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Testing the Ferguson model for the cold-hardiness of dormant grapevine buds in a temperate and subtropical valley of Chile

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Abstract

A dynamic thermal time model (DTTM) has been developed to predict cold-hardiness in dormant grapevine buds, which uses daily mean temperatures as the only input variable. However, it has been recently reported that cold-hardiness in grapevine buds depends not only on low temperatures (LTs), but also on the content of the plant phytohormone abscisic acid (ABA). An important parameter in the DTTM is the ecodormancy boundary (EDB), which represents the chilling degree days (DDc) required for the transition of the buds from endo to ecodormancy. In this study, the bud cold-hardiness of grapevines was measured by the low-temperature exotherm (LTE) in the subtropical Elqui and in the temperate Maipo valleys of Chile, and the values fitted to the DTTM. Moreover, the ABA content was determined in dormant buds throughout the dormant season. The results demonstrated that the DTTM worked better in the Maipo than in the Elqui valley, and that the content of ABA in the buds was higher in the vines grown in the Elqui valley. On the other hand, the optimized EDB parameter value varied from year to year when it was estimated as chilling accumulated, but when it was estimated as time, it remained constant in the Maipo valley. Based on the results, we conclude the following: (a) the proper functioning of the DTTM requires that the ABA content profile in the dormant buds should be kept constant from year to year, (b) the highest content of ABA in the buds collected in the Elqui valley is probably due to abiotic stresses, (c) the EDB parameter corresponds to the accumulated cold until before the buds begin their deacclimation process and not until they release from endodormancy, (d) the release of the buds from endodormancy does not depend on the accumulated cold, but on the elapsed time.

Keywords Cold-hardiness · Differential thermal analysis · Grapevine buds

Introduction

Cold-hardiness is a complex trait that involves several changes at the physiological, biochemical, and molecular levels (Wisniewski et al. 2014). The grapevine (*Vitis vinifera* L.) buds, like the buds of other deciduous fruit trees, acclimate to the cold only after they are at the state of endodormancy (Weiser 1970; Pagter and Arora 2013; Pérez and Rubio 2015; Rubio et al. 2016; Cragin et al. 2017). According to the factors that inhibits bud outgrowth, dormancy has been divided into paradormancy (PD) in which bud outgrowth is inhibited by apical dominance or correlative inhibition, endodormancy (ED) in which bud outgrowth is inhibited by intrinsic factors located within the bud, and ecodormancy (ECD) in which bud outgrowth is inhibited by environmental cues such as low temperatures (Lang 1987; Lavee and May 1997). Therefore, the entry, maintenance, and exit of the grapevine buds of the endodormancy are crucial for its process of cold acclimation (CA) and deacclimation (DA). The monitoring of the bud cold-hardiness in Thompson seedless grapevines during the autumn-winter season in the Elqui and Maipo valleys in Chile over several years gave a biphasic CA/DA curve (Rubio and Pérez 2019). During the phase of CA, the bud cold-hardiness begins to increase in mid-April, when buds are endodormant and temperatures begin to drop (Rubio et al. 2016). In the CA phase that last until the end of July, the buds become more tolerant to freezing; subsequently, a loss in freezing tolerance occurs and DA begins. This phenomenon was repeated over the years in the valleys of Elqui and Maipo in Chile (Rubio and Pérez 2019).

The DTTM has been developed by Ferguson et al. (2011) to predict the cold-hardiness of dormant grapevine buds, and

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further improved by the same authors (Ferguson et al. 2014). The DTTM uses daily mean temperatures as input variable to drive daily changes in the cold-hardiness of dormant buds, and assumes the following: (a) temperatures affect differently the cold-hardiness of buds depending if they are in the endo or ecodormancy state, (b) the transition of the buds from endo to ecodormancy requires the accumulation of a certain amount of chilling, which is represented by the parameter named ecodormancy boundary (EDB) that is calculated as chilling degree days (DDc). If the parameter EDB represents the amount of chilling required for the transition of the buds from the endo to the ecodormancy, the value of the EDB should remain constant for the same cultivar. It has recently been reported that the plant hormone abscisic acid (ABA) delays the release of grapevine buds from endodormancy (Zheng et al. 2015; Vergara et al. 2017), and that synergistically increases the cold-hardiness of grapevine buds in combination with LTs (Rubio et al. 2019). Therefore, changes in the endogenous content of ABA, either due to drought or other stress, the bud cold-hardiness could be altered and make the model not work well. Additionally, it is postulated that the EDB in the DTTM represent the time required by the buds to begin their process of deacclimation to cold, and not the chilling accumulated required to release the buds from the endodormancy, and it should be estimated by a timer instead of a cold counter.

Materials and methods

Temperature measurements

The temperature data were recorded from the automatic meteorological stations "Intihuasi" located in Elqui valley (30°2′ S, altitude 1063 m), and "La Platina" located in the Maipo valley (33°27′S, altitude 570 m) between 1 April and August 31 of 2013, 2014, 2015, 2016, and 2017. The experimental vineyards were located at 300 and 200 m distance, respectively, from the meteorological stations. Because temperatures started to decrease during early April in both valleys and the LTE did not vary before that time, temperature records started on April 1 and ended in late August before budbreak when a single exotherm was obtained. The daily mean temperature (T_{mean}) was estimated as the average of the minimum (T_{min}) and maximum (T_{max}) daily temperatures provided by the respective weather stations. Daily degree days (DDc) were calculated on base 10, which means that temperatures of 0–10 °C are considered for the calculation. The chilling accumulated during the endodormancy period for the years 2014, 2015, and 2016 calculated as DDc on base 10 were 124.4, 60.5, and 71.4 for the Maipo valley and 61.5, 31.7, and 20.4 for the Elqui valley, respectively.

Bud cold-hardiness

The cold-hardiness of Thompson seedless buds was measured by differential thermal analysis (DTA) using cane samples collected in a vineyard located in a temperate valley (Maipo 33°27′S) and in a subtropical valley (Elqui 30°2′S) throughout the dormancy period (1st April to 27th August) (Vergara and Pérez 2010) during the years 2013, 2014, 2015, 2016, and 2017. The sampled buds correspond to basal buds located between positions 5 and 10, because they are usually fertile for this variety. Although in the Elqui valley, grapevines are typically treated with hydrogen cyanamide in commercial vineyards, in this study, they were not treated for comparative reasons. Exotherms were determined in single buds by DTA as described in Rubio et al. (2016). Data were collected at intervals of approximately 2 weeks, and the LTE values for each date correspond to the average of 16 biological replicates of single buds, and bars correspond to standard deviation.

ABA determinations

Because it has been reported that the bud LTE depends on the LTs and ABA content (Rubio et al. 2019), we measured the ABA content in buds of Thompson seedless grapevines grown in the Elqui and Maipo valleys in order to better interpret the LTE results. The ABA content was determined in dormant Thompson seedless grapevine buds collected in the Elqui and Maipo valleys every 2 weeks, between mid-April and late August 2016. Approximately, 10 buds were collected per date, and separated in groups of three to obtain three biological replicas. The extraction, purification, and determination of ABA were performed as described by Vergara et al. (2017). A GC-2014 model Shimadzu gas chromatograph was used, equipped with an electron capture detector (ECD-2014, Shimadzu Corporation, Kyoto, Japan) and computer integrator for ABA quantification (Veemer et al. 1987).

Optimization of the parameters and evaluation of the DTTM

The initial parameters used in the DTTM were taken from Ferguson et al. (2014). The non-linear GRG method of the Excel Solver plug-in was used to minimize the mean square error (RMSE) between the observed and calculated LTE values, in order to select the set of model parameters that best fit the measured LTE values in each subset of data. Three subsets of data corresponding to the years 2015 and 2016 of (1) Maipo-Elqui, (2) Maipo, and (3) Elqui were used to optimize the parameters. The best fit was obtained with the Maipo 2015 and 2016 subset of data, and the optimized parameters obtained with this subset of data was subsequently used to evaluate the performance of the DTTM. For this purpose, the LTE data corresponding to the Maipo 2013 and 2014

and Elqui 2014 and 2017 were calculated with the above parameters, and the efficiency index (EF), a normalized statistic that determines the proportion of variance explained by the model (De la Fuente et al. 2015), and the root mean square (RMSE), between the observed and calculated LTE were used to measure the accuracy of the model.

EDB parameter

The limit of ecodormancy (EDB) is an important parameter in the DTTM, which represents the DDc required for the transition of the buds from endo to ecodormancy. This parameter was optimized by fixing the value of all other DTTM parameters with their optimized values (Maipo 2015 and 2016), and iterating only the EDB value until it reaches a minimum RMSE for the observed and calculated LTE values. The EDB parameter was estimated as DDc and elapsed time was optimized using the subset of Maipo 2013 and 2014, and of Elqui 2014 and 2017 LTE data.

Results

ABA content during the endodormancy of Thompson seedless grapevine buds grown in the Maipo and Elqui valleys

The content of ABA in the buds of Thompson seedless grapevines grown in the valleys of Maipo and Elqui was determined every 2 weeks between the months of April and August of 2016, which corresponds to its endodormancy period (Vergara and Pérez 2010). In both valleys, a peak of ABA was detected between the end of April (Maipo) and the beginning of May (Elqui), and the ABA content was higher in the grapevine buds cultivated in the subtropical Elqui than in the temperate Maipo valley during most of the period analyzed (Fig. 1). This difference in the content of ABA in the buds of Thompson seedless grapevines cultivated in the Elqui and Maipo valleys could be due to differences in the agronomic management of the crop, and/or differences in soil irrigation. It is necessary to highlight that in the Elqui valley, the buds were collected from different vineyards in different seasons. While in the Maipo valley, the buds were collected from the same vineyard during all the seasons, and the agronomic management of the crop was always the same.

Optimization of the DTTM parameters in the Elqui and Maipo valleys

The parameters for the DTTM were optimized using three subsets of LTE data corresponding to (1) Maipo-Elqui 2015 and 2016, (2) Maipo 2015 and 2016, and (3) Elqui 2015 and 2016. As expected, only small variations in the values of the
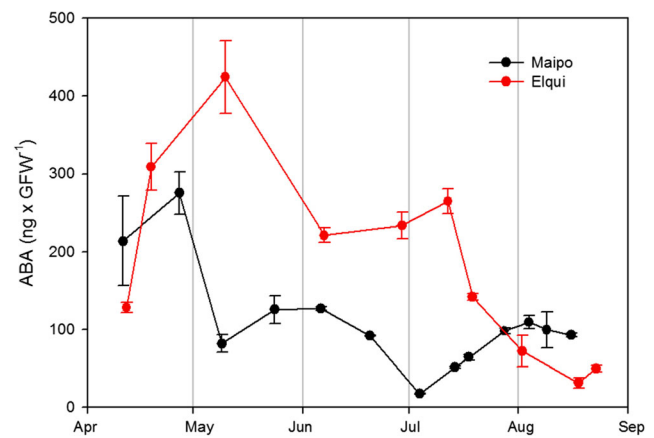

Fig. 1 ABA content in Thompson seedless buds grown in the Maipo and Elqui valleys during the year 2016 throughout their natural dormancy cycle. The ABA content was determined by gas chromatography coupled to an electron capture detector (ECD). Values correspond to the average of three biological replicates; bars represent SD

optimized DTTM parameters were observed when different subsets of LTE data were considered (Table 1). The best fit was achieved with the subset of LTE data corresponding to years 2015 and 2016 of Maipo valley (Table 1) and, therefore, those parameters were further used for the simulation studies. When the optimized parameters of the DTTM for Thompson seedless were compared with those of Cabernet Sauvignon and Chardonnay obtained in the Northern Hemisphere (Ferguson et al. 2014), a large difference in the value of the EDB parameter was observed (Table 1).

Evaluation of the DTTM in the Elqui and Maipo valleys

The LTE values for the years 2013 and 2014 of the Maipo valley and 2014 and 2017 of the Elqui valley were simulated using the optimized set of parameters established before, in order to evaluate the DTTM (Table 1). The model accuracy was tested by estimating the RMSE between the predicted versus observed LTE values, and the lowest RMSE was obtained for the Maipo 2014 and 2017 LTE data, indicating that the DTTM works better in the temperate Maipo than in the subtropical Elqui valley (Table 2).

The EDB parameter

The EDB parameter was optimized by maintaining the value of all the other parameters fixed, and iterating its value step by step until reaching the lowest RMSE between the observed and calculated LTE values. The results indicated a great variation in the value of the optimized EDB parameter between the 2 years analyzed, in the Maipo and Elqui valleys (Table 3). However, when the EDB parameter was estimated as elapsed time instead of accumulated cold, its optimized value was similar for the 2 years analyzed in the Maipo valley but different for the Elqui valley (Table 3). In addition to the RMSE,

Table 1 Optimized parameters setting for the DTTM by taking three subset of 2015–2016 LTE data of Thompson seedless grapevine grown in (1) Maipo-Elqui (ME), (2) Maipo (M), and (3) Elqui (E). *Optimized parameters for Cabernet Sauvignon and Chardonnay grown in WA, USA (Ferguson et al. 2014)

Locations	k_{a1}	k_{d1}	Th_1	k_{a2}	K_{d2}	Th_2	EDB	h_{max}	θ	RMSE
Maipo-Elqui	0.1	0.001	15.5	0.02	0.09	9.9	−20	−22	1.4	1.92
Maipo	0.1	0.001	15.1	0.08	0.27	11.2	−20	−22	1.2	1.68
Elqui	0.1	0.001	15.9	0.001	0.09	10.6	−18	−22	1.3	1.75
*WA, USA (CS)	0.12	0.08	13.0	0.1	0.1	5.0	−700	−25.1	7	1.5
*WA, USA (Char)	0.1	0.1	14.0	0.1	0.02	3.0	−600	−25.7	7	1.4

the efficiency index (EF) was calculated between the observed and calculated values (Table 3). Moreover, when the LTE was simulated with the optimized EDB values for the Elqui and Maipo valleys, the simulation for the Maipo valley (Fig. 2; Table 3) was better than for the Elqui valleys (Fig. 3; Table 3). Moreover, there was a great difference in the simulation of the LTE values of the year 2017 in the Elqui valley when the EDB was estimated as chilling accumulated (RMSE = 2.59) and as time elapsed (RMSE = 1.15) (Fig. 3; Table 3).

Discussion

Assessment of the DTTM in the Elqui and Maipo valley of Chile

The DTTM was originally developed in the northern hemisphere where low temperatures are persistent during winter. Our results in the Elqui and Maipo valleys of Chile, where winter temperatures are not so low, revealed that the DTTM works better in a temperate valley like the Maipo than in a subtropical one like Elqui. Two main reasons can explain this difference in the behavior of the DTTM. The first one is related to the climatic differences between both valleys: The temperate Maipo valley is characterized by a gradual decrease in temperatures between April and July, a phenomenon that is very regular from year to year. The subtropical

Elqui valley is characterized by a large fluctuation of winter temperatures during day and night. The second one is related to differences in crop management: The buds of the Maipo valley were always sampled from the same vineyard, which was managed similarly throughout the different seasons in which the LTE measurements were made. On the contrary, the buds of the Elqui valley were sampled from different vineyards in the different seasons in which the LTE measurements were made. Climatic and management reasons can affect the endogenous content of ABA in the grapevine buds, and as recently demonstrated, the LTE of the grapevine buds depends on both the low temperatures and their ABA content (Rubio et al. 2019). In the temperate valley of Maipo, where winter temperatures decrease similarly each year, and the bud samples were always taken from the same vineyard, it seems reasonable that the pattern of ABA's annual content does not vary significantly from year to year; therefore, the LTE will depend mainly on the low temperatures, which allows the DTTM to work well. The same can reasonably occur in the northern hemisphere where the DTTM was originally

Table 2 Evaluation of the DTTM model. The LTE values for Maipo 2013, 2014 and Elqui 2014, 2017 were calculated using the optimized set of parameters and compared with the observed LTEs

Location	year	*RMSE (M 2015–2016)
Maipo	2013	1.51
	2014	1.66
Elqui	2014	6.62
	2017	4.55

*Root mean square error (RMSE) between observed and calculated LTE values, the LTE values were calculated using optimized parameters obtained with 2015–2016 LTE dataset from the Maipo valley

Table 3 Variations of the EDB parameter estimated as (a) chilling degree days and (b) time elapsed in the Maipo valley during the years 2013 and 2014 and in the Elqui valley during the years 2014 and 2017. The set of optimized parameters for the DTTM were maintained fixed, and the EDB value was optimized step by step until reaching the lowest RMSE between calculated and observed LTE values. The efficiency index (EF), a statistic that determines the proportion of variance explained by the model, was also estimated

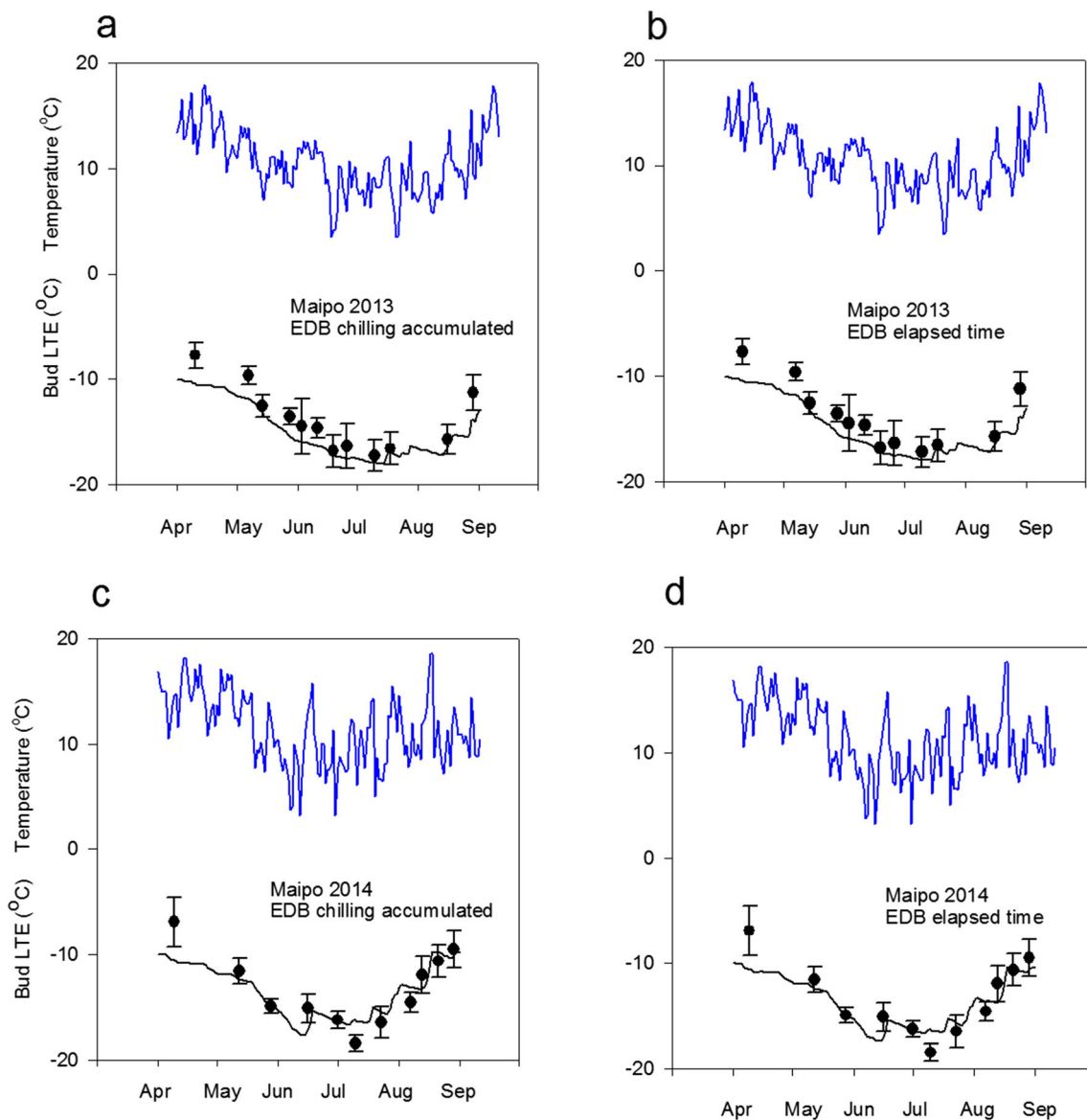| Location	year	EDB	RMSE	EF
[a]Maipo	2013	−15	1.43	0.705
	2014	−35	1.63	0.701
[b]Maipo	2013	73	1.41	0.714
	2014	70	1.60	0.701
[a]Elqui	2014	−60	2.03	−6.55
	2017	−22	2.59	−0.067
[b]Elqui	2014	120	2.18	−0.48
	2017	50	1.15	0.31

Fig. 2 Daily mean temperature, and observed and calculated bud cold-hardiness (expressed as low-temperature exotherms LTE) of Thompson seedless grown in the temperate Maipo valley in two dormant seasons 2013 (**a**, **b**) and 2014 (**c**, **d**). The EDB parameter was estimated as chilling accumulated (**a**, **c**) and elapsed time (**b**, **d**), and was optimized by fixing all the DTTM parameters (see "Material and Methods") and leaving free the value of the EDB parameter and minimizing the RMSE between the observed and calculated LTE values. The observed LTE values (points) were determined by differential thermal analysis (DTA) and represent the average of 16 buds; bars correspond to the SD

developed. On the contrary, in the subtropical valley of Elqui, where there is a great temperature fluctuation between day and night during winter, as well as the samples of the buds were taken from different vineyards in each season, the annual pattern of ABA must be different from year to year, and therefore, as the value of LTE depends on both the low temperatures and ABA content, the DTTM will not be able to predict it well; it is because it only considers daily mean temperatures as its input (Ferguson et al. 2011). Furthermore, ABA determinations will be necessary to test this hypothesis.

Cold-hardiness and ABA content in Thompson seedless grapevines grown in the Elqui and Maipo valley of Chile

It is possible that the higher ABA content found in the grapevine buds of Thompson seedless sampled in the Elqui valley relative to those sampled in the Maipo valley is due to the greater abiotic stresses that they are subjected to. This greater stress is probably due to climatic conditions and/or agronomic management, both of which can influence the water condition of the vine and thus increase its ABA content (Daszkowska-

Golec 2016). Due to the higher ABA content in the grapevine buds sampled in the Elqui valley, it should be expected that they will reach a lower LTE than those sampled in the Maipo valley (Rubio et al. 2019). However, this did not happen (Fig. 3) probably due to the existence of high temperatures that nullify the effect of low temperatures so that the chilling accumulated is significantly reduced in the Elqui valley (Pérez et al. 2008).

The significance of the EDB parameter in the DTTM

In order for the CA/DA transition of grapevine buds to occur, they must first be released from the endodormancy, and then the temperature rises. In our case, the transition of grapevine

buds from endo to ecodormancy coincides with their CA/DA transition (Rubio et al. 2016). However, in the northern hemisphere, where winter temperatures are cooler, the transition of the buds from the endo to the ecodormancy does not coincide with their CA/DA transition, and the buds remain cold acclimatized for a month or more after being released from endodormancy due to the low temperatures (Cragin et al. 2017). Under this climatic condition, the EDB values for the different genotypes varied between − 300 and − 700 °C (Ferguson et al. 2014), which is a much higher value than what we obtained in our study in the southern hemisphere. Therefore, we conclude that the EDB parameter in the DTTM corresponds to the chilling accumulated until before
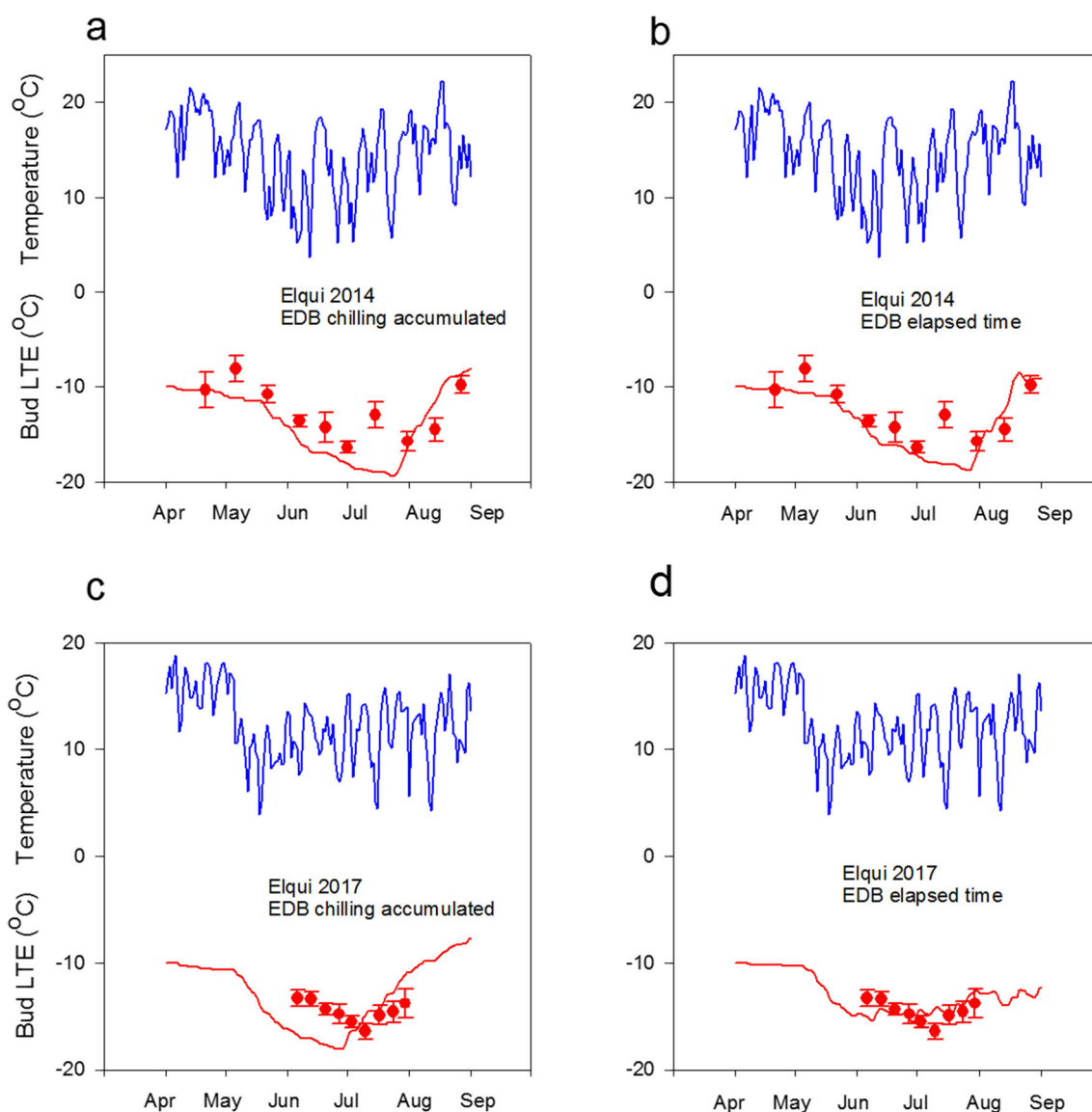

Fig. 3 Daily mean temperature, and observed and calculated bud cold-hardiness (expressed as low-temperature exotherms LTE) of Thompson seedless grown in the subtropical Elqui valley in two dormant seasons 2014 (**a**, **b**) and 2017 (**c**, **d**). The EDB parameter was estimated as chilling accumulated (**a**, **c**) and elapsed time (**b**, **d**), and was optimized by fixing

all the DTTM parameters (see "Material and Methods") and leaving free the value of the EDB parameter and minimizing the RMSE between the observed and calculated LTE values. The observed LTE values (points) were determined by differential thermal analysis (DTA) and represent the average of 16 buds; bars correspond to the SD

the beginning of the CA/DA transition of the buds and not to the chilling accumulated required for the transition of the buds from the endo to the ecodormancy, which occurs much earlier in the northern hemisphere (Cragin et al. 2017).

Estimation of the EDB parameter as chilling accumulated or time elapsed

If the EDB parameter in the DTTM corresponds to the chilling accumulated by the grapevine buds until before the beginning of its CA/DA transition, then its value will depend on the chilling accumulated necessary to release the buds of the endodormancy along with the chilling that accumulates up to the start of the temperature rise. The value of the EDB parameter is higher in the northern hemisphere as the increase in temperatures takes longer than in the southern hemisphere. For the same variety grown in the same place, the value of the EDB parameter should not vary from 1 year to the next, if the chilling required to release the buds of the endodormancy and the cold that accumulates until the beginning of the increase in temperatures remain unaltered. However, in our case, where the transition of the buds from the endo to the ecodormancy coincides with the CA/DA transition (Rubio et al. 2016), the optimized EDB value varied from year to year when it was estimated as chilling accumulated. But when the EDB was estimated as time elapsed, it remained constant in the Maipo valley during the 2 years analyzed. This could be due to the fact that the transition of the buds from the endo to the ecodormancy does not depend on the chilling accumulated but on the time elapsed. It is interesting to highlight the fact that the DTTM predicts that grapevine buds should take about 70 days to release from endodormancy in the Maipo valley (Table 3). The time it takes for the buds of other grapevine varieties grown in the northern hemisphere to release endodormancy is 60–70 days (Cragin et al. 2017), which suggests that the release of grapevine buds from endodormancy depends on time. Interestingly, the downregulation of ABA biosynthesis genes and the decrease in ABA content seem to play a key role in the loss of cold-hardiness in grapevine buds (Kovaleski and Londo 2019; Rubio and Pérez 2019). This decrease in ABA content can be developmentally regulated, and therefore, depend on time. Accordingly, in field trials conducted in the Elqui and Maipo valleys, we found that the CA/DA transition of Thompson's buds, which coincides with their transition from the endo to the ecodormancy (Rubio et al. 2016), always occurred in early July, regardless of the chilling accumulated each year (Rubio and Pérez 2019). In this study, we found that in the Maipo valley, where the value of the EDB parameter does not vary from 1 year to another when it is estimated as time, the ABA content after reaching a maximum in late April or early May decreases gradually until reaching a minimum in July, which coincided with the release of bud of endodormancy. Therefore, the time it takes for the ABA once

it reaches its maximum level to be reduced to a minimum level could be related to the time it takes for the buds to be released from endodormancy, which in our case, is represented by the EDB parameter. If the ABA levels vary from year to year due to abiotic stresses, as is probably the case in the Elqui valley, then the time it takes for the buds to release from endodormancy will vary from year to year and will be reflected in the value of the EDB parameter.

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