

The effects of leaf removal and artificial shading on the composition of Chardonnay and Pinot noir grapes

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ABSTRACT

Aims: The aim of this study was to assess the effects of leaf removal and bunch shading on the analytical composition of Pinot noir and Chardonnay (*Vitis vinifera* L.) grapes suitable for making premium sparkling wine.

Method and results: Total bunch defoliation (TD) and different treatments using shading nets (TD1L, TD2L and ND1L) were evaluated in comparison with a test with no defoliation and shading (ND) over three seasons in the southern part of Franciacorta, one of the most famous Italian sparkling wine regions. Micrometeorological variables, yield components, musts and grapes chemical composition were evaluated. Shading practices lead to a delay in ripening and they improve the acidic content of must, thus resulting in a potential improvement in the quality of juice suitable for producing sparkling wines. Furthermore, this particular type of vine canopy management leads to changes in the phenolic content of grapes.

Conclusions: From the results obtained it was possible to underline the positive effect - delaying ripening, preserving acid concentration and reducing flavonol content - of shading on the composition of Pinot noir and Chardonnay grapes suitable for making premium sparkling wine.

Significance of the study: This study shows the importance of shading, because it delays grape ripening and thereby preserves the acidic content of musts and, specifically, deals with the problem of early ripening related to the climate change now underway.

KEYWORDS

vine, shading, grape, must, leaf removal

Supplementary data can be downloaded through: <https://oeno-one.eu/article/view/2556>

INTRODUCTION

The average increases in temperature and different rainfall distributions have led to major repercussions in the agricultural sector; in the case of vines in particular, the different stages of development generally take place earlier and the time between veraison and ripening is shorter (Schultz, 2000; Jones *et al.*, 2005). This can affect grape and wine quality by increasing alcohol content and reducing aroma and acidity (Webb *et al.*, 2007; Hall and Jones, 2009). Acidity is an important determinant of sparkling wine quality and longevity (Ribéreau-Gayon *et al.*, 2000).

The effect of defoliation and shading on vine cultivation and grape quality has been widely studied in the past (Crippen and Morrison, 1986; Jackson and Lombard, 1993; Downey *et al.*, 2006). Moreover, several studies have focused on the relationship between canopy management and the variation in temperature of grapes and berries. Berries shaded by canopies show thermal behaviour very similar to that of air (Reshef *et al.*, 2017); however, different studies have demonstrated a steady increase in the temperature of exposed bunches (Spayd *et al.*, 2002), which can reach values between 7 °C and 12 °C higher than the air temperature (Kliewer and Lider, 1968; Smart and Sinclair, 1976; Bergqvist *et al.*, 2001). Bunches exposed to direct solar radiation can reach temperatures higher than 37 °C (Crippen and Morrison, 1986), thus exceeding the optimum temperature range for berry development, which has been identified as between 25 °C and 35 °C (Hale and Buttrose, 1974). This influences berry ripening and metabolism, particularly in terms of reducing titratable acidity and increasing malic acid degradation (Lakso and Kliewer, 1978; Conde *et al.*, 2007; de Oliveira *et al.*, 2019). The majority of studies to date have characterised the combined influence of solar irradiance and its accompanying climatic component, temperature - both of which are known to influence several metabolic processes - on berry composition. Berry temperature is determined by the energy balance of the fruit and is strongly affected by direct exposure to solar radiation (Cola *et al.*, 2009). At a practical level, solar irradiance is the most easily and readily controlled climatic factor (Reshef *et al.*, 2017).

For some time, studies have indeed demonstrated the effect of shading on delaying ripening

(Rojas-Lara and Morrison, 1989; Percival *et al.*, 1994; Filippetti *et al.*, 2014; Martin *et al.*, 2016) and preserving acidity, both in terms of titratable acidity (Reynolds *et al.*, 1986; Smart *et al.*, 2017) and malate concentration (Dokoozlian and Kliewer, 1996; Martin *et al.*, 2016). Some studies have associated artificial shading with an increase in pH and potassium (Smart *et al.*, 1985; Scafidi *et al.*, 2013; Martinez de Toda and Balda, 2014), although more recent studies have reported that this treatment does not significantly affect this parameter (Filippetti *et al.*, 2014).

The exposure of bunches to sunlight can also modify the content of anthocyanins (Bergqvist *et al.*, 2001; Dokoozlian and Kliewer, 1996; Downey *et al.*, 2003; Haselgrove *et al.*, 2000; Mori *et al.*, 2005; Spayd *et al.*, 2002) and other polyphenols in berries. Above a certain temperature range, both anthocyanin and polyphenol synthesis are inhibited, as reported in various previous studies (Kliewer and Torres, 1972; Price *et al.*, 1995; Pastor del Rio and Kennedy, 2006; Fernandes de Oliveira *et al.*, 2015). Nevertheless, some authors have reported that a decrease in the exposure of Pinot noir grapes to sunlight can cause a reduction in total anthocyanin concentration (Dokoozlian and Kliewer, 1996), changing the pattern and leading to lower percentages of delphinidin-glucoside, cyanidin-glucoside, petunidin-glucoside and malvidin-glucoside, with an increase in peonidin-glucoside (Cortell and Kennedy, 2006).

In general, the response of grapes to different levels of exposure, in terms of accumulation of anthocyanins and phenolic substances, also seems to be related to the cultivar's sensitivity to temperature (Fernandes de Oliveira *et al.*, 2015). Recently, the complex influence of the spatial pattern of incoming irradiance and fruit temperature on the metabolic profile within grape clusters of Cabernet Sauvignon was described in a vineyard in the Negev desert, Israel, where excess solar irradiance and midday temperatures are known to reduce grape quality. The higher irradiance increased the concentration of several amino acids and polyamines (proline, valine, leucine, GABA, putrescine and ethanolamine) and of tartaric acid in the pulp, while decreasing malic acid. Irradiance increased the concentration of phenylalanine, flavonols, naringenin-chalcone-4-O-glucoside and cyanidin-3-glucoside in the skins, while decreasing malvidin-3-glucoside,

hydroxycinnamic acids and monomeric and dimeric flavanols (Reshef *et al.*, 2017).

This paper aims to compare different canopy shading levels for *Vitis vinifera* L. cv. Chardonnay and Pinot noir suitable for producing sparkling wine. The effects of different levels of bunch exposure on the vine, must and berry composition and the relationship between treatments and micro-meteorological variables are described.

MATERIALS AND METHODS

1. Experimental trial

This research was conducted in three consecutive years (2013, 2014 and 2015) in a vineyard belonging to Azienda Agricola Castello Bonomi Tenute in Franciacorta, located in the southern part of the Franciacorta viticultural area (Lombardy Region). This geographical context is characterised by temperatures about 3 °C higher than the average for other vineyards in this winegrowing area. The vineyard was planted in 2004, cordon-trained, oriented from north to south and grass-covered.

To further confirm the results obtained, treatments were applied to two international *Vitis vinifera* L. cultivars (Chardonnay clone Entav-Inra® 96 and Pinot noir clone 292, both grafted onto Kober 5BB rootstock), which are both traditionally cultivated in this area.

Five different treatments were compared in all the years considered: a comparative test without defoliation and shading (ND), a test with total defoliation (east and west side) (TD) and three different systems adopting shading nets applied along the bunch zone; two of the shaded treatments were defoliated as for TD and covered with one layer of shading net (TD1L) or two layers of shading net (TD2L), while a third treatment was covered by only one layer of shading net, but not defoliated (ND1L).

For both cultivars, the treatments were organised into three randomised blocks, each consisting of 25 vines. The treatments were maintained in the same blocks during the whole trial period. Leaf removal and shading net application took place at about 20 % veraison and was carried out along the bunch zone (about six basal leaves removed equal to about 35 % of total leaf area), while a polyethylene UV stabilised net of approximately 95 g/m² was used for shading (shading net

OF50N provided by Retes srl). Preliminary tests were carried out in order to evaluate the percentage of global solar radiation passing through the nets. The transmittance of global solar radiation of the single layer and double layer nets was reduced by 50 % and 70 % respectively.

2. Meteorological data

Two types of measurement were carried out in all three years of the study with the aim of better understanding the effects of shading on canopy and berry temperatures. Specifically, for the cultivar Chardonnay alone, five field weather stations were installed to monitor temperature and humidity during the period between the beginning of veraison (when defoliation and shading were implemented) and harvesting. Each weather station consisted of an Onset Hobo datalogger endowed with a silicon pyranometer and an air temperature/relative humidity sensor placed in a solar shield. One station monitored atmospheric variables outside the canopy and the sensors were placed outside the vineyard at standard heights, following the recommendations of the World Meteorological Organization (2009). In the case of the other four stations, the sensors were placed at the height of the bunches (first wire level) in order to monitor the variables under the canopy for each of the four main treatments: ND, TD, TD1L and ND1L. We decided to focus on these four treatments, because they best represent the different conditions of the canopy (presence-absence of leaves; absence of artificial cover). Monitoring took place at a 5-minute time step. Subsequently, the data were aggregated to provide hourly and daily time steps.

Internal berry temperature was measured with an Onset Hobo Copper–Constantan thermocouple inserted into the berry. Data were collected with a specific datalogger. The measurements were carried out with a reduced time step of 1 min during the period, ranging from post-veraison to harvest (see Table 1) from 5 to 27 August in 2013, from 23 July to 17 August in 2014 and from 16 July to 7 August in 2015.

Inner berry temperature monitoring followed the protocol adopted in Cola *et al.* (2009): the thermocouple tip was inserted into the berry, previously pierced with a spike; the thermocouple tip was placed on a berry in the external-middle part of the cluster; the

thermocouple was relocated to a new berry every week in order to maintain optimal conditions of the living organs, so that withering could not influence the measurements. Each thermocouple was installed in a single randomised block for each treatment. No replicates of the measurements were taken.

In order to evaluate the thermal conditions of berries, the following indices were calculated for each treatment from berry temperature measurements for the period between fruit set and physiological maturity:

- GDD - Growing Degree Days, calculated from average daily berry temperature using 10 °C as a base (as with the Winkler Index (Amerine and Winkler, 1944), cumulated from fruit set to physiological maturity;
- NHH - Normal Heat Hour Index, which represents the accumulation of hourly thermal resources useful for berry maturation (Cola *et al.*, 2020), cumulated from fruit set to physiological maturity;
- HHH - High Heat Hour Index, which represents the accumulation of hourly thermal excess (Cola *et al.*, 2020), cumulated from fruit set to physiological maturity.

The main limitation of the GDD approach is the overestimation of high temperatures: a very hot summer day will show a high mean daily temperature. This translates into high GDD, meaning optimal conditions for plant growth. However, since temperature can be detrimental to biological processes, the NHH and HHH approaches (Mariani *et al.*, 2012; Cola *et al.*, 2017) measure hourly temperature (Th) based on

four cardinal temperatures: LC - low cardinal (6 °C), LOC - low optimal cardinal (24 °C), UOC - upper optimal cardinal (26 °C) and UC - upper cardinal (33 °C). LC and UC limit the cardinal range within which phenological development occurs, while LOC and UOC define the optimum for phenological development. The response function (Figure 1) translates hourly temperature into thermal effective hour: Th gives 0 NHH, if outside the cardinal range and 1 NHH, if within the optimal range. As Th moves from LC to LOC, NHH linearly increases from 0 to 1 and, similarly, NHH linearly decreases from 1 to 0 as Th moves from UOC to UC (Cola *et al.*, 2016). The values of the four parameters LC, LOC, UOC and UC proved to perform well for all the studied cultivars (Cabernet-Sauvignon, Chardonnay, Barbera and the Georgian cultivars Mtsvane Kakhuri, Rkatsiteli, Ojaleshi and Saperavi) (Mariani *et al.*, 2013; Cola *et al.*, 2014; Cola *et al.*, 2016).

It is well known that shaded berries show a thermal regime very close to air (Cola *et al.*, 2009; Berquqvist *et al.*, 2001), while the temperature of sun-exposed black berries exceeds air temperature by up to 10 °C.

Several authors have discussed the relationship between environmental temperature and ripening processes (Abeyasinghe *et al.*, 2019; Kuhn *et al.*, 2014; Mori *et al.*, 2005; Downey *et al.*, 2004; Downey *et al.*, 2003; Spayd *et al.*, 2002; Haselgrove *et al.*, 2000), while few have tried to consider berry temperature (Wu *et al.*, 2019; Lecourieux *et al.*, 2017; Greer and Weedon, 2014; Bergqvist *et al.*, 2001); it is therefore hard to understand how the temperature of air affects the temperature of clusters and then the ripening.

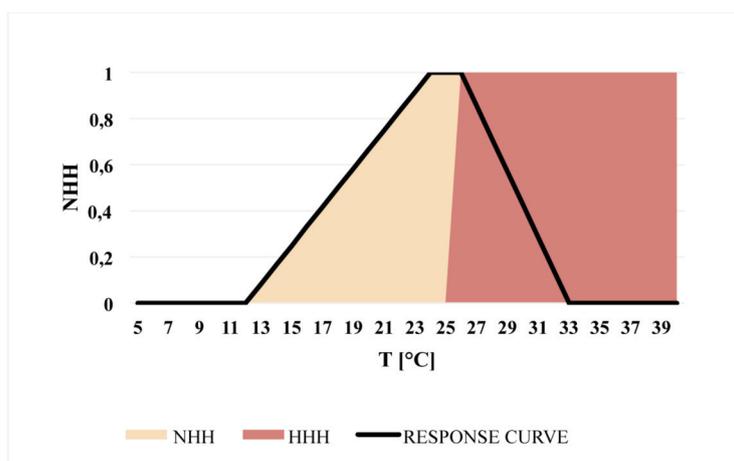


Figure 1. NHH and HHH Response curves relating thermal resources and temperature.

The NHH response curve was parameterised in order to describe the phenological development of grapevine and is strictly related to the net photosynthesis response to temperature (Greer, 2017 and Greer and Weedon, 2012). The use of the same response curve to calculate thermal resources of berries can be seen as an attempt to summarise the bulk of different physiological processes of ripening, each of which is characterised by a specific response function.

Furthermore, the three seasons were characterised according to the reference period 1988-2015, representative of the current warm phase that started in Europe at the end of the 1980s (Mariani *et al.*, 2012). The analysis was carried out with data from the weather station of Rovato, provided by the Agrometeorological Network of the Province of Brescia, located 2 km from the experimental field.

3. Yield components and the composition of grape juice

The evolution of ripening for both Chardonnay and Pinot noir was monitored by periodically sampling each of the five treatments. Seventy berries were collected from each randomised block (35 berries collected from both the eastern and the western sides and then grouped together in a single sample). The first sample was collected on the same day the shading net was installed and when defoliation took place, to obtain evidence regarding the condition of the parcels before setting up the trial. From that moment on, weekly samples were taken until harvesting time approached, when the frequency of sampling was intensified.

For both cultivars and all treatments, the harvesting time was established at about 10.5 % of potential alcohol. In this case the tests were called “fixed alcohol” and indicated with -FA at the end of the treatment code (i.e., ND-FA, TD-

FA, TD1L-FA, TD2L-FA, ND1L-FA). With the aim of having a more complete view of the ripening trend, for the Chardonnay cv alone, another harvest was carried out on the same date, when the earliest treatment reached 10.5 % potential alcohol. In this case the tests were called “fixed date” and indicated with -FD at the end of the treatment code (i.e., ND-FD, TD-FD, TD1L-FD, TD2L-FD, ND1L-FD). Each harvest date is reported in detail in Table 1.

Experimental harvesting was organised by selecting a total of 18 vines per treatment (ND, TD, TD1L, TD2L and ND1L), corresponding to 6 plants for each of the three randomised blocks. For each vine, total yield (TY), average bunch weight (AWG), yield/pruning weight (Ravaz Index - RI) and bud fertility (BF) were determined. Bud fertility was calculated from the ratio between the total number of bunches and the total number of buds, including shoots and not sprouted buds. A sample of three bunches was collected to check juice quality. These samples were then crushed and the total soluble solids concentration (TSS), pH, titratable acidity (TA) and malic acid (MA) concentration were measured in the grape juice. These measurements were determined respectively using a traditional handheld refractometer for soluble solids concentration, a Crison compact titrator analyser both for pH and TA and the enzymatic method (Hyperlab wine analyser) to determine malic acid concentration.

4. Grape anthocyanin and polyphenol composition

At harvesting, both at 10.5 potential alcohol and in the case of Chardonnay on the fixed date, 1 kg racemes sample was collected for each randomised block (for a total of three samples for each treatment). The sample was taken by collecting about 500 g from 20 clusters on each side (east and west) and immediately sent to the

TABLE 1. Harvest dates specified by year and treatment.

Year	Cultivar	Dates of treatment application	Dates of FD harvesting	Dates of FA harvesting
2013	Chardonnay	29/7	All treatments 28/08	ND 28/08; TD 28/08; TD1L 28/08; TD2L 2/09; ND1L 2/09
	Pinot noir		/	ND 22/08; TD 20/08; TD1L 23/08; TD2L 22/08; ND1L 24/08
2014	Chardonnay	16/7	All treatments 17/08	ND 17/08; TD 17/08; TD1L 17/08; TD2L 17/08; ND1L 21/08
	Pinot noir		/	ND 11/08; TD 11/08; TD1L 11/08; TD2L 13/08; ND1L 13/08
2015	Chardonnay	15/07/20	All treatments 10/08	ND 11/08; TD 10/08; TD1L 10/08; TD2L 12/08; ND1L 12/08
	Pinot noir		/	ND 5/08; TD 4/08; TD1L 4/08; TD2L 5/08; ND1L 6/08

Dates of treatment application and harvesting are reported both for Chardonnay and for Pinot noir. In case of Chardonnay harvesting dates are divided by -FD (fixed date) and -FA (fixed alcohol).

laboratory at the Edmund Mach Foundation, where it was stored at $-80\text{ }^{\circ}\text{C}$ until analysis. The sub-sampling procedure, which aimed to obtain a smaller representative sample, consisted of two steps. After removing the pedicels, a sample of 100 g of berries was randomly selected. From this a further subsample consisting of 30 randomly picked deep-frozen berries was ground under liquid nitrogen using an IKA analytical mill (Staufen, Germany) to obtain a frozen powder. A total of 3 g of the powder from each sample was extracted in sealed glass vials using 10 mL of a water/methanol mixture (30:70). After vortexing for 1 min, the samples were transferred to an orbital shaker for 15 min at room temperature. Samples were centrifuged at 1000 g and $4\text{ }^{\circ}\text{C}$ for 10 min. Extraction was repeated by adding another 5 mL of water/methanol (30:70) and after centrifugation, the two extracts were combined, brought to 20 mL with demineralised water and filtered through a $0.2\text{ }\mu\text{m}$ PTFE filter prior to analysis.

Chromatographic, separation and detection conditions were the same as those extensively validated for the quantitative analysis of phenols, as described by Vrhovsek *et al.* (2012). Briefly, Ultra Performance Liquid Chromatography separation of phenolic compounds, lasting 17 min, was performed on a Waters Acquity UPLC by means of a Waters Acquity HSS T3 column $1.8\text{ }\mu\text{m}$, $150\text{ mm} \times 2.1\text{ mm}$, kept at $40\text{ }^{\circ}\text{C}$. Mobile phase A comprised water containing 0.1 % formic acid; mobile phase B comprised acetonitrile containing 0.1 % formic acid. The flow was 0.4 mL/min. This targeted method was developed for the quantification of 60 phenolics, including benzoic acid derivatives, phenylpropanoids, coumarins, stilbenes, flavan-3-ols, flavonols, anthocyanins and thiols.

5. Statistical analysis

The statistical analysis was carried out with SPSS software (Statistical Package for Social Science). In the preliminary data analysis, outliers were deleted; i.e., observations with values greater than 1.5 interquartile ranges (IQRs) above the third quartile, or lower than $1.5 \times \text{IQRs}$ below the first quartile.

To analyse analytical and growth-productivity results, a linear mixed effects model ($p < 0.05$) was performed, including “treatment” and “block” as fixed factors and “year” as a random factor. The block was not included as a fixed

factor in ANOVA, which was carried out on phenolic and thiol variables, because a single observation was made for each block. A post-hoc REGWF (Ryan, Einot, Gabriel and Welsch F) procedure was implemented to compare the pairs of treatment means while controlling fixed and random factors.

A Principal Components Analysis (PCA) was carried out to visualise the pattern of behaviour for productive and quality variables, together with microclimatic variables (GDD, NHH and HHH). We could only apply this analysis to data obtained from Chardonnay-FD, as the thermocouple tip was inserted into the berry on this cultivar in the period between post-veraison and the fixed harvest date (-FD). We carried out this analysis on the 6 plants for each treatment and each year, in the block where the thermocouple had been installed. We therefore referred to the specific microclimatic data and the specific UPLC data of that randomised block. PCA was performed with the R package, FactoMineR, scaling data to unit variance. We also computed the correlation matrix of the microclimatic variables with the productivity and quality variables.

RESULTS

1. Weather data

Figures S1 and S2 show the temperature and rainfall in the three seasons, compared to the reference period (average for the 1988-2015 period).

In terms of temperature, the 2013 results were, in general, similar to the reference period: maximum monthly temperatures were close to normal values, with a slightly positive anomaly in February, March, April and July. The minimum temperatures were slightly above the normal values throughout the year. Yearly precipitation was slightly higher than in the reference period (1027 vs 971 mm, +5.8 %). Strong positive anomalies characterised March and May, while negative anomalies were recorded in June and September.

2014 was characterised by very high minimum temperatures during the first four months, average values in summer and high values from September to December. Maximum temperatures were close to average values, with the exception of July and August, characterised by low values. The yearly precipitation was high (1298 Vs 971

TABLE 2. GDD, NHH and LHH accumulation during Chardonnay ripening.

Index	Year	ND	ND1L	TD1L	TD
GDD	2013	338.5	314(-7.2 %)	344.2(1.7 %)	361.6(6.8 %)
	2014	394.6	362.2(-8.2 %)	407.9(3.4 %)	430.5(9.1 %)
	2015	439.5	409.9(-6.7 %)	458.4(4.3 %)	474.2(7.9 %)
NHH	2013	337.6	346.4(2.6 %)	333.2(-1.3 %)	327.2(-3.1 %)
	2014	454.1	472.8(4.1 %)	442.9(-2.5 %)	428.8(-5.6 %)
	2015	313.0	324.2(3.6 %)	308.1(-1.6 %)	301.7(-3.6 %)
HHH	2013	106.4	97.1(-8.7 %)	111.1(4.4 %)	117.2(10.2 %)
	2014	63.5	36.3(-42.8 %)	78.2(23.3 %)	97.0(52.9 %)
	2015	201.8	189.2(-6.2 %)	207.3(2.7 %)	214.5(6.3 %)

% variation compared to the ND treatment is shown.

mm + 34 %), with highly positive anomalies in January, February, July, August and November, while spring was characterised by a negative anomaly.

2015 was a fairly average year in terms of both minimum and maximum temperatures. Above-average temperatures were recorded in June, July and August. Precipitation was very low for the area, with 565 vs 971 mm (-42 %). Negative anomalies characterised the whole year, with the sole exception of October.

1.1. Micro-meteorological data

Based on data measured directly inside the berries, thermal resources during the ripening periods were analysed. An example of berry temperature monitoring is shown in Figure 2.

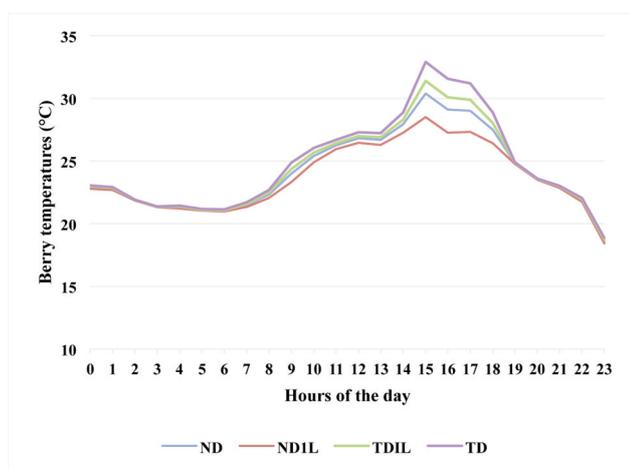


FIGURE 2. Thermal trend in berries for the four monitored treatments (2 August 2014).

Table 2 shows GDD, NHH and HHH based on inner berry temperature during ripening with the different treatments. For each season, the variability between treatments was expressed as a percentage increase/decrease compared to the ND treatment.

Regarding accumulation of GDD, variability between treatments was low, the maximum difference in 2014 when the highest level was reached by TD (+9.1 % compared to ND) and the lowest by ND11 (-8.2 %). As regards variability between years, 2013 was the year with the lowest GDD values, while 2015 showed the highest levels for all treatments (on average +31 % in 2015 compared to 2013).

The ranking from the lowest to the highest GDD value was ND11, ND, TD11 and TD in all the years. Variability in the accumulation of NHH with the treatments was low and 2014 was again the year with the highest variability: +4.1 % for ND11 and -5.6 % for TD.

As regards the years, 2014 was the year with the highest NHH accumulation, while 2015 had the lowest (on average +33.7 % in 2014 and -7.3 % in 2015 compared to 2013).

The ranking from the lowest to the highest NHH value was TD, TD11, ND and ND11 in all the years.

The ranking was reversed in comparison to GDD. This can be explained by the levels of thermal stress caused by above optimal temperatures, as shown by the HHH Index.

The higher variability of the HHH index can be explained by the lower values of the

TABLE 3. Total yield (TY), average bunch weight (AWG), Ravaz Index (RI) and bud fertility (BF) averaged over the three years of observation .

	Chardonnay-FA					Chardonnay-FD					Pinot noir-FA				
	TD	ND	TD1L	TD2L	ND1L	TD	ND	TD1L	TD2L	ND1L	TD	ND	TD1L	TD2L	ND1L
TY (kg)	2,7 ^a	2,2 ^b	2,4 ^b	2,1 ^b	2,3 ^b	2,7 ^a	2,2 ^b	2,4 ^b	2,2 ^b	2,8 ^a	2,5 ^b	3,0 ^a	2,6 ^b	2,6 ^b	2,6 ^b
AWG (g)	140 ^a	135 ^{ab}	132 ^{ab}	126 ^{ab}	123 ^b	140 ^{ab}	131 ^b	131 ^b	130 ^b	145 ^a	111 ^b	123 ^a	120 ^{ab}	122 ^a	119 ^{ab}
BF	1,1	0,9	0,9	0,9	0,8	1,1	0,9	0,9	1,3	1	1,0 ^b	1,1 ^a	0,9 ^b	0,9 ^b	0,9 ^b
RI	4,7 ^a	4,0 ^{ab}	4,1 ^{ab}	4,2 ^{ab}	3,6 ^b	4,7	4	4,1	4,4	4,3	4,1	4,9	4,6	4,7	3,9

Different letters indicate significant differences in the REGWF test ($P < 0.05$); when no letters are present no significant differences were found.

accumulated index. As a consequence, the percentage variation may be higher. 2014 showed the highest percentage variability (from -42.7 % for ND1L to +52.9 % for TD) and the lowest absolute values.

2015 was the year with the highest HHH accumulation, while 2014 had the lowest (on average +88.5 % in 2014 and -37.4 % in 2015 compared to 2013).

The ranking from the lowest to the highest HHH accumulation was ND1L, ND, TD1L and TD in all the years.

2. Growth-productivity results

The total yield (TY) and average bunch weight (AWG) results averaged over the three years of observations are shown in table 3.

TY and AWG were significantly affected by the cultivar. TD showed a higher production level compared to other treatments for Chardonnay-FA, while for Pinot noir-FA, ND showed the highest TY value. As expected, AWG behaviour was similar to TY, but the differences were only significant when comparing TD and ND1L for Chardonnay-FA. Pinot noir-FA AWG increased for ND and TD2L compared to TD.

Chardonnay-FD showed higher TY values for ND1L and TD compared to other treatments, while AWG was significantly different in ND1L and ND, TD1L and TD2L.

Considering other growth-productivity parameters, data related to the Ravaz Index (RI) and bud fertility (BF) showed minor differences.

3. Influence on ripening

As shown in Figures S3 and S4, ND1L showed a slight delay in ripening for all three years of the study for both cultivars, although in some cases

there was only a one-day delay compared to other treatments.

ND1L matured two days later in 2013 and 2014 and one day later in 2015 compared to ND, when applied to Pinot noir. In the case of Chardonnay ND1L, sugar accumulation was slower, with a

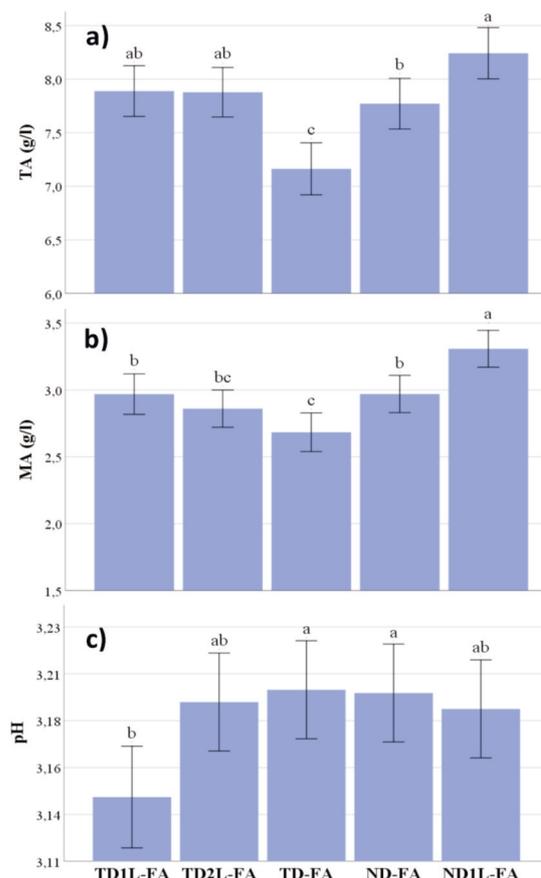


FIGURE 3. Average titratable acidity (TA) (a), malic acid content (MA) (b) and pH (c) recorded for the 3-year period for Pinot noir - FA. +/- 2 SE bar error is shown; letters above bars indicate statistical significance in the REGWF test ($P < 0.05$).

delay of five days in 2013, four days in 2014 and one day in 2015 compared to ND.

TD2L showed similar behaviour to ND1L, although in this case a delay was not recorded in all the years considered. When applied to Pinot noir, TD2L showed a delay in ripening of two days in 2013 and 2014 and one day in 2015 compared to TD, while as regards Chardonnay, a five-day delay was recorded in 2013 and a two-day delay in 2015.

TD1L showed delayed maturation in the first year of the study when applied to Pinot noir. The total defoliation treatment (TD) led to the fastest accumulation of sugar in all three years of the study.

4. Analytical results

4.1. Technological characterisation of must

4.1.1. Pinot noir

The analysis of Pinot noir must show the effect of artificial shading without leaf removal on TA and MA concentration. ND1L-FA had the highest value in terms of MA concentration compared to all other treatments (Figure 3), while TA results increased compared to TD-FA and ND-FA.

TD-FA had the lowest level of TA, while as regards MA concentration, this treatment gave similar results to TD2L-FA.

TD1L-FA had a lower pH level compared to other treatments, such as ND-FA, FD-FA and TD2L-FA and the results were similar to ND1L-FA.

4.1.2. Chardonnay

Figure 4 shows the average results obtained for for Chardonnay-FD during the 3-year period. Examining the results obtained for TSS, ND1L showed the lowest value compared to ND, TD1L and TD. In relation to TA and MA, ND1L had the highest value, although the TA results were only significant when compared to the TD-FD treatment. The pH showed similar values for TD1L-FD and ND1L-FD, while a difference was recorded between this treatment and TD1L-FD, TD-FD and ND-FD.

The results obtained for Chardonnay-FA are shown in Figure 5. ND1L-FA maintained a higher level of TA and FA, although this treatment was the last to be harvested in all the three years studied (Table 1). TD2L-FA did not preserve the acidic levels observed for the -FD

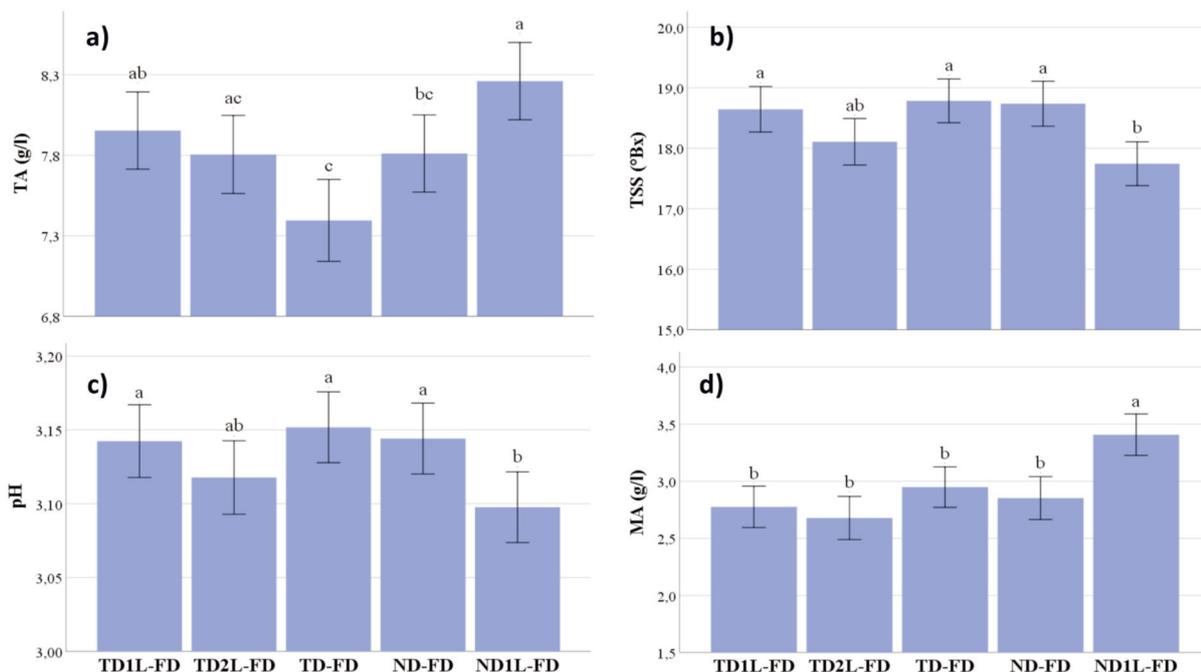


FIGURE 4. Average titratable acidity (TA) (a), total soluble solids (TSS) (b), pH (c) and malic acid content (MA) (d) for the 3-year period for Chardonnay-FD.

+/- 2 SE bar error is shown; letters above bars indicate statistical significance in the REGWF test ($P < 0.05$).

harvest; this is reflected by higher pH values compared to TD-FA, TD1L-FA and ND-FA.

4.2. Phenolic and thiol content in grapes

The average results obtained from the 3-year analysis of phenolics and thiols are shown in Table 4. The main results are described below.

4.2.1. Pinot noir

TD showed a higher content of total cinnamic acid and total flavonols compared to other treatments. Total defoliation led to an increase in Caftaric, Fertaric, Gallic and *Trans*-Coutaric acids, Quercetin, Quercetin 3-glucoside, Kaempferol-3-glucuronide, Isorhamnetin-3-glu, Kaempferol-3-glucoside, Syringetin-3-glucoside and Myricetin. Other treatments showed similar

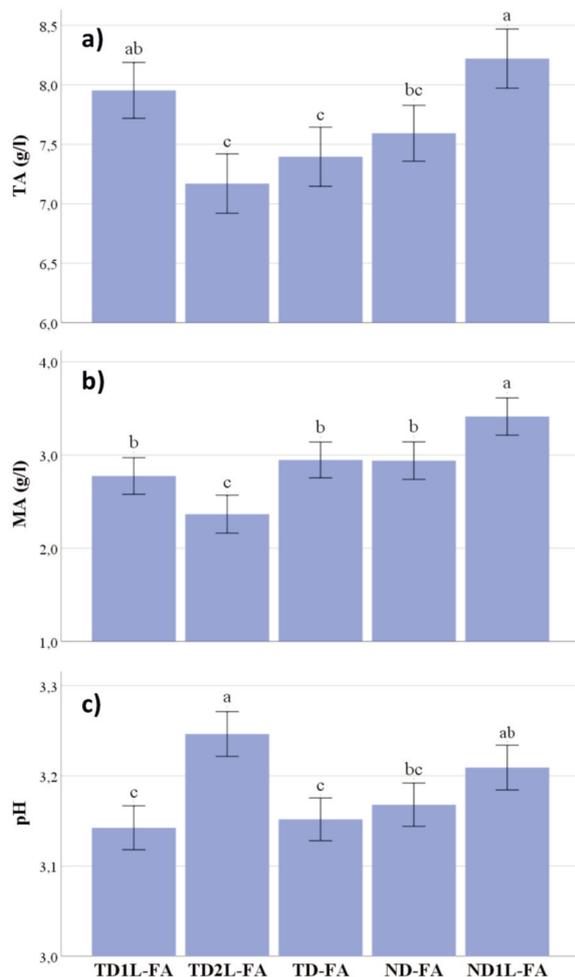


FIGURE 5. Average titratable acidity (TA) (a), malic acid content (MA) (b) and pH (c) for the 3-year period for Chardonnay-FA. +/- 2 SE bar error is shown; letters above bars indicate statistical significance in the REGWF test ($P < 0.05$).

results in terms of the total concentration of cinnamic acids and flavonols, while showing differences in the concentration of some specific phenolic compounds.

Flavanols did not show significant differences between treatments, while ND had higher values for total stilbenes compared to TD and ND1L. Total anthocyanins did not show any differences between the treatments, but some differences were observed for the pattern: peonidin-3-glucoside (PN-3-Glu) seemed to be enhanced by leaf presence (the highest values were recorded for ND and ND1L), while Delphinidin-3-glucoside (Dp-3-glu) increased with total defoliation.

High levels of shading (ND1L) led to an increase in S-glutathionylated precursor of 3-sulfanylhexasan-1-ol (GSH-3MH). This is reflected in the results obtained for total thiols.

4.2.2. Chardonnay

No significant differences were identified for harvesting carried out on “fixed dates” (-FD). Significant results were only shown for Quercetin-3-glucoside, Kaempferol-3-glucuronide, Isorhamnetin-3-glucoside and Kaempferol-3-Glucoside, for which the highest values were recorded for the TD-FD treatment compared to ND1L-FD.

The Chardonnay-FA harvest showed significant differences between treatments for total cinnamic and total flavonol concentration. Shaded treatments (TD1L, TD2L and ND1L) led to the highest values in terms of cinnamic acid concentration, while ND1L led to a lower level of flavonols. No significant differences were observed for stilbenes, while a higher level of total thiols was shown for the TD2L treatment compared to ND and TD1L. This was determined by differences identified for S-glutathionylated precursor of 3-Sulfanylhexasan-1-ol (GSH-3MH).

It is important to underline that, as shown in Table 1, both TD and TD1L were harvested on the same date, reaching the established value of 10.5 % potential alcohol at the same time corresponding to -FD harvest. For this reason, the phenolic and thiol results are the same as for -FD and -FA for these two treatments.

4.3. Relationship between microclimatic data and productivity and quality variables

PCA makes it possible to summarise multidimensional information defined by productivity and quality variables and microclimatic variables (GDD, NHH and HHH) in a lower-dimensional space. We selected 13 productivity and quality variables as quantitative parameters, (AWG, TSS, TA, MA, pH, total cinnamic acids, Quercetin-3-glucoside, Kaempferol-3-glucuronide, Isorhamnetin-3-glucoside, Kaempferol-3-glucoside, total flavonols, total flavanols and total stilbenes) on the basis of the results obtained from ANOVA, calculated for Chardonnay-FD. Considering the

eigenvalues (i.e., the percentage of overall variance explained by the principal components - PCs), we decided to carry out the analysis only with the first two PCs, preserving 68.8 % of overall dataset variability.

The biplot (Figure 6) shows that the score of observations on PC1 are positive in relation to total flavonols (specifically Kaempferol-3-glucuronide, Isorhamnetin-3-glucoside and Quercetin-3-glucoside), total stilbenes and HHH and negative in relation to TA, total flavanols, cinnamic acids, MA and NHH. Data scores on PC2 were positively correlated with total

TABLE 4. Average phenolic and thiol results obtained for the 3-year period.

Phenolics (mg/kg) and thiols (ug/ kg)	Chardonnay-FA					Chardonnay-FD					Pinot noir-FA				
	TD	ND	TD1L	TD2L	ND1L	TD	ND	TD1L	TD2L	ND1L	TD	ND	TD1L	TD2L	ND1L
Caftaric acid	96 ^b	102 ^b	119 ^a	124 ^a	118 ^a	96	99	119	114	110	158 ^a	134 ^b	124 ^b	125 ^b	126 ^b
Fertaric acid	1,60 ^b	1,57 ^b	1,70 ^b	2,06 ^a	1,74 ^{ab}	1,6	1,6	1,7	1,8	1,7	4,6 ^a	3,4 ^b	3,1 ^{bc}	3,7 ^b	2,6 ^c
Galic acid	5,9 ^b	3,2 ^c	3,5 ^c	10,3 ^{ab}	11,9 ^a	5,9	3,1	3,5	3	2,9	10,2 ^a	3,8 ^c	4,5 ^{bc}	6,2 ^b	3,7 ^c
t- Coutaric acid	16,9 ^c	20,8 ^{bc}	24,7 ^b	34,2 ^a	31,2 ^a	16,9	21,3	24,7	23,3	23,4	41,0 ^a	25,9 ^b	24,4 ^b	23,4 ^b	25,3 ^b
Ellagic acid	1,01	0,76	0,94	1,03	0,62	1,01	0,86	0,94	1,09	1,09	1,81	1,29	1,49	0,81	1,18
Total Cinnamic acids	121 ^b	128 ^b	150 ^a	172 ^a	164 ^a	121	126	150	143	139	216 ^a	169 ^b	158 ^b	160 ^b	159 ^b
Quercetin-3- glucuronide	10,2	10,9	11,2	13,8	9,1	10,2	10,5	11,2	12,1	11,8	16,9	12	15,2	12,6	12,3
dihydrokaempferol	0,47	0,4	0,32	0,43	0,36	0,47	0,4	0,32	0,35	0,19	0,49	0,34	0,38	0,48	0,44
Quercetin	0,035	0,035	0,03	0,042	0,03	0,035	0,035	0,03	0,031	0,026	0,086 ^a	0,051 ^{ab}	0,034 ^b	0,038 ^b	0,029 ^b
Quercetin-3-glu	20,7 ^a	11,8 ^{bc}	15,6 ^{ab}	17,8 ^{ab}	5,9 ^c	20,7 ^a	11,2 ^{bc}	15,6 ^{ab}	14,8 ^{ac}	7,5 ^c	19,9 ^a	7,2 ^c	11,7 ^b	7,7 ^{bc}	5,3 ^c
Kaempferol-3-glucuronide	0,41 ^a	0,24 ^{ab}	0,27 ^{ab}	0,29 ^{ab}	0,11 ^b	0,41 ^a	0,21 ^{ab}	0,27 ^{ab}	0,27 ^{ab}	0,16 ^b	0,42 ^a	0,13 ^{bc}	0,25 ^b	0,15 ^{bc}	0,11 ^c
Isorhamnetin-3-glu	0,54 ^a	0,32 ^{ab}	0,35 ^{ab}	0,37 ^a	0,14 ^b	0,54 ^a	0,33 ^{ab}	0,35 ^{ab}	0,50 ^a	0,14 ^b	3,9 ^a	1,8 ^c	2,9 ^b	2,0 ^c	1,4 ^c
Rutin	0,47	0,64	0,67	0,9	0,56	0,47	0,62	0,67	0,82	0,83	1,65	1,48	1,73	1,42	1,52
Kaempferol-3-glucoside	1,6	0,9	0,8	1,1	0,3	1,6 ^a	0,7 ^{ab}	0,8 ^{ab}	0,8 ^{ab}	0,4 ^b	0,5 ^a	0,2 ^b	0,3 ^b	0,1 ^b	0,1 ^b
Syringetin-3-glu	/	/	/	/	/	/	/	/	/	/	0,33 ^a	0,18 ^b	0,26 ^{ab}	0,18 ^b	0,21 ^{ab}
Myricetin	/	/	/	/	/	/	/	/	/	/	0,09 ^a	0,02 ^b	0,04 ^b	0,04 ^b	0,03 ^b
Total Flavonols	34,4 ^a	25,3 ^{ab}	29,3 ^a	34,8 ^a	16,6 ^b	34,4	24	29,3	29,7	21,1	44,3 ^a	23,3 ^b	32,7 ^b	24,7 ^b	21,5 ^b
Catechin	46,1	58,5	54,3	58,9	64,7	46,1	60,9	54,3	94,1	47,7	282	259	329	261	320
Epicatechin	54,8	40,9	60,6	39,2	66,5	54,8	50,4	60,6	47,5	42,9	105	127	149	149	167
Gallocatechin	1,9	1	1,3	2,1	2,3	1,9	2,2	1,3	2,3	1,9	2,6	2,7	2	1,4	4
B1	18,2 ^b	14,5 ^b	20,8 ^{ab}	41,3 ^a	31,5 ^{ab}	18,2	30,4	20,8	38	33,4	40,2	52,4	43,9	34,9	55,2
B2	25,3	19,7	32	26	33,3	25,3	24,5	32	28,6	23,9	99	76	73	93	104
Total Flavanols	146	134,6	169,1	167,6	198,4	146	168,3	169,1	210,5	149,6	530	518	597	539	650
trans- Piceide	0,31	0,53	0,42	0,54	0,43	0,31	0,54	0,42	0,52	0,47	1,54 ^{ab}	1,86 ^{ab}	1,33 ^b	2,03 ^a	1,51 ^b
cis- Piceide	0,44	0,51	0,49	0,48	0,41	0,44	0,52	0,49	0,76	0,44	2,6 ^b	4,4 ^a	3,3 ^{ab}	4,0 ^{ab}	3,0 ^b
Total Stilbenes	0,75	1,05	0,91	1,02	0,83	0,75	1,06	0,91	1,29	0,91	4,09 ^b	6,23 ^a	4,67 ^{ab}	6,05 ^{ab}	4,55 ^b
MV-3-glu	/	/	/	/	/	/	/	/	/	/	379	333	339	333	342
CN-3-glu	/	/	/	/	/	/	/	/	/	/	10,2	9,2	7,7	7,9	9,1
PN-3-glu	/	/	/	/	/	/	/	/	/	/	167 ^c	204 ^a	168 ^{bc}	200 ^{ab}	224 ^a
DP-3-glu	/	/	/	/	/	/	/	/	/	/	122 ^a	81 ^b	88 ^b	67 ^b	81 ^b
PT-3-glu	/	/	/	/	/	/	/	/	/	/	55,7	41,7	45,1	42,2	45,2
Total Anthocyanins	/	/	/	/	/	/	/	/	/	/	734	670	648	650	701
CYS-3MH	7,2	4,1	7,8	11,9	12,5	7,2	10,2	7,8	12,1	6,3	27,6	28,2	12,7	25	21,5
GSH-3MH	81 ^{ab}	66 ^b	53 ^b	115 ^a	80 ^{ab}	81	83	53	66	59	266 ^{ab}	190 ^b	234 ^{ab}	216 ^b	304 ^a
Total Thiols	88 ^{ab}	70 ^b	61 ^b	126 ^a	92 ^{ab}	88	93	61	78	65	294 ^{ab}	219 ^b	247 ^{ab}	241 ^b	326 ^a

Different letters indicate significant differences in the REGWF test ($P < 0.05$); where no letters are present no significant differences were found.

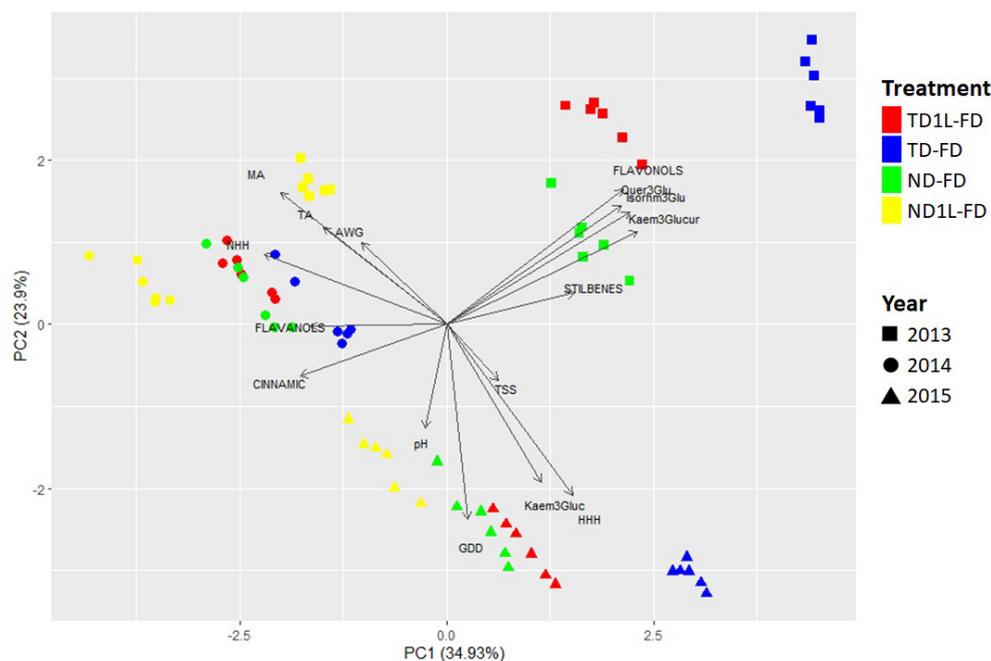


FIGURE 6. Biplot of principal component analysis of treatment parameters and microclimatic variables for Chardonnay-FD data.

Observations are plotted in a new 2-dimensional space, which is defined by the x-axis that represents the first principal component (PC1) explaining 34.9 % of overall data variance and the y-axis that is the second principal component (PC2) explaining 24.9 % of overall data variance. The points are the observations, coloured according to the treatment and differentiated by shape according to the year of observation. Arrows represent the direction of the variables, as projected onto the 2-d plane of the biplot.

flavonols, MA and Quercetin-3-glucoside and negatively correlated with Kaempferol-3-glucoside, HHH, GDD. In the two-dimensional space, the data are grouped into three sets, which represent the three survey years. Within the same year, the 4 treatments were well discriminated, always maintaining the same order according to the values on the y axis. Namely, within the same year, the observations referred to as treatment TD-FD always had PC1 scores higher than those of treatment TD1L-FD, which in turn had higher values than treatment ND-FD. Finally, the observations of treatment ND1L-FD always had PC1 scores lower than the other treatments.

This makes it possible to visually discriminate different treatment behaviour and differences between years.

Table 5 shows the correlation matrix of microclimatic data and productivity and quality variables selected for PCA. GDD and HHH was positively correlated ($\rho=0.673$), while there was a high negative correlation between NHH and HHH ($\rho=-0.859$). Kaempferol-3-glucoside was positively correlated with GDD ($\rho=0.680$) and

HHH ($\rho=0.707$). NHH was positively correlated with MA ($\rho=0.804$) and cinnamic acids ($\rho=0.684$). MA was highly negatively correlated with HHH ($\rho=-0.844$).

DISCUSSION

The effects of defoliation and shading on grape quality have been demonstrated by different authors, but the relationships between berry temperature and metabolism have not yet been fully understood. The positive effect of shading on acidity preservation is particularly interesting for sparkling wine production, because acidity is one of the most important sensory characteristics (Ribéreau-Gayon *et al.*, 2000). Grape shading can also mitigate the current problem of ripening anticipation caused by increasing temperature related to global warming (Schultz, 2000; Jones *et al.*, 2005).

This paper presents results regarding the effects of different levels of defoliation and shading. Micrometeorological characterisation was carried out by monitoring berry temperature variability in different exposure conditions. Differences among years emerged, with 2014

TABLE 5. Correlation matrix of microclimatic data and productivity and quality variables selected for PCA (n=72).

	AWG	TSS	pH	TA	MA	CINNAMIC	Quer3Glu	Kaem3Glucor	Isorhm3Glu	Kaem3Gluc	FLAVONOLS	FLAVANOLS	STILBENES	GDD	HHH	NHH
AWG	1.000															
TSS	-0.107	1.000														
pH	-0.120	0.288	1.000													
TA	0.274	-0.201	-0.212	1.000												
MA	0.437	-0.417	-0.058	0.631	1.000											
CINNAMIC	0.354	0.027	0.298	0.233	0.418	1.000										
Quer3Glu	-0.055	0.047	0.073	-0.181	-0.232	-0.309	1.000									
Kaem3Glucor	-0.161	0.031	0.069	-0.367	-0.368	-0.360	0.957	1.000								
Isorhm3Glu	-0.115	0.053	0.057	-0.248	-0.300	-0.375	0.981	0.937	1.000							
Kaem3Gluc	-0.325	0.183	0.213	-0.472	-0.626	0.053	0.078	0.294	0.051	1.000						
FLAVONOLS	-0.029	0.023	-0.069	-0.179	-0.214	-0.357	0.965	0.939	0.952	0.059	1.000					
FLAVANOLS	0.215	-0.278	0.192	0.161	0.494	0.238	-0.374	-0.349	-0.468	-0.155	-0.475	1.000				
STILBENES	-0.326	0.048	-0.032	-0.276	-0.399	-0.655	0.349	0.466	0.371	0.250	0.450	-0.270	1.000			
GDD	-0.315	0.291	0.476	-0.249	-0.497	0.166	-0.159	-0.063	-0.185	0.680	-0.326	0.092	-0.185	1.000		
HHH	-0.456	0.239	0.057	-0.467	-0.844	-0.422	0.025	0.169	0.075	0.707	-0.007	-0.370	0.330	0.673	1.000	
NHH	0.407	-0.148	0.202	0.474	0.804	0.684	-0.292	-0.416	-0.366	-0.516	-0.354	0.596	-0.594	-0.223	-0.859	1.000

Coloured boxes indicate correlation higher than 0.6 (green) and lower than -0.6 (red).

identified as the most balanced season, with high NHH values and low HHH values. The high thermal levels in 2015 (highest GDD values) were translated into high HHH levels and lower NHH levels. 2013 was in an intermediate position. PCA effectively discriminated the differences among years, while the correlation matrix revealed a positive relationship between GDD and HHH and a negative relationship between NHH and HHH. This is in agreement with the definition of GDD, HHH and NHH (Amerine and Winkler, 1944; Cola *et al.*, 2020), which associates increases in temperatures with an HHH increase at the expense of NHH. The good correlation showed by NHH and HHH regarding the ripening process suggests that the adopted response curve can provide a synthetic representation of the process.

Total defoliation (TD) was associated with a higher level of GDD and HHH and a lower level of NHH, while a higher level of shading (ND1L) gave the opposite result.

This caused a delay in ripening observed in the ND1L treatment for both analysed cultivars and

was further confirmed by the analytical results obtained from Chardonnay-FD (ND1L low TSS and pH values and high level of TA and MA). The positive effect of shading on delaying ripening is in agreement with conclusions drawn by other authors (Rojas-Lara and Morrison, 1989; Percival *et al.*, 1994; Filippetti *et al.*, 2014; Martin *et al.*, 2016) and is reflected in must and grape quality. In the correlation analysis, NHH was shown to be positively related to malic acid concentration (MA), while this variable was negatively associated with HHH. This is confirmed by the results obtained from treatment comparisons, where MA was higher for the ND1L treatment, both for Pinot noir and Chardonnay. These positive effects of shading on malic acid preservation have already been described in previous studies (Lakso and Kliever, 1978; Dokoozlian and Kliever, 1996; Conde *et al.*, 2007; Martin *et al.*, 2016; de Oliveira *et al.*, 2019). Titratable acidity (TA) followed the same behaviour as MA, showing a higher concentration in the ND1L treatment compared to the TD treatment. The better conservation of the acidic component observed for high level of shading is further confirmed by

the fact that this treatment was the last to be harvested in all three years of the study and can be supported by further studies (Reynolds *et al.*, 1986; Smart *et al.*, 2017).

The results obtained for pH showed that TD1L-FA had the lowest value both for Chardonnay and Pinot noir, while other treatments showed differing behaviour between cultivars, in agreement with other studies that have reported that shading does not significantly affect this parameter (Filippetti *et al.*, 2014).

Total yield and bunch weight showed opposite behaviour between Chardonnay-FA and Pinot noir-FA when comparing TD and ND, which is in agreement with literature that reported contrasting results with regards to the effects of leaf removal on berry size (Lemut *et al.*, 2011). In particular, the average bunch weight of Pinot noir was more affected by defoliation without shading, while in the case of Chardonnay, bunch weight seems to have been affected by the timing of the harvest: ND1L recorded the highest value for harvest at a fixed date (-FD), but this value decreased at harvesting at fixed alcohol -FA. This can probably be related to the delay in ripening associated with ND1L, which showed a delay in -FA harvest compare to -FD harvest in all the three years observed. This delay lead to a decreased in bunch weight; this reduction does not seem to indicate cause the phenomena of acid concentration the phenomena of acid concentration, as the average levels of malic acid and titratable acidity remained constant between -FD and -FA harvest for this treatment and an increase in average pH level was recorded.

The positive effect of temperature increase on flavonol accumulation (Downey *et al.*, 2004; Spayd *et al.*, 2002) can be determined from both the correlation and PCA and from the differences recorded in treatments, in which higher flavonol values were obtained in the TD-FA treatment both for Pinot noir and Chardonnay. These results confirm the findings of other previous studies on the relationship between flavonol concentration and sunlight exposure (Spayd *et al.*, 2002; Cortell and Kennedy 2006; Reshef *et al.*, 2017). The intensity of light may thus have influenced the development of quercetin-3-glucoside in particular, as demonstrated by another study by Price *et al.* (1995).

Cinnamic acids were shown to be positively related to NHH and had the opposite behaviour

in Pinot noir (positive effect of defoliation on the concentration of cinnamic acids) compared to Chardonnay (positive effect of shading in cinnamic acid concentration). Chardonnay displayed the opposite effect of irradiation to that observed for flavonols, as already observed by Rehsef *et al.* (2017).

The effects of shading and, consequently, the delaying of harvest time observed for the shaded treatments did not have a major impact on grape stilbene concentration.

The behaviour of thiols was not clearly defined, as it differed between cultivars. ND was the only treatment maintaining lower values both for Chardonnay and Pinot noir.

CONCLUSIONS

This study was carried out over the three-year period of 2013-2015. From the results obtained it was possible to underline the effect of shading on the composition of Pinot noir and Chardonnay grapes. Specifically, leaf shading combined with artificial shading (ND1L) had repercussions on ripening, slightly delaying maturation and maintaining a higher level of acidity. These characteristics in must are important in the context of sparkling wine production, because conservation of sparkling wine depends mostly on acidic composition. In terms of polyphenolic composition, total defoliation led to a higher concentration of flavonols and reduced the concentration of hydroxycinnamates in the berries, in accordance with other studies carried out on exposed grapes. ND1L also seemed to reduce flavonol content, confirming the effect of this type of shading on grape characteristics. These latter results suggest that the shading net afforded additional effective protection from irradiation, which was not entirely expected, considering that clusters are protected by several layers of leaves in the canopy, each one capable of absorbing 60–70 % of visible wavelengths (Schultz, 1996). These results also seem to be related to berry temperature, which was highest for the TD treatment and lowest for ND1L, both in terms of GDD and HHH. The knowledge gained from this study could be useful for the wine production sector in terms of adapting defoliation and shading interventions to the meteorological conditions of a specific season. In particular, the effects of shading - delaying ripening, preserving acid concentration and reducing flavonol

content - is relevant in relation to Franciacorta-specific oenological issues: indeed, sparkling wines need to be made with highly acidic grapes (in terms of high titratable acidity and malic acid content and low pH), such grapes being particularly affected by problems resulting from the climate change now underway.

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REFERENCES

- Abeysinghe, S.K., Greer, D.H., Rogiers, S.Y. (2019). The effect of light intensity and temperature on berry growth and sugar accumulation in *Vitis vinifera* ‘Shiraz’ under vineyard conditions. *Vitis*, 58, 7–16. doi: 10.5073/vitis.2019.58.7-16
- Amerine, M., & Winkler, A. (1944). Composition and Quality of Musts and Wines of California Grapes. *Hilgardia*, 15(6), 493-675. doi: 10.3733/hilg.v15n06p493
- Bergqvist, J., Dokoozlian, N., & Ebisuda, N. (2001). Sunlight exposure and temperature effects on berry growth and composition of Cabernet-Sauvignon and Grenache in the Central San Joaquin Valley of California. *American Journal of Enology and Viticulture*, 52, 1-7.
- Cola G., Failla O., & Mariani, L. (2009). BerryTone - A simulation model for the daily course of grape berry temperature. *Agricultural and Forest Meteorology*, 149, 1215–1228. doi:10.1016/j.agrformet.2009.01.007
- Cola, G., Mariani, L., Salinari, F., Civardi, S., Bernizzoni, F., Gatti, M., & Poni, S. (2014). Description and testing of a weather-based model for predicting phenology, canopy development and source–sink balance in *Vitis vinifera* L. cv. Barbera. *Agricultural and Forest Meteorology*, 184, 117-136. doi: 10.1016/j.agrformet.2013.09.008
- Cola G., Mariani L., Maghradze D., & Failla, O. (2020). Changes in thermal resources and limitations for Georgian viticulture. *Australian Journal of Grape and Wine Research*, 26(1), 29-40. doi:10.1111/ajgw.12412
- Cortell, J., & Kennedy, J. (2006). Effect of shading on accumulation of flavonoid compounds in (*Vitis vinifera* L.) Pinot noir fruit and extraction in a model system. *Journal of Agricultural And Food Chemistry*, 54(22), 8510-8520. doi: 10.1021/jf0616560
- Crippen, D., & Morrison, J. (1986). The effects of sun exposure on the compositional development of Cabernet-Sauvignon berries. *American Journal of Enology and Viticulture*, 37, 235-242.
- Conde, C., Silva, P., Fontes, N., Dias, A., Tavares, R., Sousa, M.,...Gerós, H.(2007). Biochemical changes throughout grape berry development and fruit and wine quality. *Food*, 1, 1–22.
- de Oliveira, J. B, Egipto, R., Laureano, O., de Castro, R., Pereira, G. E., Ricardo-da-Silva J. M. (2019). Climate effects on physicochemical composition of Syrah grapes at low and high altitude sites from tropical grown regions of Brazil. *Food Research International*, 121, 870-879. doi:10.1016/j.foodres. 2019.01.011
- Dokoozlian, N., Kliewer, W. (1996). Influence of light on grape berry growth and composition varies during fruit development. *Journal of the American Society for Horticultural Science*, 121(5), 869-874. doi:10.21273/jashs.121.5.869
- Downey, M.O., Harvey, J.H., Robinson, S.P. (2003). Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. *Australian Journal of Grape and Wine Research*, 9(1), 15–27. doi:10.1111/j.1755-0238.2003.tb00228.x
- Downey, M.O., Harvey, J.S., Robinson, S.P. (2004). The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Australian Journal of Grape and Wine Research*, 10(1), 55–73. doi:10.1111/j.1755-0238.2004.tb00008.x
- Downey, M., Dokoozlian, N., Krstic, M. (2006). Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. *American Journal of Enology and Viticulture*, 57, 257-268.
- Fernandes de Oliveira, A., Mercenaro, L., Del Caro, A., Pretti, L., Nieddu, G. (2015). Distinctive anthocyanin accumulation responses to temperature and natural UV radiation of two field-grown *Vitis vinifera* L. Cultivars. *Molecules*, 20(2), 2061-2080. doi: 10.3390/molecules20022061
- Filippetti, I., Movahed, N., Allegro, G., Valentini, G., Pastore, C., Colucci, E. and Intriери, C. (2014). Effect of post-veraison source limitation on the accumulation of sugar, anthocyanins and seed tannins in *Vitis vinifera* cv. Sangiovese berries. *Australian Journal of Grape and Wine Research*, 21(1), 90-100. doi: 10.1111/ajgw.12115
- Greer, H. (2017). Temperature and CO₂ dependency of the photosynthetic photon flux density responses of leaves of *Vitis vinifera* cvs. Chardonnay and Merlot grown in a hot climate. *Plant Physiology and Biochemistry*, 111, 295-303. doi:10.1016/j.plaphy. 2016.12.015
- Greer, D.H., Weedon, M.M. (2014). Temperature-dependent responses of the berry developmental

- processes of three grapevine (*Vitis vinifera*) cultivars, New Zealand. *Journal of Crop and Horticultural Science*, 42(4), 233-246. doi:10.1080/01140671.2014.894921
- Greer, D.H., Weedon, M.M. (2012). Modelling photosynthetic responses to temperature of grapevine (*Vitis vinifera* cv. Semillon) leaves on vines grown in a hot climate. *Plant, Cell and Environment*, 35, 1050–1064. doi:10.1111/j.1365-3040.2011.02471.x
- Hale, C., Buttrose, M. (1974). Effect of temperature on ontogeny of berries of *Vitis vinifera* L. cv. Cabernet-Sauvignon. *Journal of The American Society For Horticultural Science*, 99(5), 390-394.
- Hall, A., & Jones, G. (2009). Effect of potential atmospheric warming on temperature-based indices describing Australian winegrape growing conditions. *Australian Journal Of Grape And Wine Research*, 15(2), 97-119. doi: 10.1111/j.1755-0238.2008.00035.x
- Haselgrove, L., Botting, D., Van Heeswijck, R., Høj, P.B., Dry, P.R., Ford, C., Iland, P.G., 2000. Canopy microclimate and berry composition: the effect of bunch exposure on the phenolics composition of *Vitis vinifera* L cv. Shiraz grape berries. *Australian Journal of Grape and Wine Research*, 6(2), 141–149. doi:10.1111/j.1755-0238.2000.tb00173.x
- Jackson, D., & Lombard, P. (1993). Environmental and management practices affecting grape composition and wine quality - A review. *American Journal of Enology and Viticulture*, 44, 409-430.
- Jones, G., Duchêne, E., Tomasi, D., Yuste, J., Braslavka, O. and Schultz, H. *et al.* (2005). Change in European winegrape phenology and relationship with climate. In *XIV International GESCO Viticulture Congress* (pp. 54-61). Geisenheim: Groupe d'Étude des Systèmes de Conduite de la vigne (GESCO).
- Kliewer, W., & Lider, L. (1968). Influence of cluster exposure to the sun on the composition of Thompson seedless fruit. *American Journal of Enology and Viticulture*, 19, 175-184.
- Kliewer, W., & Torres, R. (1972). Effect of controlled day and night temperatures on grape coloration. *American Journal of Enology and Viticulture*, 23, 71-77.
- Kuhn, N., Guan, L., Dai, Z.W., Wu, B.H., Lauvergeat, V., Gomès, E., Li, S.H., Godoy, F., Arce-Johnson, P. & Delrot, S. (2014). Berry ripening: recently heard through the grapevine. *Journal of Experimental Botany*, 65(16), 4543–4559. doi:10.1093/jxb/ert395 Advance Access publication 27 November, 2013
- Lakso, A., Kliewer, W. (1978). The influence of temperature on malic acid metabolism in grape berries. ii. temperature responses of net dark CO₂ fixation and malic acid pools. *American Journal of Enology and Viticulture*, 28, 145-149.
- Lecourieux, F., Kappel, C., Pieri, P., Charon, J., Pillet, J., Hilbert, G., Renaud, C., Gomès, E., Delrot, S., Lecourieux, D. (2017). Dissecting the biochemical and transcriptomic effects of a locally applied heat treatment on developing Cabernet-Sauvignon grape berries. *Frontiers in Plant Science*, 8:53. doi: 10.3389/fpls.2017.00053
- Lemut, M.S., Trost, K., Sivilotti, P., Vrhovsek, U. (2011). Pinot noir grape colour related phenolics as affected by leaf removal treatments in the Vipava Valley. *Journal of Food Composition and Analysis*, 24(6), 777-784. doi:10.1016/j.jfca.2011.03.003
- Mariani, L., Parisi, S.G., Cola, G., Failla, O. (2012). Climate change in Europe and effects on thermal resources for crops. *International Journal of Biometeorology*, 56, 1123-1134, doi: 10.1007/s00484-012-0528-8
- Mariani, L., Alilla, R., Cola, G., Monte, G., Epifani, C., Puppi, G., Osvaldo, F. (2013). IPHEN - a real-time network for phenological monitoring and modelling in Italy. *International Journal of Biometeorology*, 57(6), 881-893. doi: 10.1007/s00484-012-0615-x
- Martin, D., Grose, C., Fedrizzi, B., Stuart, L., Albright, A., McLachlan A. (2016). Grape cluster microclimate influences the aroma composition of Sauvignon blanc wine. *Food Chemistry*, 210, 640-647. doi:10.1016/j.foodchem.2016.05.010
- Martinez de Toda, F., & Balda, P. (2014). Reducing the pH of wine by increasing grape sunlight exposure: A method to mitigate the effects of climate warming. *Vitis -Geilweilerhof-*, 53, 17-20.
- Mori, K., Saito, H., Goto-Yamamoto, N., Kitayama, M., Kobayashi, S., Sugaya, S., Gemma, H., Hashizume, K., 2005. Effects of abscisic acid treatment and night temperatures on anthocyanin composition in Pinot noir grapes. *Vitis*, 44(4), 161–165.
- Pastor del Rio, J., & Kennedy, J. (2006). Development of proanthocyanidins in *Vitis vinifera* L. cv. Pinot noir grapes and extraction into wine. *American Journal of Enology and Viticulture*, 57, 125-132.
- Percival, D., Fisher, K. and Sullivan, J. (1994). Use of fruit zone leaf removal with *Vitis vinifera* L. cv. Riesling Grapevines. II. Effect on fruit composition, yield and occurrence of bunch rot (*Botrytis cinerea* Pers.:Fr.). *American Journal of Enology and Viticulture*, 45, 133-140.
- Price, S., Breen, P., Valladao, M. and Watson, B. (1995). Cluster sun exposure and quercetin in Pinot noir grapes and wine. *American Journal of Enology and Viticulture*, 46, 187-194.
- Reshef, N., Walbaum, N., Agam, N. and Fait, A. (2017). Sunlight modulates fruit metabolic profile and shapes the spatial pattern of compound accumulation

- within the grape cluster. *Frontiers In Plant Science*, 8(70), 1-20. doi: 10.3389/fpls.2017.00070
- Reynolds, A., Pool, R. and Mattick, R. (1986). Influence of cluster exposure on fruit composition and wine quality of Seyval blanc grapes. *Vitis*, 25, 85-95.
- Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., & Lonvaud A. (2000). Handbook of Enology, The Microbiology of Wine and Vinifications, vol. I, Wiley, West Sussex, England.
- Rojas-Lara, B., & Morrison, J. (1989). Differential effects of shading fruit or foliage on the development and composition of grape berries. *Vitis*, 28, 199-20.
- Scafidi, P., Pisciotta, A., Patti, D., Tamborra, P., Lorenzo, R. Barbagallo, M. (2013). Effect of artificial shading on the tannin accumulation and aromatic composition of the Grillo cultivar (*Vitis vinifera* L.). *BMC plant biology*, 13:175. doi: 10.1186/1471-2229-13-175.
- Schultz, H. (1996). Leaf absorptance of visible radiation in *Vitis vinifera* L.: estimates of age and shade effects with a simple field method. *Scientia Horticulturae*, 66, 93- 102. doi:10.1016/0304-4238(96)00876-X
- Schultz, H. (2000). Climate change and viticulture: A European perspective on climatology, carbon dioxide and UV-B effects. *Australian Journal of Grape and wine Research*, 6(1), 2-12. doi: 10.1111/j.1755-0238.2000.tb00156.x
- Smart, R., & Sinclair, T. (1976). Solar heating of grape berries and other spherical fruits. *Agricultural Meteorology*, 17(4), 241-259. doi: 10.1016/0002-1571(76)90029-7
- Smart, R., Dick, J., Gravett, I. and Fisher, B. (2017). Canopy management to improve grape yield and wine quality - principles and practices. *South African Journal Of Enology and Viticulture*, 11(1), 3-17. doi: 10.21548/11-1-2232
- Smart, R., Robinson, J., Duen, G. and Brein, C. (1985). Canopy microclimate modification for the cultivar Shiraz. II. Effects on must and wine composition. *Vitis*, 24, 119-128.
- Spayd, S., Tarara, J., Mee, D. and Ferguson, J. (2002). Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *American Journal of Enology and Viticulture*, 53, 171-182.
- Vrhovsek, U., Masuero, D., Gasperotti, M., Franceschi, P., Caputi, L., Viola, R. and Mattivi, F. (2012). A Versatile Targeted Metabolomics Method for the Rapid Quantification of Multiple Classes of Phenolics in Fruits and Beverages. *Journal of Agricultural and Food Chemistry*, 60(36), 8831-8840. doi: 10.1021/jf2051569
- Webb, L., Whetton, P. and Barlow, E. (2007). Modelled impact of future climate change on the phenology of winegrapes in Australia. *Australian Journal Of Grape And Wine Research*, 13(3), 165-175. doi: 10.1111/j.1755-02
- World Meteorological Organization (2009). Guide to climatological practices—WMO 100. 3d ed. (World Meteorological Organization: Geneva, Switzerland).
- Wu, J., Drappier, J., Hilbert, G., Guillaumie, S., Dai, Z., Geny, L., Delrot, S., Darriet, P., Thibon, C., Pieri, P. (2019). The effects of a moderate grape temperature increase on berry secondary metabolites. *OENO One*, 53(2). doi:10.20870/oeno-one.2019.53.2.2434