Seasonal differences in *Vitis vinifera* L. cv. Pinot noir fruit and wine quality in relation to climate

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**Aims:** A better understanding of the relationship between weather conditions and wine quality would provide tools for assessing the impact of climate change and the potential for adaptation. Most studies rely on assessing wine quality by the price per bottle or by an overall ranking and then establishing general relations to weather conditions. However, such an approach may imply the addition of bias by variable winemaking techniques overcoming vintage effects. The aim of our study was therefore to implement a controlled conditions approach using grape samples from a single vineyard and a standardized micro-scale winemaking technique to produce wines in similar conditions for each vintage over more than a decade. We hope that this data will allow new insights into responses to climatic differences.

**Methods and results:** From 2005 to 2015, data was collected from a vineyard of Hochschule Geisenheim University planted with *Vitis vinifera* L. cv. Pinot noir grafted on rootstock SO4 in four field replicates. Weather conditions were recorded together with the major phenological stages, yield, infection of the bunches by *Botrytis cinerea* bunch rot, and pruning weight. Key primary juice compounds were analyzed and berry phenolics in skins and seeds were determined before harvest. Micro-scale winemaking was developed to produce wines in standardized conditions. The repeatability of the method to assess the extraction of anthocyanins and tannins was shown to be 2–10% and 8–12%, respectively, depending on grape maturity stage. Sugar accumulation was coupled to warmer conditions during the maturation period, and high temperatures after *véraison* decreased the concentration of malic acid in the juice. The accumulation of primary amino acids (N-OPA) in the juices seemed positively related to warmer conditions between bud break and flowering. Increased temperature, especially before *véraison*, accompanied by a lack of precipitation was related to an accumulation of tannins in fruit and wine, with a higher accumulation in skins than seeds. The temperature-sensitive anthocyanin accumulation in grapes was coupled to warmer conditions after *véraison*. These differences in anthocyanin concentration could also be observed in the wine.

**Conclusions:** High-quality vintages were linked to warmer than normal growing seasons and below normal precipitation.

**Significance and impact of the study:** The use of a micro-scale winemaking technique represents an innovative tool to provide detailed information in a controlled and reproducible way. A better understanding of the interaction between weather conditions and berry/wine compounds will help with developing improved winemaking techniques and better adapting to future impacts of climate change.

**KEYWORDS**
climate change, tannins, anthocyanins, water deficit, micro-scale winemaking
INTRODUCTION

Many studies have linked increases in temperature to earlier phenological events in grape berry development, with the potential to greatly affect fruit and wine characteristics (Duchêne and Schneider, 2005; Petrie and Sadras, 2008; Webb et al., 2011). A sound understanding of the climatic contribution to wine quality would provide tools for assessing the future impact of climate change on vintage quality. The price of a bottle of wine is used generally as an indicator for wine quality (Ashenfelter et al., 1995; Jones and Storchmann, 2001) or an overall rank of each vintage between wine reviewers (Jones and Goodrich, 2008) or as a consensus ranking (Baciocco et al., 2014), with all the bias it could imply. From the literature review, a hypothesis was proposed that high-quality vintages were linked to warmer than normal growing seasons with high heat accumulation and below normal precipitation, as observed for American Viticultural Areas (Jones et al., 2005; White et al., 2006; Nicholas et al., 2011). In this study, we used data gathered under controlled conditions from a single vineyard for the analysis of grape berry phenolics, and a micro-scale winemaking technique to produce wines in similar conditions for each vintage over 11 years. The aim of the study was to link the impact of seasonal weather conditions to berry composition, i.e. phenolics in skins and seeds, followed by analysis of the wine produced using a standardized micro-scale winemaking approach for all vintages.

MATERIALS AND METHODS

1. Growth conditions and experimental setup

A rootstock trial was established in 2003 at Hochschule Geisenheim University in the Rheingau Region (Germany; 49°98’77.9»N 7°93’98.5»E) (Blank et al., 2018). Vitis vinifera L. cv. Pinot Noir, clone Gm1-1 grafted on Selection Oppenheim 4 (SO4) rootstock was used for the purpose of the study, which covered a period of 11 years from 2005 to 2015. Four replicates of 14 vines in a randomized block design were selected. Temperature (mean, max, min, in °C), precipitations (mm), wind speed (ms⁻¹) at two heights (cm) and solar radiation (h) were recorded daily by a station located approximately 400 m away from the site of the experiment. The heat summation of growing degree days from the first day of the year (DOY) was calculated on a 10 °C basis (ΣGDD₁₀°C). Reference evapotranspiration (ET₀) was estimated using the FAO-56 Penman-Monteith equation (Allen et al., 1998). The major phenological stages were assessed using the BBCH system (Lorenz et al., 1994).

2. Modeling of vine and soil water balance of a vintage

Soil water balance was calculated according to the model of Hofmann et al. (2014) which is based on the model of Lebon et al. (2003), accounting for the presence of cover crop (Celette et al., 2010). Soil water holding capacity (SWC) was relatively low for this vineyard (125–150 mm; Löhnhertz et al., 2004). The fraction of transpirable soil water (FTSW) was calculated to quantify plant water deficit, and the vine predawn leaf water potential (ψpd) was modeled according to the relationship between ψpd and FTSW (Lebon et al., 2003; Gruber and Schultz 2005).

3. Bunch rot estimation through Botrytis cinerea

An estimate of the level of Botrytis cinerea bunch rot was determined visually on 100 bunches per replicate according to a scoring system with seven classes. From this scoring, disease incidence was calculated as the number of bunches that were infected out of all that were assessed, and disease severity was calculated as the intensity of disease affecting the bunches.

4. Description of vigor induced by the rootstock

The mass of wood removed during pruning (pruning mass) and the mass of grapes harvested (fruit yield) were recorded for each of the four replicates.

5. Yield juice primary compounds

At harvest, five bunches per replicate were selected to determine primary juice compounds as total soluble solids (TSS; °Brix), pH and titratable acidity, malic and tartaric acid concentration (by FTIR, FT2 WineScan Flex) and the concentration of primary amino acid N-OPA (Dukes and Butzke, 1998).

6. Grape skin and seed extracts and winemaking

At harvest, 100 berry samples (50 berries per canopy side) were collected from each field.
replicate around 21°Brix and frozen at -20 °C. A 20-berry sub-sample was later selected for the analysis of phenolic compounds and the remaining 80-berry sub-sample was used for micro-scale winemaking as described elsewhere (Lafontaine et al., 2011). Grape skin and seeds were analyzed separately according to the method of Harbertson et al. (2002) for phenolics in grape berries. For the micro-scale winemaking, fermentors were created from 250 mL jars and lids with air-locks, with a stainless-steel screen to plunge down the pomace. The wine was fermented for six days on skins and the pomace was pressed after a two-week post-fermentation period.

6. Phenolics in grapes and wines

The analysis of tannins was performed on skin/seed extracts or wine samples according to Harbertson et al. (2002) with a bovine serum albumin (BSA) protein solution. All measurements were performed with a VIS spectrophotometer (Odyssey, Hach Lange GmbH, Düsseldorf, Germany). Tannin concentration was calculated as mg CE (catechin equivalent) using a standard curve. For the analysis of anthocyanins, skin extracts or wine samples were analyzed according to Heredia et al., (2006). Anthocyanin concentrations were calculated as Malvidin-3-O-glucoside (M3OG) equivalents.

7. Statistical analysis

The open source R 3.3.1 statistical computing environment (R Foundation for Statistical Computing, Vienna, Austria) was used for all ANOVA and graphs with the platform Eclipse Mars Release (4.5.2) (Eclipse Foundation, Ottawa, ON, Canada) and its plug-in StatET 3.5 (WalWare-Team, Dortmund, Germany). Differences between the seasons means were compared using the Tukey HSD test (P=0.01 or P=0.05). Multiple linear regressions (MLR) and model reduction through a stepwise selection of predictor variables (forward, backward and both) were conducted using the R package MASS (Venables and Ripley, 2002) and its function stepAIC. The R package FactoMineR (Lê et al., 2008) was used for principal component analysis (PCA) on variables scaled to unit variance.

RESULTS

1. Climatic conditions and phenology

Bud break date (usually April 23) was 13 days earlier in 2011 and 2014. The Grapevine Flowering Véraison (GFV) model (Parker et al., 2011) could predict the flowering date (R²=0.755) with a root-mean-square error (RMSE) of 4.65 days. The sums of growing degree days (ΣGDD 10°C) and precipitations were calculated according to the specific stages of development. The water deficit of the plant was quantified as the modeled daily predawn water deficit (ψpd) during vine

FIGURE 1. Modeled predawn water deficit (ψpd).
Vine predawn leaf water potential (ψpd) modeled from the fraction of transpirable soil water (FTSW) (Lebon et al., 2003; Gruber and Schultz 2005) for the periods of (a) bud break (Bb) to flowering (Fl) ψpdBb-Fl, (b) flowering (Fl) to véraison (V) ψpdFl-V, and (c) ψpdV-Hvéraison (V) to harvest (H) ψpdV-H. A ψpd of -0.6 MPa was retained as the limit for severe stress (Hofmann and Schultz, 2015). The central box in each box plot indicates the interquartile range and the bold line indicates the median; whiskers indicate the 10th and 90th percentiles of the daily ψpd. The mean is marked with a square.
development (Figure 1). All vintages were above a ψpd of -0.6 MPa during bud break and flowering (Figure 1a) but water deficit was high for the 2006 season during the period of flowering to véraison (Figure 1b). Conversely, water deficit was high during the period véraison to harvest for the 2009 season (Figure 1c). In the PCA of the climate parameters (Figure 2), the first two components accounted cumulatively for 68.63% of the total variance of the data. The first PC (PC1) separated years with different predawn water deficits (ψpd) before véraison as median ψpdBB-Fl (33.38 %) and ψpdFl-V (29.95 %), whereas PC2 emphasized differences for the ΣGDD_{10°C} after flowering as ΣGDD_{Fl-V} (31.46 %) and ΣGDD_{V-H} (35.98 %). It was possible to form five groups according to the similarity of their weather conditions (1: 2009; 2: 2011–2012; 3: 2005, 2007, 2008; 4: 2010, 2013, 2014, 2015 and 5: 2006) mostly separated according to differences in the ΣGDD_{10°C} after flowering on PC2.

2. Pruning mass and yield

Warm and dry conditions during the flowering véraison period for groups 1, 4 and 5 resulted in a higher amount of wood removed at pruning. The yield range over the 11 seasons was between 6 100 kg ha⁻¹ (2010) to more than double that at 15 100 kg ha⁻¹ (2015) (Table 1). Wet conditions around the flowering period favored fruit set, while warmer conditions together with higher precipitation during the maturation period resulted in a higher yield. However, higher infestation levels of Botrytis cinerea (2006, 2007, 2010) negatively impacted yield and required a selective harvest.

3. Juice primary compounds

As the grapes were destined for red wine production, special attention was directed to Botrytis infection; the aim was to limit the severity to under 5% and therefore the date of harvest was moved forward to avoid further infestation. The TSS for 2006 (average 23.59°Brix) were unusually high (Table 1). Although targeted final TSS was 21°Brix, TSS for the seasons 2009, 2011, 2012 (groups 1 and 2) was also significantly higher, with a lower total titratable acidity (TA) and malic acid in the musts. Lower TA was obtained under warmer conditions during the maturation period, as was the concentration of malic acid in the musts. The accumulation of primary amino acid N-OPA in the juices was independent of maturity differences (R²=0.172) and was extremely low for group 4. The accumulation of primary amino acid N-OPA in the juices seemed positively correlated to the ΣGDD_{10°C} accumulated between bud break and flowering (R²= 0.748).

4. Anthocyanin concentrations in berries

Anthocyanin concentrations in the skins was negatively correlated to N-OPA in the musts (R²=0.475) and was enhanced by increased heat accumulation during the period of flowering to
TABLE 1. Results for Pinot noir on SO4 for 2005–2015.

<table>
<thead>
<tr>
<th>Pruning mass [kg tree⁻¹]</th>
<th>Yield [kg tree⁻¹]</th>
<th>Botrytis incidence [% of grape]</th>
<th>Botrytis severity [% of grape]</th>
<th>Soluble Solids [°Brix]</th>
<th>pH</th>
<th>TA [g L⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>39.12 ± 4.59 ab</td>
<td>138.82 ± 18.80 ab</td>
<td>13.01 ± 0.82 c d</td>
<td>72.02 ± 0.16 e f g</td>
<td>19.54 ± 0.23 a e</td>
<td>3.24 ± 0.02 a</td>
</tr>
<tr>
<td>2006</td>
<td>44.73 ± 4.38 ab</td>
<td>155.44 ± 47.92 ab</td>
<td>15.75 ± 0.29 e f</td>
<td>84.40 ± 0.05 a c d</td>
<td>23.59 ± 0.26 a</td>
<td>2.31 ± 0.08 a</td>
</tr>
<tr>
<td>2007</td>
<td>34.15 ± 4.32 ab</td>
<td>94.95 ± 21.89 ab</td>
<td>12.53 ± 12.34 abc</td>
<td>4.12 ± 2.67 abc d</td>
<td>20.81 ± 1.07 ab c d</td>
<td>3.17 ± 0.07 a b</td>
</tr>
<tr>
<td>2008</td>
<td>36.27 ± 4.19 ab</td>
<td>88.57 ± 32.47 ab</td>
<td>17.25 ± 24.57 abc</td>
<td>1.08 ± 2.13 abc de</td>
<td>21.22 ± 0.68 abc d e f</td>
<td>3.05 ± 0.02 b c</td>
</tr>
<tr>
<td>2009</td>
<td>42.31 ± 4.58 ab</td>
<td>114.06 ± 36.21 ab</td>
<td>16.13 ± 14.11 abc</td>
<td>1.02 ± 0.07 e f g</td>
<td>21.73 ± 0.46 ab c d e f</td>
<td>3.24 ± 0.09 c d e f</td>
</tr>
<tr>
<td>2010</td>
<td>30.18 ± 3.29 ab</td>
<td>61.23 ± 9.82 ab</td>
<td>16.13 ± 3.06 a b</td>
<td>5.19 ± 1.39 a b c</td>
<td>18.62 ± 0.22 a</td>
<td>3.14 ± 0.04 ab</td>
</tr>
<tr>
<td>2011</td>
<td>24.23 ± 4.44 ab</td>
<td>130.83 ± 7.40 ab</td>
<td>17.75 ± 18.82 ab</td>
<td>1.21 ± 2.17 ab c d</td>
<td>21.62 ± 1.24 ab c d e f</td>
<td>3.22 ± 0.04 a b c d</td>
</tr>
<tr>
<td>2012</td>
<td>20.30 ± 3.46 ab</td>
<td>121.87 ± 15.84 ab</td>
<td>17.35 ± 14.11 abc</td>
<td>1.06 ± 0.07 e f g</td>
<td>21.70 ± 0.91 ab c d e f</td>
<td>3.14 ± 0.04 ab c d e f</td>
</tr>
<tr>
<td>2013</td>
<td>45.72 ± 3.34 ab</td>
<td>75.47 ± 4.83 ab</td>
<td>21.06 ± 16.27 ab</td>
<td>1.39 ± 13.54 a b c</td>
<td>21.42 ± 0.33 ab c d e f</td>
<td>3.01 ± 0.01 ab c d</td>
</tr>
<tr>
<td>2014</td>
<td>46.71 ± 4.87 ab</td>
<td>125.42 ± 14.48 ab</td>
<td>15.28 ± 13.78 a c d</td>
<td>0.88 ± 0.20 a b c d</td>
<td>19.65 ± 0.67 ab c d e f</td>
<td>2.97 ± 0.05 c d e f</td>
</tr>
<tr>
<td>2015</td>
<td>39.39 ± 4.88 ab</td>
<td>151.16 ± 16.18 ab</td>
<td>6.75 ± 0.16 c d f</td>
<td>0.31 ± 0.28 a b c d</td>
<td>20.01 ± 0.84 ab c d e f</td>
<td>2.91 ± 0.08 a b c d e f</td>
</tr>
</tbody>
</table>

5. Anthocyanins in wines

Wine anthocyanins (Figure 3b) were mostly explained by the groups formed, which were separated in PC2 according to differences in the ΣGDD, when considering the groups formed according to the weather conditions, higher anthocyanin concentrations in the berries were observed in the 2009 season (group 1). Indeed, high water deficits after véraison for the 2009 season limited the Pinot noir berry weight. An MLR analysis was performed and the concentration of anthocyanins in the berry skins was higher for seasons with limited yields and lower berry weight and skin weight (Table 2).
weight, while a lower yield and higher Brix led to an enhanced extraction of the anthocyanins in the wine. However, the most important contributor for the prediction of the anthocyanin extraction in the wine was the water stress experienced during the period of maturation, which influenced the concentration in the wine. A higher water stress during this period coupled with warmer conditions led to wine with enhanced color.

6. Tannins in berries

Berry tannin concentration was correlated to total phenolic concentration for seeds (R²=0.662) and skins (R²=0.963). Tannin concentration in seeds ranged from 24.30 to 45.14 mg g⁻¹ seed with an average 36.51 mg g⁻¹ and an even greater variability in skins from 9.37 to 40.14 mg g⁻¹ skin. Tannin concentration was negatively correlated to N-OPA in musts for seeds (R²=0.291) and skins (R²=0.492). Furthermore, tannin concentration was negatively related to the ΣGDD₁₀°C (between bud break and flowering) in seeds (R²=0.529) and positively in skins (R²=0.264). Consequently, warmer conditions during bud break and flowering favored the accumulation of tannins in skins compared to seeds.

An MLR analysis was performed and 95% of the variation in tannin concentration in skins could be explained by an enhanced accumulation due to warmer conditions and higher water stress during the period between flowering and véraison (Table 3). However, a negative contribution of berry weight, skin weight per berry, pH and yield were also observed.
Prediction of anthocyanins through an MLR analysis and model reduction through a stepwise selection of predictor variables (forward, backward and both) for anthocyanin concentrations in wines.

**TABLE 3.** Multiple linear regression analysis for the prediction of wine anthocyanins.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Estimate</th>
<th>sd</th>
<th>t value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_1$</td>
<td>0.602</td>
<td>0.329</td>
<td>1.831</td>
<td>0.042 *</td>
</tr>
<tr>
<td>$X_2$</td>
<td>0.105</td>
<td>0.185</td>
<td>0.571</td>
<td>0.023 *</td>
</tr>
<tr>
<td>$X_3$</td>
<td>0.039</td>
<td>0.166</td>
<td>0.238</td>
<td>&lt;0.001 ***</td>
</tr>
<tr>
<td>$X_4$</td>
<td>-55.331</td>
<td>116.046</td>
<td>-0.477</td>
<td>0.033 *</td>
</tr>
<tr>
<td>$X_5$</td>
<td>-36.384</td>
<td>76.886</td>
<td>-0.473</td>
<td>0.025 *</td>
</tr>
<tr>
<td>$X_6$</td>
<td>159.321</td>
<td>39.458</td>
<td>4.038</td>
<td>0.001 ***</td>
</tr>
<tr>
<td>$X_7$</td>
<td>1.264</td>
<td>0.770</td>
<td>1.641</td>
<td>0.051 *</td>
</tr>
<tr>
<td>$X_8$</td>
<td>-0.384</td>
<td>0.166</td>
<td>-2.322</td>
<td>0.009 **</td>
</tr>
<tr>
<td>$Y$</td>
<td>12.175</td>
<td>36.236</td>
<td>0.336</td>
<td>0.027 *</td>
</tr>
<tr>
<td>$X_{10}$</td>
<td>1513.600</td>
<td>278.833</td>
<td>5.428</td>
<td>0.011 *</td>
</tr>
<tr>
<td>$X_{13}$</td>
<td>8.217</td>
<td>4.148</td>
<td>1.981</td>
<td>0.045 *</td>
</tr>
<tr>
<td>$X_{19}$</td>
<td>17.874</td>
<td>3.149</td>
<td>5.676</td>
<td>0.001 ***</td>
</tr>
</tbody>
</table>

Prediction of tannins through an MLR analysis and model reduction through a stepwise selection of predictor variables (forward, backward and both) for tannin concentration in berries (upper tables) and wines (lower table) for Pinot noir.

**TABLE 4.** Multiple linear regression analysis for the prediction of skin tannins.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Estimate</th>
<th>sd</th>
<th>t value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_1$</td>
<td>0.008</td>
<td>0.035</td>
<td>0.228</td>
<td>0.034 *</td>
</tr>
<tr>
<td>$X_2$</td>
<td>0.059</td>
<td>0.020</td>
<td>2.910</td>
<td>&lt;0.001 ***</td>
</tr>
<tr>
<td>$X_3$</td>
<td>-0.023</td>
<td>0.018</td>
<td>-1.266</td>
<td>0.023 *</td>
</tr>
<tr>
<td>$X_4$</td>
<td>-15.951</td>
<td>12.939</td>
<td>-1.233</td>
<td>0.029 *</td>
</tr>
<tr>
<td>$X_5$</td>
<td>29.343</td>
<td>8.558</td>
<td>3.429</td>
<td>&lt;0.001 ***</td>
</tr>
<tr>
<td>$X_6$</td>
<td>-15.190</td>
<td>4.383</td>
<td>-3.466</td>
<td>0.032 *</td>
</tr>
<tr>
<td>$X_7$</td>
<td>-0.403</td>
<td>0.086</td>
<td>-0.498</td>
<td>&lt;0.001 ***</td>
</tr>
<tr>
<td>$X_8$</td>
<td>-0.057</td>
<td>0.018</td>
<td>-3.201</td>
<td>0.045 *</td>
</tr>
<tr>
<td>$X_9$</td>
<td>-2.671</td>
<td>3.912</td>
<td>-0.683</td>
<td>0.041 *</td>
</tr>
<tr>
<td>$X_{10}$</td>
<td>-154.214</td>
<td>24.700</td>
<td>-6.243</td>
<td>&lt;0.001 ***</td>
</tr>
<tr>
<td>$X_{14}$</td>
<td>-8.490</td>
<td>7.085</td>
<td>-1.198</td>
<td>0.018 *</td>
</tr>
</tbody>
</table>

Prediction of tannins through an MLR analysis and model reduction through a stepwise selection of predictor variables (forward, backward and both) for tannin concentration in berries (upper tables) and wines (lower table) for Pinot noir.

7. Prediction of tannin concentration in berry skin [mg g$^{-1}$ berry skin]

*Prediction of tannins through an MLR analysis and model reduction through a stepwise selection of predictor variables (forward, backward and both) for tannin concentration in berries (upper tables) and wines (lower table) for Pinot noir.

Furthermore, 86% of the variation in tannin concentration in seeds could be explained with a positive contribution of higher ΣGDD$_{10^\circ C}$ between bud break and flowering and by dry condition during this period (Table 5). However, other factors positively contributed to yield, berry weight and average seed weight. Although the timing of influence seemed different for seeds and skin, in general, warmer conditions between flowering and véraison were coupled with an accumulation of tannins in berries and the effect was enhanced under water deficit during this period, especially in 2005. The final concentration in berries was related to the concentration in skins and seeds, but also to berry anatomy, with a positive contribution of number of seeds per berry and seed weight and a negative contribution of berry weight, which was related to the water deficit during flowering and véraison. The temperature sum between bud break and flowering was by far the largest contribution.

Furthermore, warmer conditions during the period between flowering and véraison, most relevant for tannin production, resulted in an
enhanced accumulation of tannin in skins ($R^2=0.597$). When considering the groups formed according to the weather conditions, higher tannin concentrations in the skins were observed for group 4 (Figure 4b), mainly separated by PC1 (Figure 2) and related to water deficit before **v**éraison. The years with more tannins in seeds were also the years with more tannins in skins, with the exception of 2005, 2015, 2011, which contained more seed tannin compared to skin tannins. When considering the tannin concentration in the fruit, it was not only the concentration per se, but also the relative quantity of the grape material that played a role in the latter extraction in the wine. Furthermore, the number of seeds and the seed size (related to seed weight) is of importance. The high concentration of tannins in the seeds for these three seasons was less the result of a higher number but more the result of much smaller seeds, leading to a higher extraction surface. The number of seeds per berry and the seed weight were correlated to the amount of precipitation during the early stages of bud break and flowering ($R^2=0.724$, $R^2=0.686$, respectively).

### Tannins in wines

As the wines were produced using micro-scale fermentation, each field replicate could be separately investigated under similar conditions for all seasons and an ANOVA comparing the phenolic extraction in wine in relation to the season was applied. When considering the groups formed according to the weather conditions, higher tannin concentrations in the wines were observed for groups 2 and 4 (Figure 4c), mostly separated by PC1 (Figure 2) and related to water deficit before **v**éraison. The prediction of tannin concentration in wines ($R^2=0.567$) showed a positive contribution of

### TABLE 5. Multiple linear regression analysis for the prediction of seed tannins.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Estimate</th>
<th>sd</th>
<th>t value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X1$</td>
<td>$\Sigma GDD_Bb-Fl$</td>
<td>-0.168 $\pm$ 0.045</td>
<td>-3.774</td>
<td>&lt;0.001 ***</td>
</tr>
<tr>
<td>$X3$</td>
<td>$\Sigma GDD_Fl-V$</td>
<td>-0.071 $\pm$ 0.023</td>
<td>-3.025</td>
<td>0.018 *</td>
</tr>
<tr>
<td>$X4$</td>
<td>$\Sigma PD_Bb-Fl$</td>
<td>20.141 $\pm$ 16.353</td>
<td>1.232</td>
<td>&lt;0.001 ***</td>
</tr>
<tr>
<td>$X6$</td>
<td>$\Sigma PD_V-H$</td>
<td>-3.728 $\pm$ 5.539</td>
<td>-0.673</td>
<td>0.443 *</td>
</tr>
<tr>
<td>$X7$</td>
<td>Wood weight (kg ar$^{-1}$)</td>
<td>-0.014 $\pm$ 0.109</td>
<td>-0.126</td>
<td>0.01 **</td>
</tr>
<tr>
<td>$X8$</td>
<td>Yield (kg ar$^{-1}$)</td>
<td>0.072 $\pm$ 0.023</td>
<td>3.181</td>
<td>&lt;0.001 ***</td>
</tr>
<tr>
<td>$X9$</td>
<td>Average berry weight (g berry$^{-1}$)</td>
<td>20.517 $\pm$ 4.945</td>
<td>4.149</td>
<td>0.012 *</td>
</tr>
<tr>
<td>$X11$</td>
<td>Number of seeds per berry</td>
<td>2.799 $\pm$ 2.777</td>
<td>1.008</td>
<td>0.076 .</td>
</tr>
<tr>
<td>$X12$</td>
<td>Average seed weight (g seed$^{-1}$)</td>
<td>455.523 $\pm$ 202.036</td>
<td>2.255</td>
<td>0.027 *</td>
</tr>
</tbody>
</table>

Prediction of tannins through an MLR analysis and model reduction through a stepwise selection of predictor variables (forward, backward and both) for tannin concentration in berry seeds.

### FIGURE 4. Tannin in skin and seeds and its extraction into wine.

The central box in each box plot indicates the interquartile range and the bold line indicates the median; the whiskers indicate the 10th and 90th percentiles. A 20-berry sample per replicate was analyzed according to the method of Harbertson et al. (2002). BFW, berry fresh weight. (a) Seed tannins (mg CE g$^{-1}$ BFW), (b) Skin tannins (mg CE g$^{-1}$ BFW), (c) Wine tannins (mg CE L$^{-1}$).
berry weight, skin weight per berry, pH and yield; there was a negative impact of seed tannin concentration but a positive impact of skin tannin concentration (Table 6).

As previously observed, water stress during the period before véraison enhanced the extraction of tannins in the wines. The total tannin concentration in the berries was not correlated to the concentration in the wines (n=44, R²=0.191), seeds (n=44, R²=0.384), or skins (n=44, R²=0.179) alone. Using this micro-scale fermentation, it was possible to move the analyses to a mass balance approach. The observed extraction rate of grape tannins into the wines was calculated by comparing the concentration in wine to the initial (total) fruit tannin content and was, on average, low at 10%. Large differences were observed between the seasons in the extraction rate, which was between 5.75% (2015) and 13.85% (2012). A negative correlation was observed between the extraction rate and the ΣGDD 10°C between flowering and véraison (R²=0.567). A higher temperature during flowering and véraison had not only a positive influence on the tannin concentration in berries also but seemed to have a negative influence on their extractability into wine (data not shown).

A PCA was performed on the whole data set and most of the variability (45.41%) was explained by the first two principal components (PC1 28.22%, PC2 17.19%) (Figure 5). PC1 could discriminate the years by their tannin concentration in seeds (7.55%) and skins (10.72%) and was mainly dominated by water deficit (ΨPD_Bb-F, ΨPD_Fl-V, ΨPD_V-H) (15.09%) explaining the differences in the wine tannins.

PC2 could explain the differences in wine anthocyanins with the ΣGDD10°C between véraison and harvest (5.01%) influencing the acidity (TA 13.62%, malic acid 13.84%) and described by berry size (11.03%).

**DISCUSSION**

1. Climatic conditions and phenology

Some models are available relating grapevine phenology to climate parameters (García de Cortázár-Atauri, 2006; Parker et al., 2011; Santos et al., 2011; Cola et al., 2014). The GFV model (Parker et al., 2011) uses the temperature summation of daily average temperature from the first 60 DOY (t0) in the Northern Hemisphere and a baseline minimum temperature threshold (Tb) set at 0°C for the prediction of 50% flowering (stage 23 on the modified E-L scale; Coombe, 1995) and 50% véraison (stage 35 on the modified E-L scale), and bud break (Nendel et al., 2010) was used to fit phenological models within this study. We found a good prediction of the GFV for flowering date within our data, showing that warmer conditions positively affect the appearance of the flowering event. Therefore, daily raw weather data are reorganized into periods that coincide with average phenological stages (Jones and Davis, 2000).

2. Yield

We found that the amount of precipitation during the period between budbreak and flowering had a negative influence on yield quantity for the season, influencing the inflorescence differentiation and berry set in relation to the “coulure climatique” phenomenon. There was

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**TABLE 6.** Multiple linear regression analysis for the prediction of tannins in wines.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Estimate</th>
<th>sd</th>
<th>t value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>X3 ΣGDD_V-H</td>
<td>-0.285</td>
<td>± 1.116</td>
<td>-0.255</td>
<td>0.006 **</td>
</tr>
<tr>
<td>X4 ΨPD_Bb-Fl</td>
<td>1073.819</td>
<td>± 731.219</td>
<td>1.469</td>
<td>0.009 **</td>
</tr>
<tr>
<td>X7 Wood weight (kg ar⁻¹)</td>
<td>4.251</td>
<td>± 4.462</td>
<td>0.953</td>
<td>0.026</td>
</tr>
<tr>
<td>X8 Yield (kg ar⁻¹)</td>
<td>1.169</td>
<td>± 1.158</td>
<td>1.009</td>
<td>0.010  **</td>
</tr>
<tr>
<td>X9 Average berry weight (g berry⁻¹)</td>
<td>177.854</td>
<td>± 281.023</td>
<td>0.633</td>
<td>0.044  *</td>
</tr>
<tr>
<td>X10 Average skin weight (g berry⁻¹)</td>
<td>611.995</td>
<td>± 2312.313</td>
<td>0.265</td>
<td>&lt;0.001 ***</td>
</tr>
<tr>
<td>X14 pH</td>
<td>4.450</td>
<td>± 7.085</td>
<td>1.112</td>
<td>0.018  *</td>
</tr>
<tr>
<td>X19 Tannins (mg g⁻¹ berry seeds)</td>
<td>-5.311</td>
<td>± 8.863</td>
<td>-0.599</td>
<td>0.023  *</td>
</tr>
<tr>
<td>X20 Tannins (mg g⁻¹ berry skin)</td>
<td>14.241</td>
<td>± 11.201</td>
<td>1.271</td>
<td>0.021  *</td>
</tr>
</tbody>
</table>

Prediction of tannins through an MLR analysis and model reduction through a stepwise selection of predictor variables (forward, backward and both) for tannin concentration in wines.
Increasing heat accumulation in grapes would be temperature sensitive (Pereira et al., 2006; Mori et al., 2007, Tarara et al., 2008) and we found that anthocyanin concentrations in skins was enhanced with increasing heat accumulation ΣGDD10°C before véraison. This is in concordance with previous studies on Pinot Noir sites, which showed that warm temperatures during early berry development appeared to increase phenolic concentrations (Nicholas et al., 2011). Increased anthocyanins were correlated with warm days and cool nights during the period between bud break and flowering (Nicholas et al., 2011). However, in our study, decreasing heat accumulation after véraison had a positive influence on anthocyanins accumulation in skins. This was also found for Pinot Noir where anthocyanins were decreased by increasing heat during the final ripening period (Nicholas et al., 2011). However, anthocyanin concentrations in berries as mg g⁻¹ BFW, relevant from an oenological point of view, were mostly enhanced by yield and berry size, as a result of an early water deficit. Indeed, mild water deficits have a direct and/or indirect effect on active sugar accumulation in berries, which causes a change in the ratio between the fruit dry matter and the sugar concentration. This is due to a lower water content in grapes when the water status is reduced. In consequence, the concentration of the compound per unit of dry matter is higher in grapes with low water status (Matthews and Anderson, 1989; Sweetman et al., 2009; Rienth et al., 2011). Enhanced anthocyanin concentrations in skins were mostly correlated with warm days and cool nights during the period between véraison and harvest dates. Some attempt to predict yield according to historical weather data (De la Fuente et al., 2015), during which warm temperatures (dry and stable atmospheric conditions) were required for balanced crop yield and wine quality (Jones and Davis, 2000; Santos et al., 2011).

3. Juice primary compounds

Positive correlations were found between total acidity and flowering, véraison and harvest dates, indicating that delayed phenology means higher relative acidity (Jones and Davis, 2000). However, our results were slightly different as Botrytis incidence was one driving factor for the harvest date. Although comparable in TSS, the concentration of malic acid in musts was lower in warmer conditions, and the seasons 2007, 2008, 2010, which showed high concentrations of malic acid, were extremely cool during the maturation period and had high precipitation levels during the early period of development (until véraison). One of the clearest relationships between temperature and fruit composition is that high temperatures lead to a reduction of the concentration of organic acids (Kliewer, 1973), as malic acid degradation is temperaturesensitive (Ruffner, 1982; Sweetman et al., 2009; Rienth et al., 2016). Ambient temperature during Stage III of berry ripening is the major driver of malic acid degradation in grapes (Ruffner, 1982). In most cases, no TA changes have been observed in the must from moderately water-stressed vines (Matthews and Anderson, 1989), but the malate/tartrate ratio was in general lower because of the malate breakdown in vines with low water status (Matthews and Anderson, 1989). Malic concentration decreased in a linear manner with water deficit stress (van Leeuwen et al., 2009).

4. Berry and wine anthocyanins

It is well established that anthocyanin accumulation in grapes would be temperature sensitive (Pereira et al., 2006; Mori et al., 2007, Tarara et al., 2008) and we found that anthocyanin concentrations in skins was enhanced with increasing heat accumulation ΣGDD10°C before véraison. This is in concordance with previous studies on Pinot Noir sites, which showed that warm temperatures during early berry development appeared to increase phenolic concentrations (Nicholas et al., 2011). Increased anthocyanins were correlated with warm days and cool nights during the period between bud break and flowering (Nicholas et al., 2011). However, in our study, decreasing heat accumulation after véraison had a positive influence on anthocyanins accumulation in skins. This was also found for Pinot Noir where anthocyanins were decreased by increasing heat during the final ripening period (Nicholas et al., 2011). However, anthocyanin concentrations in berries as mg g⁻¹ BFW, relevant from an oenological point of view, were mostly enhanced by yield and berry size, as a result of an early water deficit. Indeed, mild water deficits have a direct and/or indirect effect on active sugar accumulation in berries, which causes a change in the ratio between the fruit dry matter and the sugar concentration. This is due to a lower water content in grapes when the water status is reduced. In consequence, the concentration of the compound per unit of dry matter is higher in grapes with low water status (Matthews and Anderson, 1989; Sweetman et al., 2009; Rienth et al., 2011). Enhanced anthocyanin concentrations in skins were mostly correlated with warm days and cool nights during the period between véraison and harvest dates. Some attempt to predict yield according to historical weather data (De la Fuente et al., 2015), during which warm temperatures (dry and stable atmospheric conditions) were required for balanced crop yield and wine quality (Jones and Davis, 2000; Santos et al., 2011).

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as for its impact on accumulation in berries. Previous studies have shown that water deficit increased the concentration of fruit anthocyanins in Cabernet Sauvignon (Koundouras et al., 2009; Holt et al., 2010; Casassa et al., 2015) and Merlot (Bindon et al., 2011; Bucchetti et al., 2011) which concurs with our results. There would be a direct influence on anthocyanin concentration (Roby et al., 2004; Ju et al., 2019). Indeed, anthocyanin content increased in a linear manner with water deficit stress (van Leeuwen et al., 2009). Water deficit has been considered to directly enhance the accumulation of anthocyanins, probably by upregulating genes responsible for its synthesis (Castellarin et al., 2007; Chaves et al., 2010). Gene regulation of the anthocyanin pathway was known to be affected by the timing of imposition of water deficit (Castellarin et al., 2007). Anthocyanins have been previously reported to increase with water deficit, especially when water limitation is applied prior to véraison (Bindon et al., 2011). An early exposure of water stress before véraison led to increased anthocyanin accumulation, with an increased sugar accumulation that accelerates anthocyanin synthesis in our own and other experiments (Castellarin et al., 2007), probably due to ‘sucrose boxes’ in the promoters of LDOX and DFR genes (Gollop et al., 2001, 2002).

However, in our case the late water deficit during the maturation period had no significant and no direct influence on skin anthocyanin accumulation, which was also seen elsewhere (Casassa et al., 2015). It seemed that the influence of a late water stress was more indirect through a limitation of berry weight and a higher contribution of berry skin (Roby et al., 2004).

Vine water status is known to influence fruit composition through an indirect effect on berry size, and therefore the ratio of skin to pulp, which increases in the smaller berries of vines subjected to water deficits (Kennedy et al., 2002; Roby et al., 2004). Indeed, anthocyanin concentration increased with water deficit stress (van Leeuwen et al., 2009) but with a simultaneous decrease of berry weight. In general, mild water deficits were shown to have a positive impact on wine quality in red varieties and, in our case, the most important contributor for the prediction of anthocyanin extraction in wine was the water stress experienced during the period of maturation. The impact of water deficit on wine anthocyanin concentration is not as clear as for its impact on accumulation in berries (Casassa et al., 2015). In our case, higher levels of water stress during this period coupled with warmer conditions led to wine with enhanced color. The concentration of anthocyanins in the skins was not the only significant predictor of the concentration in wine, and was enhanced by higher amounts of skin per berry and higher Brix. Therefore, it seemed that the anthocyanin concentrations, per se, was increased by warm temperatures during early berry development and water deficits limiting berry size development. The extractability of the anthocyanins into wine depended greatly on the amount of organ to extract, with higher maturity depending on the water stress experienced during the period of maturation.

5. Berry and wine tannins

We observed that higher ΣGDD°C before véraison resulted in an increase of the tannin concentration in the fruit, with a higher accumulation in skins than seeds. The mechanisms for the regulation of tannin biosynthesis are not quite fully understood, but it is well known that their synthesis occurs between flowering and véraison (Kennedy et al., 2002) and is impacted by temperature variation (Pastor del Rio and Kennedy, 2006; Cohen et al., 2012). The influence of the temperature on tannin composition in berries remains unclear but an increase of berry tannins under higher temperatures was reported (Cohen et al., 2012). This was also observed in our study. Indeed, an increase in tannin concentration for both Pinot Noir skin and seeds was already associated with warmer conditions between fruit set and véraison (Pastor del Rio and Kennedy, 2006), although others found that the period between bud break and flowering was the major driver (Nicholas et al., 2011). However, the warm conditions during the period of tannin biosynthesis was coupled in our study with the lowest precipitation rates and higher water deficits. Tannin concentration in the skin was shown to increase under water deficit (Ojeda et al., 2002; Kennedy et al., 2002; Bucchetti et al., 2011) while limited evidence exists on the effect of water deficit on seed tannins (Kennedy et al., 2000; Geny et al., 2003; Koundouras et al., 2009, Genebra et al., 2014). Two studies performed with the same variety (although in different environments) did not show any significant effects of water deficit on seed tannins (Kennedy et al., 2000; Geny et al., 2003). Indeed, we observed that the increase of
the tannin concentration in fruit during warmer early berry development was enhanced with water deficit before véraison. In previous studies, water deficit did not alter the concentration of seed tannins in Shiraz (Roby et al., 2004) and Cabernet Sauvignon (Koundouras et al., 2009) despite its impact on berry weight. An increase in skin tannin with a water deficit appears to result more from the berry size than from direct effects on phenolic biosynthesis (Roby et al., 2004). We showed that the final concentration in fruit was depending on berry size, with a lower concentration for smaller berries, which was related to the water deficit during flowering and véraison. Seed tannin concentration is also determined by seed weight and the number of seeds per berry (Harbertson et al., 2002; Pastor del Rio and Kennedy, 2006). It appears that in our study the influence of water stress impacted berry size, limiting the number of seeds per berry and seed weight, which was highly related to the water deficit experienced during the early stages of berry development between bud break and flowering. There are conflicting reports on how vine water status affects seed weight and number, as some studies found increases caused by water deficit (Roby and Matthews, 2004) and others found no effect (Castellarin et al., 2007).

As previously observed, water stress during the period before véraison enhanced the extraction of tannins in wines. Likewise, Casassa et al. (2015) showed that wine tannins were 14% higher for the early water deficit treatment, and both vine water status and the growing seasons affected the proportion of tannin extracted from seeds, whereas none of these two factors affected the proportion of tannins extracted from skins. We also observed differences in the tannin extraction rate between the seasons, with a large range of 5.75% to 13.85%. An incomplete extraction of 20–40% of tannin from grape into wine has already been observed (Smith et al., 2007) as only a portion of skin (40–50%) and seed (16–44%) tannin would be extracted into wine (Harbertson et al., 2009). In our case, tannin concentration in the wines was not completely related to tannin concentration in the berries and depended on the season. We found a negative correlation between the extraction rate and the ΣGDD \textsubscript{10°C} between flowering and véraison (R\textsuperscript{2}=0.567). This may be due to interactions between phenolics and cell-wall components of yeast lees (Adams and Scholz, 2007) such as polysaccharides and/or mannoproteins (Hanlin et al., 2010).

**CONCLUSION**

The main focus of our study was to investigate the influence of climate condition on berry and wine composition, considering the different stages of phenology. A large number of climatic variables have been linked to vintage fruit quality parameters. A strong relationship exists between improved grape quality and water deficit prior to véraison. However, in our study it seems that the influence of water deficit was mostly indirect, through modification of berry size and the contribution through the seeds. Cool, wet winters and springs followed by warm, dry summers are key factors in the development of a high-quality wine. This is in concordance with our results, as anthocyanin concentrations in skins was enhanced with increasing heat accumulation ΣGDD\textsubscript{10°C} before véraison, together with an increase of the tannin concentration in fruit, with a favored accumulation in skins over seeds. Furthermore, an increasing heat accumulation ΣGDD\textsubscript{10°C} before véraison influenced the extraction rate of the tannins, e.g. the extractability of the compounds into wine.

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