sensory replications.

smoky/honey intensities while masking the citrus/mineral and green/vegetative nuances. The fact that ethyl acetate had only a masking effect but was not perceived as such with its solvent character despite concentrations exceeding the odour threshold in a model wine by a factor of four can be rationalised by the recently published huge increase of the odour threshold of ethyl 2-methyl butanoate ester when established in a model wine (11.5 µg/L) and a regular white wine (2332.8 µg/L) (Gottmann *et al.*, 2023). The deviation in ethyl acetate among the treatments is unlikely to be caused by bacteria or *Hanseniasporum uvarum* metabolism, as only sound grapes were processed, and a strong *Saccharomyces cerevisiae* yeast strain was inoculated. However, different levels of phytochemicals may have contributed to the hyperosmotic stress, which triggers minor ethyl acetate formation in *Saccharomyces cerevisiae* (Bely *et al*., 2005; Ferreira *et al.,* 2006).

Based on the above-discussed results (ANOVA, PCA, MFA, TDS), it seems likely that enhanced fruity odour and retronasal perception due to elevated ester concentrations may have contributed to a more intense sweet perception, although residual sugar varied only by less than 1 g/L, having no sensory impact (Table 1). According to day-to-day experience, fruits with a higher ripeness and sugar content correlate with increased fruity ester concentrations. Vice versa, it is well known that a stronger sweet taste does enhance retronasal fruitiness perception (Matysiak and Noble, 1991).

This hypothesis is corroborated by our examination of the wines by GC-olfactometry (not reported) that ethyl esters generally exhibited fruit characteristics of sweet fruits such as strawberry in ethyl butanoate and pineapple in ethyl hexanoate. This interaction between fruity esters and sweet perception is also backed by our TDS results in Figure 4, where wines of increased fruitiness showed a stronger sweet dominance early on and a lower and later onset of acidity dominance in the course of the temporal examination. In case of the early defoliation treatment, the application of reflecting $Ca(OH)_2$ and kaolin particles into the grape zone enhanced the perception of fruitiness and ester concentrations, while the detrimental dull/ATA attribute was suppressed.

Another hypothesis, especially regarding the late defoliation treatments, can be derived based on the lower right half of the MFA (Figure 5). 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) is solely responsible for the formation of the petrol off-flavour, while the structurally very similar vitispirane yields a camphor, woody and herbaceous flavour (Ziegler, 2020). The degradation of TDN precursors from the carotenoid catabolism is strongly accelerated by low pH values (Gök *et al*., 2022). Thus, the observed correlation between sour taste and TDN concentration in the MFA could be explained by the acidic hydrolysis of the TDN progenitors. However, due to a narrow range of pH values between 3.1 and 3.3, sourness perception did not vary significantly and, thus, was not included in the MFA exploration (Figure 5). Overall, the enhanced TDN and vitispiran concentration due to later defoliation at berry closure can be explained by a stronger carotenoid degradation.

The observation in the TDS analysis over time that late defoliation yielded less sweet and fruity dominance during the first ten seconds is mostly due to more dominant sourness and later astringency. Vice versa, enhanced perception of sweetness, which was also triggered by a higher concentration of fruity esters, and fruitiness by itself may have lowered the dominance of sourness. Interestingly, the application of $Ca(OH)_2$ did not result in lowered acidity perception due to the formation of neutral calcium tartrate or calcium malate salts.

Carrying viticultural field replicates forward as grape processing/fermentation replicates and finally even as sensory/analytical replications yielded a joined evaluation of the field, fermentation, and sensory variance. Despite the accumulated variance, many sensory and analytical parameters varied significantly due to different treatments, although the experimental error increased. This highlights the robustness of the here reported significant findings.

Further vintages should be observed to confirm the results of the study and draw more conclusions on the relationship between sunburn damage, preventive measures and aroma composition of white wine. Further on, additional treatments, such as the use of shading nets, should be investigated. The application of reflecting particles should be improved by using more efficient wetting agents, which will achieve a more uniform and complete distribution of the reflected particles on the grape surface.

CONCLUSION

Assessing the results of the warm vintage 2020, the defoliation measures had a larger impact on the sensory characteristics of the wines than the application of the reflecting particles, similar to the reports of previous studies by Ou *et al*. (2010). Early defoliation resulting in an early toughening of the grapes creates wines with less fruity aroma but with a tendency towards an increased smoky taint and the atypical ageing flavour reminiscent of acacia flower and fusel alcohols. The effects of the early defoliation were mitigated by applying the reflecting particles, and the application even increased the fruity aroma and gave a sweeter taste impression. Late defoliation reduced both the green notes of the non-defoliated treatment as well as the smoky taint and atypical ageing note occurring with early defoliation. However, these wines exhibited a slight petrol taste confirmed by increased levels of TDN (1,1,6-trimethyl-1,2-dihydronaphthalene) and vitispirane. In contrast, early defoliation promoted the formation of floral compounds such as linalool, 2-phenylethanol, and β-damascenone. Thus, the study supports Friedel *et al*. (2015), stating that defoliation measures greatly influence the aroma composition of the wine.

The year 2020 was a very hot and dry year, with a total of 20 hot days between June and August measured at the

experimental station. It may serve as a model for future weather scenarios in case of ongoing climate change. Thus, the reported findings will serve the wine industry well to act in the prevention of increasing incidents of sunburn in the future.

In light of these results, early defoliation in combination with the application of reflecting particle suspensions such as kaolin or $Ca(OH)_2$ not only lowers the sunburn risk but also contributes to wines with an enhanced sweet taste. The application of reflecting particles diminishes the dull and ATA impressions observed in grapes from early defoliated grapes and also leads to a more fruity smell of the resulting wines. Late defoliation, however, increases the sunburn risk and results in wines with a more mature aroma that is not diminished by applying a kaolin suspension, while $Ca(OH)_2$ yielded more citrus and green-flavoured wines. While defoliation is a long-term measure, protection by reflecting particles is a short-term treatment, which can be applied shortly before a forecasted heat wave occurs.

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