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# **SPATIAL VARIABILITY OF PHENOLOGY IN TWO IRRIGATED GRAPEVINE CULTIVAR GROWING UNDER SEMI-ARID CONDITIONS**  3 **N. Verdugo-Vásquez<sup>a</sup>, C. Acevedo-Opazo<sup>a\*</sup>, H. Valdés-Gómez<sup>a</sup>, M. Araya-Alman<sup>a</sup>, B. Ingramb, I. García de Cortázarc , B. Tisseyred**   $\frac{4}{5}$

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### **ABSTRACT**

 Knowledge and monitoring of the grapevine phenology during the season are important requirements for characterization of productive regions, climate change studies and planning of various production activities at the vine field scale. This work aims at studying the spatial variability of grapevine phenology at the within field scale. It was conducted on two fields, one of cv Cabernet Sauvignon of 1.56 ha and the other of cv Chardonnay of 1.66 ha, both located in Maule Valley, Chile. Within each vine field, a regular sampling grid was designed, to carry out weekly measurements of phenology and maturation. The main results show that there is a significant spatial variability in the phenological development and maturation at the within field scale for both fields. This variability is spatially organised and temporaly stable from the beginning of the season (post-budburst) to harvest and over the years. A cluster analysis allowed us to define two clearly contrasted zones in terms of phenology and maturation in both fields, explained by the microclimate. The magnitude of difference between zones varied from 4 to 9 days depending on phenological stages and from 5 to 43 days for maturation. These differences are similar and comparable to that observed at larger scales or under scenarios of climate change. These results highlight the necessity to better take into account this variability to improve sampling and to base decisions of production activities (spraying, harvest, pruning, etc.) application on more relevant information. Further investigations should determine the environmental factors that determine the observed spatial variability.

 **Keywords**: *Vitis vinifera*, berry maturity, within field variability, temporal variability, management zones, climate change.

 

 

### **Abbreviations**

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### **INTRODUCTION**

 Grapevine phenology is the study of the stages of growth as budburst, flowering and veraison, which are repeated every season and depend mainly on climatic and hormonal factors (Mullins et al. 1992; Jones and Davis 2000). Knowledge and monitoring of the different phenological stages of the grapevine during the season are important requirements for planning of various production activities at the vine field scale (Mullins et al. 1992). For example, for the management of powdery mildew in the vine there are control methodologies based on the monitoring of the pathogen and plant phenology so as to make phytosanitary applications at the most susceptible phenological stages (flowering and fruit set), thereby reducing the number of phytosanitary treatments (Campbell et al. 2007; Bramley et al. 2011). Likewise, for irrigation management it has been observed that the application of regulated deficit irrigation on specific phenological periods of post-setting and post-veraison optimizes the vegetative growth, the yield and the final quality of the berries (Ojeda et al. 2002; Acevedo-Opazo et al. 2010). Moreover, knowing the phenological development of the vine

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 together with its fruit ripening makes it possible to optimize the harvesting process, often supporting a differentiated management of vine fields (Trought and Bramley 2011).

 The importance of monitoring the phenology of the vine as a decision support information has motivated numerous investigations at different spatial scales, for example, at meso scale (vineyards, more than 200 ha surface) models that predict phenological events have been developed and can be used to plane farming operations at this scale (Ortega-Farías et al. 2002; García de Cortázar-Atauri et al. 2009; Caffarra and Eccel 2010; Nendel 2010; Parker et al. 2011; Sadras and Petrie 2012). Moreover, phenological process based models have also been used to assess the impact of climate change on the phenological development of grapevines at macro scales (regions) (Marta et al. 2010; Moriondo et al. 2010; Caffarra and Eccel 2010, 2011). There have also been studies on the spatial variability of the phenology of grapevines at the macro scale, in order to determine optimum pedo-climatic zones for the production of quality grapes (Tesic et al. 2001) and to establish spatial patterns of evolution of berry maturity in different areas of Australia (Petrie and Sadras 2008).

 With regard to the spatial variability of climate at the meso scale, in recent years the TERADCLIM (Quénol and Bonnardot 2014) and ADVICLIM (Quénol et al. 2014) projects modeled spatial variability of climate (temperature) in the main vine production areas of the world. Assuming the availability of relevant calibrated model linking climate variables to vine phenology, these projects may constitute relevant piece of information to model the spatial variability of vine phenology.

 Generally speaking, the findings reported in these researches have large spatial scale applicabilities (macro and meso scales), which is at odds with the needs of growers for whom the basic unit of management is the vine field (surface less than 5 ha, micro scale) which is characterized by a same variety, training system and management practices.

 In recent years, several authors have observed that in agriculture and especially in viticulture, there is significant spatial variability at the micro scale (within field level) in the production variables such as plant water status (Acevedo-Opazo et al. 2010; Acevedo-Opazo et al. 2013), vegetative expression (King et al. 2014), yield (Bramley and Hamilton 2004; Tardaguila et al. 2011) and the quality components of the berries (Bramley and Hamilton 2004; Baluja et

 al. 2013). This observed variability was mainly attributed to differences in soil (Tardaguila et al. 2011).

 In this light, one wonders whether the vine field is a unit of homogeneous management in relation to the evolution of its phenological cycle during the season. If this is not the case, then the methods traditionally used by wine producers to characterize the phenological stage of their vine fields would not be appropriate to represent the spatial variability of the fields. Indeed, in practice, wine industry does not carry out more than two phenological observations per field. These two observations are assumed to be representative of the whole vine field and sometimes to other neighbouring fields of the same variety. Thus, traditional methods could result in inadequate decisions of interventions.

 To our knowledge, studies on spatial variability on climate and phenology have focused at macro or meso scale (surface > 200 ha) (Bonnefoy et al. 2012; Irimia et al. 2015; Quénol 2013; Quénol et al. 2014), without considering the micro scale (spatial scale < 5 ha). The aim of this study is therefore to investigate the spatial variability of grapevine phenology at the within field scale, answering the following questions: i) is there a spatial variability in the phenological development of the vine at the field scale? ii) is the spatial variability observed stable over time? and iii) is it worth to define management zones in relation to the phenology of the fields?

### **MATERIALS AND METHODS**

### **Experimental Fields**

 The study was conducted in two fields, one of cv. Cabernet Sauvignon of 1.56 ha (field 1) and the other of cv. Chardonnay of 1.66 ha (field 2), both located in the Panguilemo Experimental Station of the University of Talca (Maule Valley), Chile (Fig. 1). The characteristics of both fields are summarized in Table 1. The region is characterized by Mediterranean climatic conditions, while the soil belongs to the Talca and San Rafael series (Ultic Haploxeralfs) (Soil Survey Staff 1999). Both vineyards were managed according to the conventional agricultural practices used in the commercial vineyards of central Chile in terms of canopy management, fertilization, pest and disease control, pruning and irrigation, over all the seasons of the study period. Within each vine field a regular sampling grid was designed, one with 18 measurement sites (25x25 m) for field 1 (Fig. 2 a) and one with 19

 measurement sites (25x25 m) for field 2 (Fig. 2 b). Each site of the grid was represented by four consecutive plants. The borders of the fields and sampling sites within each field were geo-referenced with a differential global positioning system receiver (DGPS) (Trimble, Pathfinder ProXRS, Sunnyvale, California, USA) and stored as Eastern and Northern coordinates (Datum WGS84, UTM projection, Zone 19S) to perform the mapping and spatial analysis.

 An automatic weather station (Adcon Telemetric, A730, Klosterneuburg, Austria) installed under reference conditions, at 300 m from the vineyards, provided data such as air temperature, relative humidity, solar radiation, precipitation, wind speed and direction of the wind at 15-minute intervals. The sensors were installed at 2.5 m above the soil surface, except for the temperature and relative humidity sensors which were located 1.5 m above the soil 12 surface. This information was used to estimate reference evapotranspiration  $(ET_0)$ , using the Penman-Monteith equation (Allen et al. 2006) and the sum of growing degree-days (GDD), from 1st May to harvest and for each phenological period considered (i.e. flowering or veraison). Sum of GDD was calculated as the sum of the daily difference between the average air temperature minus 10°C (Winkler 1974).

 Additionally, considering that the main variable that affects phenological development of the grapevine is the temperature (Chuine et al. 2013; Quénol et al. 2014), 8 temperature sensors (Dickson, LogTK500, USA) were installed in the field 1 (cv Cabernet Sauvignon) with the aim to characterize the spatial variability of temperature at the within field scale.

 The sensors were located according to a preliminary analysis of the 2009-10 season. Two zones of phenology were identified. Assuming temporal stability of these zones, 8 sensors (4 sensors for each zone) were installed (Fig. 3) at 1.5 m above the soil surface and on vine rows. Temperature was recorded every 30 minutes in the period of September (before budburst) until October (pre-flowering) during the 2011-2012 season, corresponding to a total of 37 days of measurements.

- - **Measurements**

*a) Grapevine Phenology* 

 The phenological observations were made from budburst to veraison, every 7 days in 30 shoots chosen systematically among the 4 vines of a site of the grid. The same shoots were

 

 always measured date after date over a season. On each site of the grid, mean value of phenology measured over the 30 shoots was calculated. Phenology was estimated using the Eichhorn and Lorenz phenological scale as modified by Coombe (Coombe 1995) (Table 2). It considers measurements of leaf number, length of shoots (cm) and berry diameter (mm). The phenological scale assigns a number to each phenological measurement (expressed in units of Phenological Scale, PS, Table 2), allowing us to represents phenology as a numeric variable. For data analysis it was used only the three major phenological stages (post- budburst, flowering and veraison). Veraison for cv Cabernet Sauvignon was considered by berries colour change, while for cv Chardonnay it was considered by berry softness and colour change simultaneously.

### *b) Maturation (expressed as total soluble solids, TSS)*

 From veraison to harvest, measurements of total soluble solids (TSS) were performed as indicator of grapevine maturity. TSS was chosen as the most typical indicator used to define harvest time. This measurement was done by a thermo-compensating refractometer (BRIX30 model, Leica, USA), by randomly selecting eight clusters from each site of the grid. For each cluster two berries were sampled at the top, the middle and the bottom of the cluster. Therefore, TSS value of each site of the grid and each date corresponded to a sample of 48 berries. Three sampling dates were considered, called Pre-Ha 1 (25 days before harvest), Pre- Ha 2 (12 days before harvest) and Ha (Days of the harvest). For both fields, harvest (Ha) was defined at 22°Brix. This last value corresponding to the optimal maturity at harvest in our conditions.

**Analysis method** 

 For main phenological stages and maturity dates, basic statistics such as standard deviation, maximum/minimum values and coefficient of variation (CV) were calculated. These statistics were expressed in units of Phenological Scale (PS) and TSS (ºBrix) respectively for phenology and maturation.

 A variographic analysis was performed to study the spatial structure of the phenological data and the maturity at each date for both experimental fields. Omnidirectional semivariograms were calculated according to the methodology proposed by Webster and Oliver (2001). The

1 three basic parameters of the semivariogram were obtained: nugget  $(C_0)$ , sill  $(C_0+C_1)$  and range (a), which define the degree and scale of spatial variation among the observations. The computation of a semi variogram is usually not recommended with a number of points as low (Webster and Oliver 1992). However, for this experiment, the quality of data collected (manual measurements averaged over a large number of individuals) and the significant spatial organisation of the fields allowed us to consider such an analysis. From semivariogram parameters the degree of spatial dependence (DESP) also called the Cambardella index, was derived by using the ratio between the nugget and the total semivariance of the semivariogram (sill), expressed as a percentage (Cambardella et al. 1994), Eq. 1.

11 
$$
DESP(\%) = \left[\frac{C_0}{C_0 + C_1}\right] * 100
$$
 (1)

 The Cambardella index allowed us to compare the relative size of the nugget effect for each 13 date of the experiment. Thus, the values of  $DESP \le 25\%$  indicate a strong spatial 14 dependence;  $25\% \leq DESP \leq 75\%$  indicate a moderate spatial dependence, and the values 15 of  $DESP > 75\%$  correspond to a weak spatial dependence (Wu et al. 2008). In addition to DESP, the mean correlation distance (MCD) (Han et al. 1996) was estimated (Eq. 2).

17 
$$
MCD(m) = \frac{3}{8} * \left[ \frac{C_1}{C_0 + C_1} \right] * a
$$
 (2)

 The MCD provided an estimate of the distance at which the data have a high spatial dependence (Han et al. 1996).

*Mapping spatial distribution* 

 Maps with both phenological and maturation variables were done in order to visualize the phenology and maturation of the fields. To this end, the method of interpolation Block- Kriging as proposed by Baluja et al. (2013) was used. The scale of the maps was defined using an equidistant range, with two intervals.

 *Analysis of the Temporal Stability of the Spatial Variability (TSSV) of the grapevine phenology and maturation* 

 To quantify the TSSV of both phenology and maturation, the Kendall coefficient of concordance (W) was calculated according to the methodology described by Tisseyre et al. (2008). W ranges from 0, in the case of no temporal stability (total desagreement in site order

 between dates), to 1 in the case of temporal stability (Saporta 1990). This analysis was carried out in two ways:

 - to quantify the TSSV of phenology and maturity over the seasons, it was performed separately on the main phenological stages (post-budburst, flowering and veraison) and on the maturation (Pre-Ha 1, Pre-Ha 2 and Ha),

 - to quantify the intra-season TSSV, it was performed on the main phenological stages and maturation dates measured within each season.

*Zoning of the vine fields based on grapevine phenology and maturation* 

 For each field, the zoning of both phenology and maturation was conducted with a cluster analysis using the centroid squared euclidean distance (Flores 2005). For phenology zones most relevant phenological stages of the vineyard management (post-budburst, flowering and veraison) were considered while three main dates (Pre-Ha 1, Pre-Ha 2 and Ha) were considered for maturation. For both fields and each dates, two clustering operation were therefore performed, one related to phenology and the other one to maturation. The clustering was conducted to provide two classes for each field under consideration. Considering the high spatial organisation of our data, this clustering method was expected to highlight within field zones. In the rest of the document, zones will refer to the classes resulting from the clustering.

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*Phenological and maturation characterization expressed in days* 

a) Grapevine Phenology

 In order to provide a practical interpretation of the spatial variability in the phenology observed between budbreak and veraison, the observed phenological stage was turned into chronological days for both fields. This transformation was performed through the implementation of a predictive model of phenology following to the methodology proposed by Ortega-Farías et al. (2002). This model uses the monomolecular Mitscherlich equation (Thornley and Johnson 1990), (Eq. 3). It estimates the phenological stage (expressed in units of Phenological Scale, PS) in terms of thermal accumulation from the time of the budburst. Based on observed values and meteorological data, the model was calibrated for each cultivar. Resulting model allowed us to estimate the growing degree-days required to fulfill a certain phenological stage. Fig. 4 represents the simulation of the proposed model for cv

 Cabernet Sauvignon and cv Chardonnay. On Fig. 4, vertical lines cutting x-axis correspond 2 to the sum of GDD necessary to reach flowering  $(PS = 23)$ . This approach is similar to that used in studies conducted by Jorquera-Fontena and Orrego-Verdugo (2010) who studied the effect of climate change on the phenological development of the vine.

$$
5 \tPs = Ps_f - (Ps_f - Ps_i)e^{-k(sGDD)} \t(3)
$$

7 Where:  $Ps =$  current phenological stage (PS),  $Ps_f =$  last phenological stage corresponding to 8 PS = 38,  $Ps_i$  = first phenological stage corresponding to PS = 4,  $k$  = rate of phenological 9 development and  $sGDD = sum$  of Growing degree-days ( $\degree$ C) from the date corresponding to *Psi* to the date of *Ps*.

 Knowing observed GDD of each day from climatic data, Eq. (3) allowed us to transform PS values in a number of days necessary to reach the phenological stage (Ps) under consideration. At the field level, this approach was used to determine the number of days 15 required to achieve a given phenological stage (*Dat<sub>Fi</sub>*) considering the mean of PS observations on all sampling sites. It was also used to determine the number of days required 17 to achieve a given phenological stage for a within field zone (*Dat<sub>Zi</sub>*) by considering the mean of PS observations belonging to the considered zone. For a given phenological stage, this methodology was used to determine the difference, expressed in days between the average of the whole field and the specific zones of the same field (Eq. 4).

= − (4)

24 Where:  $Data_{Fi}$  = estimated date of the phenological stage *i* for the whole field,  $Data_{zi}$  = estimated date of the same phenological stage *i* for the within field zone *Z.* Phenological 26 stages *i*, corresponds to  $i =$  post-budburst, flowering and veraison.

b) Maturation

 For maturation the three dates (Pre-Ha 1, Pre-Ha 2 and Ha) were transformed into days through the implementation of a maturity index (MI). The MI quantifies the increase in TSS (°Brix) for each degree-day accumulation (Eq. 5):

$$
1 \tMI = \frac{SS_H - SS_M}{\sum_{j=1}^{n} GDD} \t{5}
$$

2 Where: MI = maturity index ( ${}^{\circ}$ Brix d  ${}^{\circ}$ C<sup>-1</sup>); *SS<sub>H</sub>* = total soluble solids measured at harvest ( $\text{Brix}$ ); *SS<sub>M</sub>* = total soluble solids measured at post-veraison (15 days after veraison, TSS  $\frac{4}{5}$  >15°Brix); GDD = growing degree day (°C d<sup>-1</sup>); *j* corresponds to the measurement day of *SSM* and *n* to the day of *SSH*.

 This approach assumes that after a period of rapid increase in TSS (at veraison), TSS increases linearly over time (Sadras and Petrie 2012). The MI was estimated for both fields, 8 obtaining a specific value for each cultivar. *SS<sub>M</sub>* and *SS<sub>H</sub>* were estimated by averaging values measured over the whole field for each field. MI was used to estimate the number of days explaining observed difference in maturity between the whole field and the within field zones. (Eq. 6).

13 *Difference in days* = 
$$
\frac{SS_{Fi} - SS_{Zi}}{M1 * GDD_A}
$$
 (6)

14 Where:  $SS_{Fi}$  = average total soluble solids of all sampling sites of the fields at date *i* ( $\text{Brix}$ ); 15 *SS<sub>Zi</sub>* = average total soluble solids of the sites belonging to zone *z* at the same date *i* ( $\text{Prix}$ ), *i* is the date of measurement corresponding to Pre-Ha 1, Pre-Ha 2 and Ha. *MI* = Maturity 17 Index ( ${}^{\circ}Brix$  d  ${}^{\circ}C^{-1}$ ); *GDD<sub>A</sub>* = Daily mean of GDD from post-veraison (15 days after 18 veraison) to harvest  $({}^{\circ}C \, d^{-2})$ .

### **Software and Tools**

 For the classical statistical analysis, the Statgraphics Plus 5.1 (StatPoint Inc., Virginia, USA) software was used, while for the geostatistical analysis, the *GS*+ version 9.0 (Gamma Design Software, LLC, 2008) software was used. For the design of the maps the 3DField (version 2.9.0.0., Copyrigtht 1998 – 2007, Vladimir Galouchko, Russia) software was used. Finally, the Matlab Software (The Mathwork Inc., Natick, MA, USA) was used for the calculation related to phenology model.

### **RESULTS AND DISCUSSION**

**Climate characterization** 

 The climatic characterization for all the study seasons is presented in Table 3. The mean air 2 temperature during the period between 1<sup>st</sup> May and the harvest ranged between 15 and 15.7 °C. The third season (2011-12) presents the highest temperature, showing this season was 4 warmer (with mean temperature values above 20  $^{\circ}$ C) during the period from flowering to harvest. These temperatures lead to higher sum of growing degree-day values, which 6 fluctuated between 1455 and 1640  $^{\circ}$ C d<sup>-1</sup> for field 1 (cv Cabernet Sauvignon) and between 1385 and 1523 °C d<sup>-1</sup> for field 2 (cv Chardonnay). These values are well above the 1150 and  $1300 \text{ °C } d^{-1}$  considered adequate to properly mature respectively, cv Chardonnay and cv Cabernet Sauvignon (Gladstones 1992). Regarding the precipitation, these were mainly similar during the last three seasons and 23% lower during the first season (2009-2010). As 11 a result of the low rainfall and evapotranspiration, a climate water deficit  $(P-ET_0)$  was

 observed, which fluctuated between 464 and 553 mm for the different seasons; the first season showed the lowest water deficit. Finally, the climatic conditions of the seasons studied can be considered similar, except the 2012-2013 season which presents higher sGDD value.

### **Non-spatialized analysis of the grapevine phenology and maturation**

 Main statistics of the phenology and the maturity are presented in Table 4 and Table 5 respectively. For field 1, the phenological stages of post-budburst, flowering and veraison presented a range of variation between 1 and 6 units on PS for the 4 seasons. Meanwhile, for field 2, these ranges varied between 2 and 5 units of PS (Table 4). For both fields the stage of veraison presented the lowest dispersion during seasons (smallest range). For the 21 maturation period, ranges of variation varied from 3.6 to 6.0 °Brix for field 1 whereas for field 2 they varied from 2.7 to 4.9 °Brix (Table 5). With regards to the variability between seasons, the range values remained similar over the seasons, suggesting that there is a temporal stability in the dispersion of the phenological stages and maturation.

 The coefficients of variation (CV) for both fields show that the phenological stage with the greatest variation during all the seasons was post-budburst, with values that ranged between 8.3 to 12.8% and 6.4 to 15.6% for field 1 and field 2 respectively. The veraison presented the lowest variability. In both fields and over all seasons of the experiment, CV value decreases from post-budbreak to veraison. This result may be due to the proposed scale (Table 2) which is less sensitive to the changes observed at veraison. During maturation, CV values did not show great variations within each season.

 

 Regarding the literature dealing with spatial variability at the within field scale, to our knowledge, no reference on CV values for the phenological stage is available. Concerning the maturation, especially for harvest, Baluja et al. (2013) obtained CV values for total soluble solids measured during the harvest ranging between 5.3 and 7.5% for cv Tempranillo in Spain. These values are similar to that observed in the present study. Similar range of variation were also observed by Tisseyre et al. (2008) and Bramley (2005) for TSS at harvest. This first analysis highlight variability in the phenological development and maturation at the within field scale. Next sections aim at studying whether this variability is organised spatialy and if the observed variability is significant enough to justify site specific managements.

### **Spatial variability of the grapevine phenology and maturation**

 The semivariogram model which was generally better adjusted to the different phenological 13 stages and maturation dates was the Gaussian model, with  $r^2$  values ranging between 0.67 and 0.99 (Table 6 and 7). A significant variation is also observed in the range of the semivariogram which fluctuated between 22.8 and 150 m (Table 6 and 7). The range is in almost all cases higher than the sampling distance (25 m). This indicates the sampling distance is large enough to highlight the spatial variation. Range of the semi variogram exceeds in almost all cases length of the fields, showing the presence of a non-stationary phenomenon.

 For all seasons, all phenological stages and almost all maturation dates, a strong spatial dependence (DESP) was observed (Table 6 and 7). Indeed values of DESP are lower or equal to 25%. These results agree with those obtained by Baluja et al. (2013) at least for TSS measured at harvest.

 The identification of a non-random spatial structure, for all the seasons throughout phenology and maturation could be explained by the environmental factors of the fields, such as the soil conditions and topography and their resulting effect on the microclimate of the canopy (Tesic et al. 2001). Thus, more research should be conducted to determine more precisely which factor or set of factors impact the within field microclimate and determine the spatial structure observed in phenology and maturation.

 Fig. 5 and Fig. 6 show the spatial distribution of the main phenological stages and maturation respectively for field 1. Fig. 7 and Fig. 8 show the spatial distribution of the main

 

 phenological stages and maturation for field 2. For both fields and almost all the maps, two well-contrasted zones resulting from the clustering method can be observed. For field 1, more advanced phenological stages and maturation are observed in the North-Western part of the field. This zone is rather flat and is characterised by a deeper soil and less inclination. Meanwhile, for field 2 more advanced phenological stages and maturation occur in the southern part of the field. This latter zone is located in a slightly lower position, characterized by a higher total soil water availability (data not shown).

 

### **Relationship with canopy temperature**

 Regarding results of temperature sensors (Fig. 9) a relationship between phenology and canopy temperature is clearly highlighted. There are important differences in temperature between zones (defined in Fig. 3) throughout the 37 days of measurements (Fig. 9 a) for field 1. Sensor located in zone 1 (Fig. 3) showed higher temperature and GDD (Fig. 9 b), which may explain more advanced phenology and maturation stages (Fig. 5 and Fig. 6). The difference in temperature between zones was 0.4 °C per day and 15 GDD (between DOY 257 and 293).

 Variability observed in both phenology and maturation (Fig. 5 and 6) may correspond to zones where microclimatic conditions are different. In our conditions, at within field scale, we can hypothesize that stable factors as differences in soil type, slope, topography and vegetative expression, could explain these differences in microclimatic conditions (temperature) and consequently differences in grapevine phenology and maturation. Then, determination of environmental factors which drive grapevine phenology and climate variability at the within field scale could constitute interesting investigations to map spatial variability of grapevine phenology at this scale.

### **Temporal variability of the grapevine phenology and maturation**

 The spatial distribution of all maps (Fig. 5, 6, 7 and 8) presents fairly constant and well defined spatial patterns. These latter remain stable within each season and between seasons. This stability is observed for both fields. Baluja et al. (2013) reported a high temporal stability of spatial patterns of the TSS measured at harvest over 3 seasons in a plot of 2.2 ha of the cultivar Tempranillo in Navarra, Spain. In turn, results obtained by Bramley (2005)

 and Tisseyre et al. (2008) show that spatial patterns were not as stable over time for the same variable. In this way, the temporal stability is probably a characteristic of the specific conditions of each of the experimental sites, such as the weather and soil conditions and the choice of harvest date (Tisseyre et al. 2008; Baluja et al. 2013). It is worth mentioning that, in the present investigation, the environmental conditions over the seasons were quite similar 6 (Table 3) and that the harvest date was considering when mean of the field reached 22  $\textdegree$ Brix for each seasons, which would explain the stability of the observed patterns.

 

> The Kendall coefficient of concordance W (Table 8 and 9) was used to quantify the TSSV of phenological stages and maturation. Results of TSSV between seasons are shown in Table 11 8. Observed W values are high ( $W > 0.5$ ) and statistically significant for both fields either for phenology or maturation. This result corroborates the temporal stability observed on maps presented previously (Fig. 5, 6, 7 and 8).

 Results of within season TSSV are shown in Table 9. For both fields, observed W values are 15 high and statistically significant either for phenology ( $W > 0.54$ ) or maturation ( $W > 0.78$ ). This high TSSV observed within each season suggests that information generated at the beginning of the season (post-budburst) can be used at a later date to characterize the spatial variability of both phenology and maturation.

## **Zoning of the vine fields according to their phenology and maturation**

 The cluster analysis allowed us to define two clearly contrasted zones in terms of phenology (Fig. 10 a and b) and maturation (Fig. 10 c and d). As expected, zones obtained from the cluster analysis were very similar to zones observed on Fig. 5 to 8. Regarding the phenology, zone 1 (cluster 1) represents 78% and 42% of the area of the field 1 and 2, respectively (Fig. 10 a and b). For the maturation, zone 1 (cluster 1) represents 89% and 58% of the area of the field 1 and 2, respectively. Zone 1 presents the most advanced phenological stages (highest values of PS) and most advanced maturation (highest values of TSS), for both fields. Zones presented in Fig. 10 (a, b, c and d) integrates all the dates of the seasons. Therefore they highlight possible management zones. High similarity is observed between zones defined for phenological stage and maturity for both fields. The zone 1 of field 1 differ only in 1 site of the grid between phenology and maturation, while for field 2, it differs in 3 sites. This

 suggests a strong relationship between the phenological development of the grapevine and the maturation. Parker et al. (2014) observed that differences in maturation (expressed in TSS) at harvest were the result of changes observed at the onset of maturation, during veraison. Therefore, variability of phenology, for example at veraison, may constitute a relevant decision support to define harvest zones of different quality early in the season.

 In order to verify the opportunity to manage specifically zones identified on our experiments, analysis of the delay in term of days has been performed. This analysis is presented in the next section.

 

### *Grapevine phenology and maturation expressed in days*

 From an operational point of view, vinegrowers need to quantify the difference in days among the different phenological stages and maturation of the zones so as to plane their work. Thus, the numeric phenological stage was turned into chronological days through the implementation of a predictive model of phenology (Ortega-Farías et al. 2002). The 15 calibration of this predictive model (Eq. 3) with our data led to a fit of  $R^2=0.99$  for both cultivars. In the same way the maturity index (MI) used to turn into chronological days the 17 difference in maturation (TSS,  $\textdegree$ Brix) yielded a value of 0.01 and 0.02  $\textdegree$ Brix d  $\textdegree$ C<sup>-1</sup> for the cv Cabernet Sauvignon and cv Chardonnay, respectively. Differences between MI values is explained by the precocity of each cultivars.

 Fig. 11 present results of the difference between within field zones relative to the mean field considered as reference level equal to 0. Positive values refer to advance in phenological stage and maturation, while negative values refer to delay in these parameters.

 For field 1 differences between each zone of the fields ranges from 3 to 5 days depending on the phenological stage considered and from 35 to 36 days depending on the maturity dates considered (Fig. 11 a). Regarding zone 2, it presents the highest deviation from the mean of the field. This is because zone 2 represents a small part of the field, therefore mean field is logically, closer to the mean of the zone 1.

 For field 2 (Fig. 11 b), observed differences in post-budburst (3 to 4 days) increase slightly in subsequent phenological stages, showing the veraison as the phenological stages that presents the highest difference (5 days) with respect to the mean field (value 0). For maturation, differences between 3 and 5 days were observed. In general, differences observed

 

 for field 2 are much smaller than those observed for field 1. This suggest that factors that explain observed differences in both fields may be different or present a lower magnitude of variation in field 2. The magnitude of difference (in days) between zones varied from 4 to 7 days for the phenological stages of field 1, with flowering presenting the higher difference. Regarding the maturity of field 1, the difference between zones varied from 42 to 43 days. For field 2, difference varied from 6 to 9 days for phenology, with veraison presenting the higher difference between zones. For maturity of field 2, this difference varied from 5 to 8 days. The variability of the phenological stages expressed in days (Fig. 11) was higher for veraison in both fields. Indeed it can be related to the subjectivity of measuring this stage (associated with colour change estimated visually), therefore the definition of zones within field could reduce variability to estimate this phenological stage, improving the accuracy of the estimation. With respect to maturation, variability in days was higher for field 1.

#### **General discussion**

 This work showed that there is a significant spatial variability in the phenological development and maturation within the studied vine fields. This variability was spatially organised and temporally stable from the beginning of the season (post-budburst) to harvest and over the years. This suggests that factors explaining this phenomenon would be related to stable parameters of the environment affecting microclimate conditions. This effect may be emphasized by the perennial specificity of grapevines. It was shown, for one field that the phenology and maturation zones correspond to zones where the microclimate was different. The observed differences in the microclimate of the field 1 can be explained by stable environmental factors (soil characteristics, slope, soil texture, presence of groundwater and compacted strata that limit the growth of the root, etc.), ie, the spatial variability of stable environmental factors produced differences in the microclimate of each zone, which affected the phenology and maturity of the grapevine. This explains the observed high stability of zones between different seasons for field 1. There is no information on factors that determine the spatial variability of both microclimate conditions and phenological development at the within field scale in the litterature.

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 These results generate new questions concerning the modelling of the spatial variability of the grapevine phenology at the within field scale. Two approaches may be considered for further experiments: a) microclimate monitoring from wireless temperature sensors network, such as works carried out at the meso scale level (Quénol et al. 2014). However, it is important to define the number of sensors to be installed, location of those in the field and the maintenance cost of the system which may limit its practical application (Kunz and Tatham 2012; Primicerio et al. 2013). b) Characterisation of environmental factors at a high spatial resolution. This approach corresponds to using high spatial resolution data (topography, soil characteristics, slope) with low operational costs, for example, obtained by unmanned aerial vehicle (Matese et al. 2015). Assuming these factors explain the spatial variability of microclimate and the resulting variability in phenology and maturity, an empirical model combining observations and high resolution spatial data could be considered to model the microscale spatial variability of the phenology. The methodology used for the water status in grapevines (Acevedo-Opazo et al. 2010) could be a relevant approach in this case.

 The observed within field differences may have important implications for crop management. The goal of the following section is to identify the opportunity to manage the observed within field variability for each phenological stages as well as for maturity.

 For post-budburst, observed magnitude of variation may have practical applications concerning spring freeze event. Susceptibility of the buds to spring frost depends on the phenological stage of buds (Friend et al. 2011). On a controled experimentation, Friend et al. 23 (2011) shown effect of frost was different when occuring on two treatments characterized by a difference of 1 PS (corresponding to 4 days in our study). A significant increase in primary shoots death for the most advanced treatment (at budburst) was observed while reduced dammages were observed in delayed treatment. Differenciate effect of frost had a significant impact on yield at harvest. In our study, observed difference of 4 to 8 days (Fig. 8 a and b) corresponding to 2-3 PS (Fig. 3 and 5) for post-budburst could lead to significant differences between zones in case a frost event occurs at this period. Practicaly, the delineation of phenology zones is hardly manageable at this stage since spring frost events are hardly predictible. However, when it occurs, differential effect of the frost may be of importance to

 understand the resulting within field yield variability and to consider site specific management operations later in the season.

 Flowering is considered as a critical stage for disease development such as powdery mildew and botrytis (Campbell et al. 2007). Therefore, this stage determines the application date of specific pesticides to control these diseases (Bramley et al. 2011). Thus, a delay in fungicide application, waiting for the slowest zone of the field to reach the flowering stage, could impact negatively on the proper control of the most advanced zones. In our study, the average differences between the zones were 7 days for field 1 and 6 days for field 2. To our knowledge, there is no information in the literature dealing with the effect of application time on diseases control. However our study provides new background information on the spatial variability of flowering. This results opens opportunities to better understand the success or failure of control strategies and the resulting within field variability of diseases. Information on the spatial variability of the incidence and severity of diseases in vineyards is scarce. Bramley et al. (2011) found that incidence and severity of the two main diseases (powdery mildew and botrytis) was spatially variable at the within field scale. The same authors observed that diseases development was associated to the topography (slope), they hypothetised that topography explained differences in microclimate more or less favorable to diseases development. Regarding our results, it can also be hypothesized that topography and the resulting microclimate could affect the date of flowering. Considering homogeneous pesticides application over the vineyards, protection associated with the applications may vary according to spatial variability of flowering. Therefore, spatial variability of diseases may be due to either differences in phenology (and resulting pesticides efficiency) or to environmental factors that directly affect diseases development (Valdés-Gómez et al. 2011). These considerations highlight the necessity to take into account the spatial variability of phenology (and especialy flowering) to better understand factors that affects diseases variability. Specific experiments based on phenology maps and differencial application of pesticides should be considered to properly analyse the spatial variability of diseases development at the within field scale.

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 Regarding the period going from fruit setting to veraison, it is of critical importance to manage irrigation. Spatial variability of phenology may be a relevant decision support to consider site specific management zones of regulated deficit irrigation strategies in such a way to optimize the final quality of the grapes (Ojeda et al. 2002; Acevedo-Opazo et al. 2005; Girona et al. 2009; Acevedo-Opazo et al. 2010). For veraison, Parker et al. (2014) observed that differences in veraison remain stable until harvest affecting the final wine quality. It confirms our results of the temporal stability of the zones of phenology and maturity. Therefore, phenology maps may be useful to determine maturity zones at the within field scale. These maturity zones are the basis of a decision support to manage grape quality at harvest by considering, for exemple differential harvest over time and/or space (Bramley 2005). Note that in our experiment, differential harvest would be of great interest in the field 1. Indeed, for this field, zone 2 never reaches the expected maturity. This is probably due to the specific soil conditions associated to inadequate irrigation management (Acevedo-Opazo et al. 2013). In this case, zone 2 could be harvested separately.

 Identifying the spatial variability of the phenology could be a useful decision support to suggest management strategies that seek to homogenize the vine field phenology. Authors have reported that a late winter pruning operations delayed by 4-5 days the date of budburst (Dunn and Martin 2000). Thus, site specific pruning operation could reduce differences in phenological development. More generaly, our study points out the interest in delineating phenology zones to optimise field sampling and to improve the efficiency of the various agricultural operations and decisions during the season.

 This work focused on the variability of the phenology at the within field scale. For terroir delineation or climate change monitoring purposes, other studies focused on the phenology and its variability at meso scale (Barbeau et al. 1998; Webb et al. 2007). At the regional scale (denomination of Loire Valley, France), Barbeau et al. (1998) found differences in dates of budburst, flowering and veraison ranging from 6 to 8 days on cv Cabernet Franc. At the same scale Webb et al. (2007) in Australia and Valdés-Gómez et al. (2011) in Chile, reported the results of simulations on the effect of climate change on the phenological development of the grapevine. Under different climate change scenarios, these authors reported advances in

 budburst date ranging from 4 to 12 days for cv Cabernet Sauvignon. It is interesting to note 2 that phenology variability observed at larger scales or under scenarios of climate change is similar and comparable to those obtained at the within field scale. This observation raises new questions:

 - on the sampling strategy aiming at characterising grapevine phenology of a field at this scale. Indeed, regarding the observed within field variability, sampling quality is of paramount importance to guarantee that differences in phenology is related to macro scale factors and not to micro scale (within field) effects,

 - on management practices adaptation to climate changes. Our work showed that observed within field variability encompasses change in phenology estimated from climate change scenarios. This may demonstrate the robustness of current vine fields to climate change since within field zones may still be well adapted to the expected climate change.

### **CONCLUSIONS**

 To our knowledge, this is the first study highlighting within field spatial variability in the phenological development of the grapevine and maturation simultenaously. In our conditions, this variability proves to be spatially organised and temporal stable since advanced and delayed zones remain stable throughout the growing season (from post- budburst to harvest). It was possible to identify two well-contrasted zones of phenology and maturity within each vine field. Observed magnitude of variation was similar to the one observed at larger scales or under scenarios of climate change. These results should be considered for the sampling strategy of phenology and maturation at within field scale and for planning production activities. Differences in phenology may explain the spatial variability observed in other variables such as yield and disease development. Further investigations should determine the environmental factors which drive the observed spatial variability in phenology and maturity, and if possible propose predictive models that consider this variability.

 

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### **CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

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- **Fig. 3** Location of temperature sensors in the field 1 (cv Cabernet Sauvignon). Where: Z1
- represents zone 1 and Z2 represents zone 2
- **Fig. 4** Phenology model simulation for cv Cabernet Sauvignon (Model Cab) and cv
- 4 Chardonnay (Model Cha). Where: Lines represent the sum of GDD necessary to fulfill the state 23 in PS (Flowering).  $R^2$ : Represents the degree of fit between phenological stage (PS)
- state 23 in PS (Flowering).  $R^2$ : Represents the degree of fit between phenological stage (PS) and growing degree days (GDD)
- **Fig. 5** Maps of spatial distribution of the main phenological stages, field 1, 2009-10, 2010-
- 11, 2011-12 and 2012-13 seasons. a) Post-Budburst, b) Flowering and c) Veraison. a), b) and c) expressed in units of phenological scale (PS)
- **Fig. 6** Maps of spatial distribution of the maturation, expressed in total soluble solids (°Brix),
- field 1, 2009-10, 2010-11, 2011-12 and 2012-13 seasons. a) Pre-Ha 1 (25 days before harvest), b) Pre-Ha 2 (12 days before harvest) and c) Ha (Harvest)
- **Fig. 7** Maps of spatial distribution of the main phenological stages, field 2, 2011-12 and
- 2012-13 seasons. a) Post-Budburst, b) Flowering and c) Veraison. a), b) and c) expressed in units of phenological scale (PS)
- **Fig. 8** Maps of spatial distribution of the maturation, expressed in total soluble solids (°Brix),
- field 2, 2011-12 and 2012-13 seasons. a) Pre-Ha 1 (25 days before harvest), b) Pre-Ha 2 (12 days before harvest) and c) Ha (Harvest)
- **Fig. 9** Air temperature (a) and growing degree-days (b) for each zone (zone 1 and 2) from
- the field 1 (cv Cabernet Sauvignon). Each point on the figure represents the average of 4 sensors for each zone
- **Fig. 10** Mapping of the cluster analysis for field 1 (a and c) and field 2 (b and d). a) and b)
	- cluster analysis of the main phenological stages. c) and d) cluster analysis of the maturation (total soluble solids)
- **Fig. 11** Days difference between the defined zones (cluster analysis) according to the average of the fields, for the main phenological stages and maturation. (a) field 1 and (b) field 2. The dashed line represents the reference level (0). Each point on the figure represents the average of all seasons considered for each cultivar (4 for field 1 and 2 for field 2). Vertical bars represent the standard deviation
	- **TABLES**
- 

 

**Table 1** Field characteristics of the Cabernet Sauvignon and Chardonnay experimental fields



 



 $a$ : at the beginning of the experiment.

VSP: Vertical shoot positional

#### 5 **Table 2** Representation of the phenological scale of Eichhorn and Lorenz as modified by 6 Coombe (Coombe 1995)



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 1 2 3

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9 **Table 3** Summary of thermal time and main climatic variables, which characterize the 10 growing conditions over the 4 years of experiment. Variables are presented by 11 phenological stage (mean of the field) with Bu: Budburst, Fl: Flowering, Ve: Veraison 12 and Ha: Harvest. Cab: cv Cabernet Sauvignon and Cha: cv Chardonnay 13



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1 Mav-Ha <sup>*</sup>	15.7	15.7	1523	1385	464.6	464.6	1018	956	$-553$	$-491$
2012-2013	Cab	Cha	Cab	Cha	Cab	Cha	Cab	Cha	Cab	Cha
1 May-Bu	9.2	9.1	171	157	323.3	323.3	180	-66	143	158
Bu-Fl	14.6	14.1	297	268	85.7	85.7	220	201	$-134$	$-115$
Fl-Ve	194	19 0	597	589	41.1	41.1	338	349	$-297$	$-308$
$Ve-Ha^*$	19.0	20.0	575	509	10	10	268	237	$-258$	$-227$
$1$ May-Ha <sup>*</sup>	15.5	155	1640	.523	460.1	460	1006	952	-546	-492

 $T_{mean}$ : average mean temperature; sGDD: Sum of growing degree-days; P: Precipitation; ET<sub>0</sub>: Evapotranspiration. \* Harvest: the harvest was undertaken when the berries presented 22°Brix of total soluble solids.

10 **Table 4** Main statistics of grapevine phenology for the two fields studied

Season		Min	Max			Range		<b>SD</b>		CV(%)		
2009-10		Cab	Cab			Cab		Cab		Cab		
Post-Bu		5					0.65		10.5			
F1		20		23			0.87		3.9			
Ve		34		35			0.34		1.0			
2010-11		Cab		Cab		Cab		Cab	Cab			
Post-Bu		5				$\mathfrak{D}$	0.47		8.3			
Fl		20		26				1.47	6.1			
Ve		34		35				0.26	0.8			
2011-12	Cab	Cha	Cab	Cha	Cab	Cha	Cab	Cha	Cab	Cha		
Post-Bu	5	9	8	11	3	2	0.89	0.64	12.8	6.4		
Fl	21	22	25	25	4	3	1.35	0.69	5.6	3.0		
Ve	33	33	35	35	$\overline{c}$	2	0.54	0.52	1.5	1.5		
2012-13	Cab	Cha	Cab	Cha	Cab	Cha	Cab	Cha	Cab	Cha		
Post-Bu	7.0	7.0	11	11	4	4	1.05	1.36	12.1	15.6		
Fl	18	20	21	25	3		0.77	1.46	3.9	6.5		
Ve	34	33	35	35		2	0.28	0.97	0.8	2.9		

11 Min:minumun, Max: Maximun, SD: Standard deviation, CV: Coefficiente of variation, Range: Range of variation. Post-<br>12 Bu: Post-Budbreak, Fl: Flowering, Ve: Veraison. Post-Bu, Fl and Ve expressed in PS. Cab: cv Cabernet 12 Bu: Post-Budbreak, Fl: Flowering, Ve: Veraison. Post-Bu, Fl and Ve expressed in PS. Cab: cv Cabernet Sauvignon, field 1. Cha: cv Chardonnay, field 2.

**Table 5** Main statistics of maturation, expressed in Total Soluble Solids (TSS), for the two fields studied two fields studied

10	two fields studied	
	S <sub>anon</sub>	

14



17 Min:minumun, Max: Maximun, SD: Standard deviation, CV: Coefficiente of variation, Range: Range of variation. Pre-Ha<br>18 1: 20 days before Harvest, Pre-Ha 2: 12 days before Harvest. Ha: Harvest. Pre-Ha 1, Pre-Ha 2 and Ha

18 1: 20 days before Harvest, Pre-Ha 2: 12 days before Harvest. Ha: Harvest. Pre-Ha 1, Pre-Ha 2 and Ha expressed in °Brix.<br>19 Cab: cv Cabernet Sauvignon, field 1. Cha: cv Chardonnay, field 2.

19 Cab: cv Cabernet Sauvignon, field 1. Cha: cv Chardonnay, field 2.

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# 1  $\frac{2}{3}$

**Table 6** Parameters of semivariogram adjusted for each phenological stage evaluated in the seasons

in the seasons



 $C_0$ : Nugget.  $C_0 + C$ : Sill. a: Range. DESP (%) Degree of spatial dependence. G: Gaussian, Ex: Exponential, Sp: Spherical, 6 L: Linear. Post-Bu: Post-Budbreak, Fl: Flowering, Ve: Veraison. Post-Bu, Fl and Ve expressed in PS. Cab: cv Cabernet Sauvignon, field 1. Cha: cv Chardonnay, field 2.

### 10 **Table 7** Parameters of semivariogram adjusted for each maturation date evaluated in 11 the seasons



1



1 Co: Nugget. Co + C: Sill. a: Range. DESP (%) Degree of spatial dependence. G: Gaussian, Ex: Exponential, Sp: Spherical, L: Linear. Pre-Ha 1: 25 days before Harvest, Pre-Ha 2: 12 days before Harvest. Ha: Harvest. Pre-Ha 1 2 L: Linear. Pre-Ha 1: 25 days before Harvest, Pre-Ha 2: 12 days before Harvest. Ha: Harvest. Pre-Ha 1, Pre-Ha 2 and Ha expressed in °Brix. Cab: cv Cabernet Sauvignon, field 1. Cha: cv Chardonnay, field 2.

# 4

### 5 **Table 8** Kendall Coefficient of Concordance (W) for phenological stage and maturation 6 throughout all the seasons (4 for field 1 and 2 for field 2)



#### 7 8

9 **Table 9** Kendall Coefficient of Concordance (W) for phenological stages and maturation

10 within the seasons



## 12

0 10 20 30 40

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### **FIGURES**

### **Figure 1**



Figure 2



**Figure 3** 



Figure 4



Figure 5



















Figure 10





