


# Macro- and microclimate conditions may alter grapevine deacclimation: variation in thermal amplitude in two contrasting wine regions from North and South America

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**Abstract** Low temperature is a limiting factor that affects vineyard distribution globally. The level of cold hardiness acquired during the dormant season by *Vitis* sp. is crucial for winter survival. Most research published on this topic has been generated beyond 40° N latitude, where daily mean temperatures may attain injurious levels during the dormant season resulting in significant damage to vines and buds. Symptoms of cold injury have been identified in Mendoza (32–35° S latitude), a Southern Hemisphere wine region characterized by a high thermal amplitude, and warm winds during the dormant season. These symptoms have usually been attributed to drought and/or pathogens, but not to rapid deacclimation followed by injurious low temperatures.

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Because local information on meteorological events as probable causes is scarce, this research was designed to test and study this assumption by comparing macro-, meso-, and microclimatic data from Mendoza, Argentina, and eastern Washington, USA. The goal was to unveil why freezing damage has occurred in both regions, despite the existence of large climatic differences. Because environmental parameters under field conditions may not correspond to data recorded by conventional weather stations, sensors were installed in vineyards for comparison. Microclimatic conditions on grapevines were also evaluated to assess the most vulnerable portions of field-grown grapevines. In order to better understand if it may be possible to modify cold hardiness status in a short period with high thermal amplitude conditions, deacclimation was induced using a thermal treatment. Hence, despite the fact that Mendoza is warmer, and temperatures are not as extreme as in Washington, high daily thermal amplitude might be partially involved in plant deacclimation, leading to a differential cold hardiness response.

**Keywords** Cold hardiness · Deacclimation · Thermal amplitude · Grapevine · Mendoza · Washington state

## Introduction

Climate affects the distribution of vineyards, resulting in localization of the main areas of viticulture between 30 and 50° N and 30–40° S. These latitudes define areas corresponding approximately to isotherms between 10 and 20 °C. As a perennial temperate plant, the physiology of this climbing plant is adapted to a marked seasonality modulated by temperature. Thus, warm conditions during the growing season (GS, spring to autumn) are crucial for plant development and fruit production, while during autumn-winter, the cessation of growth

leads to a dormant season (DS). During the DS, air temperature usually falls below the freezing point, causing damages to vulnerable tissues. This situation is one of the main causes of yield losses and/or plant death in grapevines (Fennell 2004).

Two factors, plant cold hardiness (CH) and temperature, interact to determine tissue damage by freezing. Most research has focused on CH, since this phenomenon constitutes a dynamic and complex trait acquired in response to a shortening photoperiod and declining temperature in autumn (Howell and Shaulis 1980; Fuchigami et al. 1982; Wisniewski et al. 1996). Three different stages are identified for CH: (i) acclimation, when the plants gain CH; (ii) deacclimation, when the plants lose CH; and (iii) re-acclimation, when the plants regain CH after temporary deacclimation (Levitt 1980). Rates of acclimation and deacclimation vary dynamically during the DS, and are reversible (Damborská 1978; Wolf and Cook 1992; Gu et al. 2002). CH is a reversible process; therefore, a cold hardy plant can deacclimate and then re-acclimate depending on temperatures. This process differs with species, cultivar, phenology, organ, weather (Xin and Browse 2000; Gusta and Wisniewski 2013; Pagter and Arora 2013), and crop management (Wample and Wolf 1996).

Temperature, the second factor, is a complex parameter and is highly variable in time and space, affecting biological systems directly (Eagles 1989; Sage and Kubien 2007). This factor can be considered at different spatial scales: macroclimate (a regional scale of tens to hundreds of kilometers), mesoclimate (a vineyard scale of tens to hundreds of meters), and microclimate (the specific environment around any plant) (Robinson 2006). Traditionally, the following limited number of parameters has been measured to estimate thermal condition at the three mentioned scales: (i) maximum temperature ( $T_{max}$ ), (ii) minimum temperature ( $T_{min}$ ), (iii) daily mean temperature ( $T_{mean}$ ), (iv) daily thermal amplitude (TA), and (v) relative humidity (RH). These parameters are traditionally obtained from conventional weather stations (CWS), which according to international standards consist in a white woody shelter located at 1.4 m above the soil and sensors confined mostly under the shield to protect them from the influence of precipitation and direct radiation. Moreover, these stations are often located at a considerable distance from a vineyard. This combination of elements often leads to under- or overestimation of the real thermal environment to which a plant is subjected to which may result in misinterpretation and/or minimization of the influence of temperature on plant damage.

In the Northern Hemisphere, most vineyards beyond 40° N are affected by recurrent climatic contingencies due to freezing damage. This implies a direct impact on the growth management (Mills et al. 2006; Cragin et al. 2017; Hamman et al. 1996; Fennell 2004; Keller and Mills 2007), and a permanent real-time monitoring and measurement of CH (<http://wine.wsu.edu/research-extension/weather/cold-hardiness>). On the other hand, in the Southern Hemisphere, most vineyards are

located in maritime regions (e.g., New Zealand, Chile, Australia, and South Africa) with low incidence of freezing damage (Mullins et al. 1992). Therefore, there are scarce regional data concerning CH in this hemisphere, as regards vineyards or any other temperate fruit crops (Supplementary Fig. 1). Mendoza Province (MZA), the main grape producing region of Argentina, is an exception to this environmental condition, as evidenced by a high level of continentality and altitude (Table 1).

Typical symptoms of cold injury include lack of and/or uneven bud break, death of canopy, under soil plant re-sprouting, and trunk cracking (Brusky Odneal 1983; Goffinet 2004). In Mendoza, these symptoms have also been observed but have been attributed to drought and pathogen attack because freezing damage is not considered to be a critical factor in vineyards. This occurs firstly, because there is little available information about the range of CH in grapevine cultivars from local vineyards, which are usually extrapolated from studies conducted in the Northern Hemisphere. Secondly, most of the microclimatic field conditions are estimated by extrapolation from data generated in CWS and not from temperature sensors installed at a plant level. Finally, variability among years, in winter temperature, makes it difficult to associate the dormant with the symptoms of cold damage by producers and agronomist.

**Table 1** Comparison of climatic and geographical condition between MZA and WA

Item	MZA	WA
Latitude (°)	32–35 S	46–49 N
asl (m)	766	117
Köppen-Geiger classification	BW.k—arid, desert, cold arid	BS.k—arid, steppe, cold arid
Annual precipitation (mm)	213	204
Pluvial regime	Summer	Winter
Annual temperature average (°C)	16.4	12
Sea of influence	Pacific	Pacific
Range of influence	Los Andes	Cascade
Wind condition	Lee	Lee
Mean altitude of range (m)*	4000	1300
Width (km)*	150	70
Distances foothills to vineyards (km)	20	130
Continuity	Continuous	Discontinuous
Continental index (Ivanov)	Continental climate (165.3)	Slightly continental climate (122.7)
Continental index (Conrad; %)	26.9	10.6

Sources: Climatic-data.org, 2017; Google Earth, 2017

\*measurement at the study region

MZA is located at the center-western side of the country near the foothills of the Andes Range, whereas the wine growing region of eastern Washington (WA), USA, is an area located between the Cascade Ranges to the west and the Rocky Mountain Range to the east (Table 1). Both regions show orographic similarities and are subject to periodic warm winds from the Pacific Ocean locally named Zonda in Argentina and Chinook in the USA. These winds are strong and warm and are associated with adiabatic compression upon descending the eastern slopes of mountain ranges (Norte and Simonelli 2016). Both, Zonda and Chinook, mainly occur during the DS, provoking unseasonal warming periods. Moreover, after the windy days are often followed by hard freezing events. However, it is not clear how these atemporal warming events influence CH.

This research aimed to compare climate conditions between MZA and WA during the DS in order to understand differences and similarities and unveil why there is freezing damage in both regions despite their differences. This comparison was performed at three scales: (i) macroclimate, using the 10-year climate data recorded from CWS ( $T_{\max}$ ,  $T_{\min}$ , HR, and TA); (ii) mesoclimate, evaluated by comparing daily field temperature measurements with data recorded by CWS in order to evaluate its reliability and accuracy to predict the real field conditions; and (iii) microclimate, evaluated at plant level, to determine the most vulnerable portions of the plant to damage. The second objective was to evaluate how TA during the DS may determine loss of CH in cold-acclimated plants. For this purpose, short-term experiments with acclimated plant exposed to different TA treatment for 24 h were performed in order to measure the rate of deacclimation following exposure of grapevine to different TA.

## Materials and methods

### Macroclimate conditions in MZA and WA

The meteorological conditions during the vineyard DS (autumn-winter period) at the regions of MZA and WA were evaluated for 10 years (2002–2012) using data collected in two official weather stations: a DACC (Direction of Climate Contingencies) station located at INTA La Consulta, San Carlos, Mendoza Province, Argentina (33° 43' 10" S lat; 69° 69' 0.6' 16" W long) and a Washington State University, AgWeatherNet (<http://weather.wsu.edu>), station located at the Irrigated Agriculture Research and Extension Center (IAREC) Headquarter, Washington, USA (46° 15' 25" N; lat, 119 44' 25" W long). To standardize data from Northern (NH) and Southern Hemispheres (SH), the beginning of autumn was considered as day 1 of the DS. Daily parameters evaluated in this study were  $T_{\max}$ ,  $T_{\min}$ , TA, and RH. DS was broken down into three different stages: early DS

corresponding to April–May in SH and October–November in NH, middle DS corresponding to June–July in SH and December–January in NH (around the winter solstice), and late DS corresponding to August–September in SH and February–March in NH.

### Comparison between temperature measurements in the field and CWS

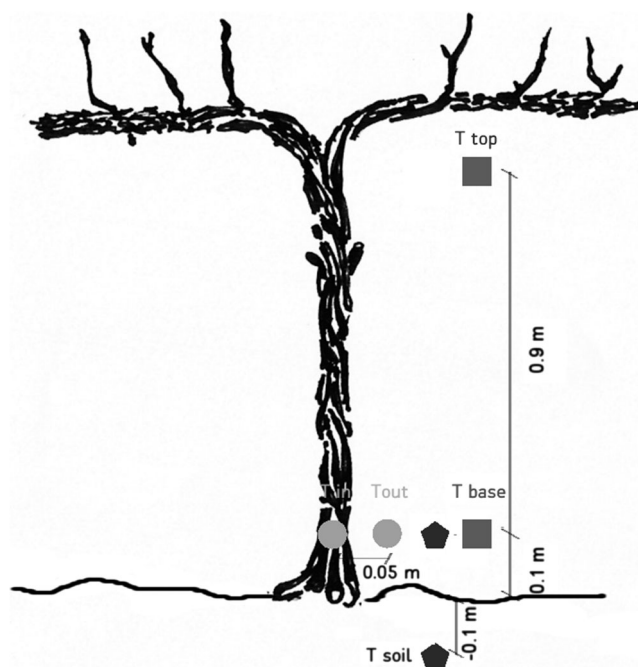
The characterization of field microclimatic conditions was done in vineyards belonging to the Plant Physiology Department of the Faculty of Agricultural Sciences (UNCuyo) in Luján de Cuyo, MZA (33° 0' 29 60" S latitude; 68° 52' 21" W longitude) and to the IAREC in Prosser, WA (46.2° 15' 25" N latitude, 119 44' 25" ° W longitude) during the DS of 2013 and 2012, respectively.

To determine temperature differences between field and CWS from both locations, the same equipment was used to record field data (Thermochron DS1922L-F5 iButton temperature loggers, Maxim Integrated, San Jose, CA, with a measurement range of  $-40$  to  $+125$  °C, and accuracy  $\pm 0.5$  °C). At each vineyard site, three sensors were installed adjacent to grapevine trunks at 40 cm above the soil and protected from solar radiation by reflector panels. Temperatures were recorded hourly during February–March 2012 in WA, and during July–August 2013 in MZA and used to calculate daily  $T_{\min}$  and  $T_{\max}$ . Data were recorded for at least 15 days in the middle DS, and repeated three times in each location. To compare temperatures at field conditions with those registered from CWS located nearby the vineyards (500 m approximately), daily maximum and minimum temperatures for the same time period were also obtained. In the case of vineyards from MZA, data were obtained from the weather station of the Faculty of Agricultural Sciences (33° 00' 18" S latitude; 68° 52' 13" W longitude) whereas for those from WA, the weather station was the IAREC Headquarter described previously.

### Plant microclimatic conditions

In order to characterize microclimatic conditions close to grapevines during the DS, three independent assays were conducted, as indicated in Fig. 1: (a) air thermal differences between the top ( $T_{\text{top}}$ ) and the base ( $T_{\text{base}}$ ) of the plant were measured with sensors located at 1 and 0.1 m above the soil, respectively; (b) above the soil versus below the soil thermal differences with sensors installed near the plant at 0.1 m above the soil ( $T_{\text{base}}$ ) and 0.1 m below soil ( $T_{\text{soil}}$ ); and (c) internal trunk temperature ( $T_{\text{in}}$ ) versus air temperature near the plant ( $T_{\text{out}}$ ), with sensors installed 0.1 m above the soil, one inside the trunk and the other placed at 0.05 m from the plant.

Because iButtons could not be placed in the trunk, thermocouples were set up in the internal portion of the trunk by drilling a hole of 1 mm in diameter and 10 mm deep. Then,



**Fig. 1** Grapevine microclimate characterization during the dormant season in Mendoza, Argentina (MZA) and Washington, USA (WA). Three independent assays were conducted to determine (a) air thermal differences between the top ( $T_{top}$ ) and the base ( $T_{base}$ ) of the plant, with sensors (filled circle) located at 1 and 0.1 m, respectively; (b) soil versus air thermal differences with sensors (filled square) installed near the plant at 0.1 m above the soil ( $T_{base}$ ) and  $-0.1$  m below soil ( $T_{soil}$ ); (c) internal trunk temperature ( $T_{in}$ ) versus air temperature near the plant ( $T_{out}$ ) with sensors installed 0.1 m above the soil, one inside the trunk and the other at a distance of 0.05 m from the plant

a T-type thermocouple (copper-constantan; measurement range  $-40$  to  $+125$  °C, accuracy  $\pm 0.1$  °C) was inserted at the level of the trunk phloem (5 mm deep) and then covered with tape. Thermocouple data were collected by data loggers of different trademarks but with the same functions: Cava Devices, four channels in Argentina and Campbell Scientific CR10 in USA. All temperature measurements were done in three different adjacent plants of *Vitis vinifera* cv. Malbec in MZA and *Vitis labrusca* cv. Concord in WA. Plants of each cultivar had trunks of similar diameter (between 4 and 5 cm) and were of similar age (8 to 10 years old). Daily measurements were divided into two parts, day and night, and daytime measurements were taken from 8:30 a.m. to 7:00 p.m. in MZA and from 7:30 a.m. to 5 p.m. in WA.

### Effect of high TA on deacclimation of buds and cane tissues

For this experiment, 12-year-old field-grown grapevines *Vitis vinifera* cv. Chardonnay and cv. Merlot were sampled in the IAREC vineyards. Twenty plants per cultivar were selected, and we cut off one full cane from each above the fourth node to preserve the more basal nodes for spur pruning. Only the

buds 4 through 8 were used and divided into buds and internode sections, which were mixed and randomized for freezing treatments ( $n = 20$  for buds section and  $m = 8$  for internodes). Each cane was split into two parts: the bud and the internode sections. The bud section consisted in the bud itself and nearly 2 mm of subtending cane tissue above and below the bud (Andrews et al. 1984; Wolf and Pool 1987). The internode section of the cane consisted in 2-cm-long cane tissue without the bud section. Plant material was obtained after sunrise (9:00 a.m.). Canes were placed in plastic bags with a wet paper towel inside to prevent dehydration. Also, during both transport and preparation of samples in the laboratory, temperatures were maintained slightly above 0 °C to avoid heating.

Three treatments simulating different temperature changes prior to freezing conditions were applied to determine the deacclimation temperature of buds and internode sections. In all treatments, the temperature at the sampling time was around 0 °C. The treatments were (a) control: sections without any thermal treatment and evaluated after collection; (b) low TA: sections maintained at 7 °C in a fridge for 24 h; and (c) high TA, maintained at 30 °C in a heated cabinet for 24 h.

Following thesis treatment, CH of grapevine buds and cane tissues from each treatment was estimated by the differential thermal analysis (DTA) method, based on the low-temperature exotherms (LTE). This method was showed by Mills et al. (2006) and correlated very closely with visual damage of cane phloem and xylem, based on the browning tissue. Briefly, the DTA consists of a chamber of thermoelectric modules (TEM) integrated with a commercially available programmable freezer and data acquisition system (DAS). TEM consist in 40 channels of Peltier plate model CP1.4-127-045L (Melcor Corporation, Trenton, NJ) that senses exotherms produced when water or tissues freeze in each channel and converts this thermal signals at an output voltage (mV). The programmable freezer model T2C (Thermal Product Solutions, Williamsport, PA) was equipped with a temperature controller model 44212; (YSI, Dayton, OH) that senses freezer temperatures. All measurements from individual TEM channels and temperature were acquired and recorded at 15-s intervals in DAS, that consist in a multi-parameter data logger Keithley model 2700-DAQ-440 (Keithley Instrument, Cleveland, OH). The low-temperature exotherms corresponding to 10% phloem injury and 10% xylem injury, respectively, were determined as shown in Supplementary Fig. 2. The DTA data for grapevine bud and internode sections had previously been shown to correlate closely with visual damage as determined by tissue browning (Mills et al. 2006).

Samples were placed directly on TEM chambers. From each treatment, 20 buds were placed with the cut surface facing up; whereas in the case of cane tissues, eight of such cane pieces were placed directly on each TEM. The rate of descend for freezing simulation was the standard for DTA method being 4 °C/h (Mills et al. 2006). It was demonstrated that this

descend rate is statistically similar over cold injuries if compared with the real rates observed in field (Haynes et al. 1992).

Lethal temperatures for buds were reported as bud  $LT_{10}$ , (i.e., lethal temperatures at which 10% of the buds are killed; Andrews et al. 1984). For internode sections, lethal temperatures were reported as phloem  $LT_{10}$  and xylem  $LT_{10}$ .

### Statistical analysis

The statistical analysis was performed with R (R Core Team 2013) and Infostat software v. 2016 (Di Rienzo et al. 2016). For the analysis of macroclimatic conditions of the two contrasting locations (MZA and WA), a generalized linear mixed model (GLMM) method was used. The final model included the standardized day of year and location as fixed effect, and year as a random effect. Data were partitioned according to the DS subperiod (early DS, middle DS, and late DS). Significant differences between mean values of Tmax, Tmin, TA, and RH were determined by the DGC test with  $\alpha < 0.05$  (Di Rienzo et al. 2002).

To compare data obtained from field sensors against data from CWS, a paired *t* test with  $\alpha < 0.05$  was used. The daily differences between the weather station and the field sensors of Tmax (Tmax diff = Tmax<sub>station</sub> - Tmax<sub>field</sub>) and Tmin (Tmin diff = Tmin<sub>station</sub> - Tmin<sub>field</sub>) were estimated. The same analysis was applied for TA.

Daily Tmax, Tmin, and TA measured in the microclimatic analysis were analyzed with the same methodology employed to analyze macroclimatic conditions. The time (in minutes) was considered as a fixed effect whereas temperature measured by sensors was considered as a random effect, and the data were partitioned in day-night values. Moreover, in the analysis of  $T_{in}$  versus  $T_{out}$  gradient, the average day was estimated using a generalized linear model (GLM).

For the analysis of differences in CH between tissues, treatments, and cultivars, repeated measures one-way ANOVA were used. When effects were significant, multiple comparisons were performed using Duncan's post hoc test.

## Results

### Comparison of macroclimatic conditions between MZA and WA

The average of daily temperature during the DS in the 10-year period analyzed was higher in MZA (9.8 °C) than in WA (5.2 °C). Moreover, average Tmax was always higher MZA than in WA (Tmax<sub>MZA</sub>-Tmax<sub>WA</sub> are 6.2, 11.3, and 5.8 °C during the early, middle and late DS, respectively). By contrast, Tmin was relatively similar at the two locations and only showed significant differences during the middle DS (Tmin<sub>MZA</sub>-Tmin<sub>WA</sub> are -2.1, -1.2, and -0.1 °C during the

early, middle, and late DS, respectively). Major differences were observed during the middle DS (Fig. 2a).

MZA was relatively constant throughout all the DS, oscillating around 60% (characterized by a dry winter season). On the other hand, RH in WA started to increase during the early DS peaked at almost 90% in the middle DS (characterized by high frequency of rains and snowfall) to decrease gradually during late DS until reaching similar values than those registered in MZA (Fig. 2b).

The seasonal progression of TA was closely related to that of RH (Fig. 2c). Consequently, in MZA, the TA was relatively constant throughout the DS (around 16 °C) but with a significant higher interannual variation (MZA<sub>var(n-1)</sub> = 27.6 °C and WA<sub>var(n-1)</sub> = 11.3 °C;  $p < 0.0001$ ). Unlike in MZA, the TA in WA showed a gradual decline during early DS, reaching minimum values of nearly 6 °C during the middle DS, to continue with a gradual increase during the late DS.

### Comparison between temperature measurements in vineyard and CWS

Close correlations were found between data obtained from field sensors and CWS in both MZA and WA (MZA: Tmax<sub>field</sub> vs. Tmax<sub>station</sub>  $r^2 = 0.91$  and Tmin<sub>field</sub> vs. Tmin<sub>station</sub>  $r^2 = 0.92$ ; WA: Tmax<sub>field</sub> vs. Tmax<sub>station</sub>  $r^2 = 0.90$  and Tmin<sub>field</sub> vs. Tmin<sub>station</sub>  $r^2 = 0.92$ ). However, at both sites, the TA registered close to the plants (field conditions) was significantly higher than that registered by the CWS (Fig. 3). In WA, both field TA and CWS TA were lower than in MZA.

The Tmax and Tmin differences registered between the field and the CWS revealed that in none of the cases that the difference the TA was equal to zero. However, the CWS tended to underestimate Tmax and overestimate Tmin (Fig. 3). Field plants were exposed to higher Tmax values than those recorded by CWS: mean Tmax were 3.04 and 2.52 °C higher in the field than in MZA and WA weather stations, respectively. Also, mean values of Tmin were 1.70 and 0.71 °C lower in the field than CWS values from MZA and WA, respectively. It is important to note that rather large differences (e.g., 10 °C) were sometimes found between field and CWS-recorded temperatures (circled symbols in Fig. 3).

### Plant microclimatic conditions

The analysis of plant microclimatic conditions close to the base of the trunk (10 cm above the soil level) and near the top (100 cm above the soil level) of dormant grapevine canopies revealed that during the day, there was no vertical temperature gradient, while at night, the base of the plant is experienced to lower temperatures, a situation that leads to a higher TA at the base than at the top of the plant (Table 2(A)). This pattern was similar in both MZA and WA vineyards. However, differences were larger in MZA, with a night

temperature gradient of  $-2.03$  °C between base and top, and a TA difference of  $3.93$  °C, compared to a night temperature gradient of  $-0.22$  °C and TA differences of about  $1.25$  °C in the WA vineyard.

Soil temperatures were also significantly different from with the temperatures registered at the base of the plant (Table 2(B)). In this case, similar results were obtained in MZA and WA, with the soil being colder than the air near the base during the day ( $T_{\text{base}}-T_{\text{soil}} = -4.87$  and  $-4.49$  °C in MZA and WA, respectively) and warmer during the night ( $T_{\text{base}}-T_{\text{soil}} = 2.71$  and  $3.13$  °C in MZA and WA, respectively). A similar effect was also observed in TA, where the soil exhibited less significant diurnal temperature variation than the air near the trunk base ( $TA_{\text{base}}-TA_{\text{soil}} = -11.82$  and  $-10.84$  °C in MZA and WA, respectively).

Significant differences were also observed between the temperatures inside ( $T_{\text{in}}$ ) and outside ( $T_{\text{out}}$ ) the vine trunk with a similar response at both sites (Fig. 4). Whereas  $T_{\text{in}}$  was slightly higher than  $T_{\text{out}}$  during the night, during the daytime, the trunk was warmer than the air. This nighttime difference was  $1.3$  °C in MZA and  $2.7$  °C in WA. On the other hand, was observed that at sunrise, the air temperature of the surrounding air increased faster than the trunk, but at midday, the temperature of the trunk was greater than of the air. This pattern is maintained until around 8:00 p.m., the moment from which  $T_{\text{in}}$  and  $T_{\text{out}}$  became similar.

### Effect of TA on deacclimation rates of buds and cane tissues

In order to determine whether tissue deacclimation may be induced by differences in TA, field acclimated cane internodes and buds were subjected to Low-TA ( $7$  °C), High-TA ( $30$  °C), and control ( $0$  °C) during 24 h of artificial thermal treatments in two cultivars.

The  $LT_{10}$  of Chardonnay buds and internode tissues did not differ between the control and Low-TA. Conversely, there was a significant effect of the High-TA treatment. The tissue that was most vulnerable to deacclimation was the internode xylem, followed by the phloem, and finally the bud (Table 3).

In Merlot, neither buds nor xylem buds and xylem showed significant differences between any treatments. However, High-TA led to loss of phloem CH in cv. Merlot (Fig. 5, Table 3).

## Discussion

Most information concerning CH in grapevines in MZA is currently extrapolated from information generated from different regions of the NH, because there is no local information. In WA, however, cold injury is a common phenomenon with severe economic consequences. In fact, different models

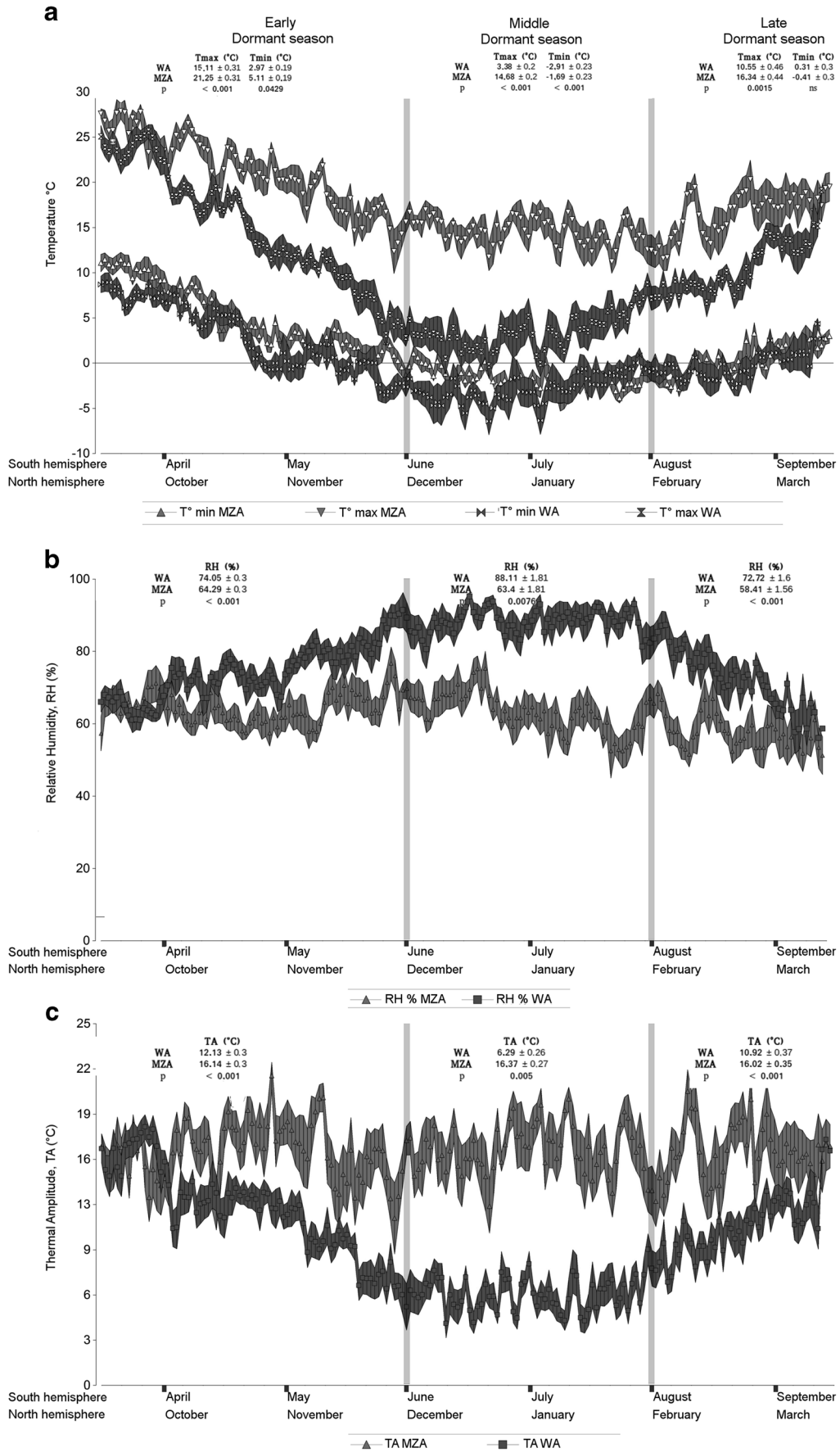
**Fig. 2** Ten years of macroclimatic records (2002–2012). Mendoza, Argentina (MZA;  $33^{\circ} 43' 10''$  S latitude;  $69^{\circ} 06' 16''$  W longitude) and Washington, USA (WA;  $46^{\circ} 15' 25''$  N latitude,  $119^{\circ} 44' 25''$  W longitude). (a) Comparison of daily maximum (Tmax) and minimum (Tmin) temperatures, (b) relative humidity (RH), and (c) thermal amplitude (TA) during the early, middle, and late dormant season of grapevines (October–March in USA and April–September in Argentina). Significant differences between mean values were determined by the DGC test with  $\alpha < 0.05$  ( $n = 10$  years)

have been generated in order to predict cold damage during the DS (Ferguson et al. 2011, 2014). These models were applied in regions where the temperature remains relatively low and stable during DS (Proebsting et al. 1980; Jiang and Howell 2002). However, the same models did not perform well under the climatic conditions of MZA (Gonzalez Antivilo; unpublished results), suggesting that the model does not capture important aspects of climatic variation among growing regions and the importance to generate local information and fine CH monitoring in order to understand the causes of freezing damage in this region.

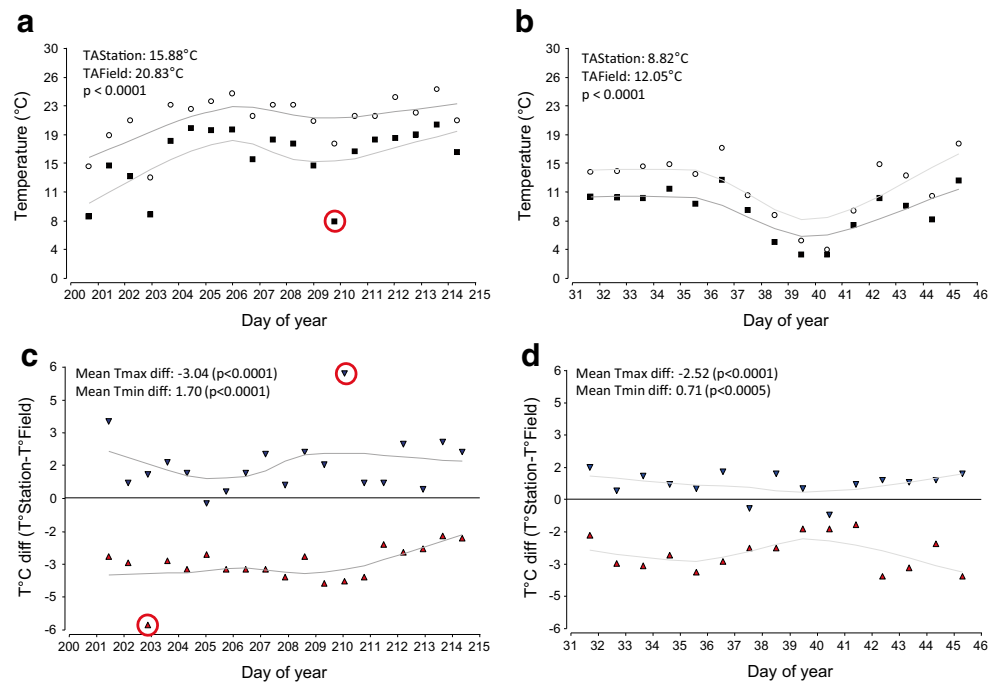
Both MZA and WA are viticulture regions of global importance and share some climatic similarities, grape cultivars, as well as irrigation, diseases, and other vineyard management. Geographically, both sites have in common the proximity to mountains, episodes of Föhn wind, similar bioclimatic indexes according to Köppen-Geiger (cold, arid, and desert), and annual precipitation around 200 mm (Table 1). Also, both regions show signs of frost damage. However, a comparison of 10-year meteorological records from both regions revealed contrasting differences with potential influence in the physiology of *Vitis* sp. plants.

The use of Tmean to compare climatic characteristics among regions and its association with biological parameters (i.e., photosynthesis, CH) are widely accepted. According to this bioclimatic index, the DS of MZA is  $\sim 5$  °C warmer than that of WA in. However, deepening the remainder parameters that define thermal characteristics, it is possible to generate another interpretation during the DS from both sites: (i) despite latitude differences, there are slight differences in average Tmin ( $< 1.5$  °C); (ii) mean Tmax is considerably higher in MZA than in WA, reaching maximum differences during the middle DS with mean values of  $12$  °C and often higher daily differences; (iii) the average TA in MZA is  $\sim 16$  °C with high interannual variation throughout the whole DS period, whereas in WA, the TA reaches a minimum during the middle DS ( $\sim 6$  °C); (iv) moreover, in WA, but not in MZA, there are some days when Tmax did not exceed  $0$  °C (Fig. 2). Considering the vulnerability of *V. vinifera* to deacclimation induced by unseasonal high temperatures (Ferguson et al. 2011, 2014), these climatic differences could lead to occasional cold damage even in MZA vineyards.

The presence of the mountain barriers produces a considerable influence on the regional climate, deeply influencing



**Fig. 3** Comparison of microclimate variation between field conditions and official weather station in Mendoza, Argentina (MZA; *left*) and Washington, USA (WA, *right*). (a) and (b) show the daily thermal amplitude variation measured in the field (*empty circles*) and by the weather stations (*filled squares*). (c) and (d) show differences in Tmax (*filled triangles*) and Tmin (*filled inverted triangles*) between the weather station and the field ( $T_{diff} = T_{Station} - T_{Field}$ ). Mean values of thermal amplitude, Tmax diff, and Tmin diff are also shown. *Circled symbols* indicate extreme values. Measurements were conducted during the dormant season of 2013 and 2012 in MZA and WA, respectively



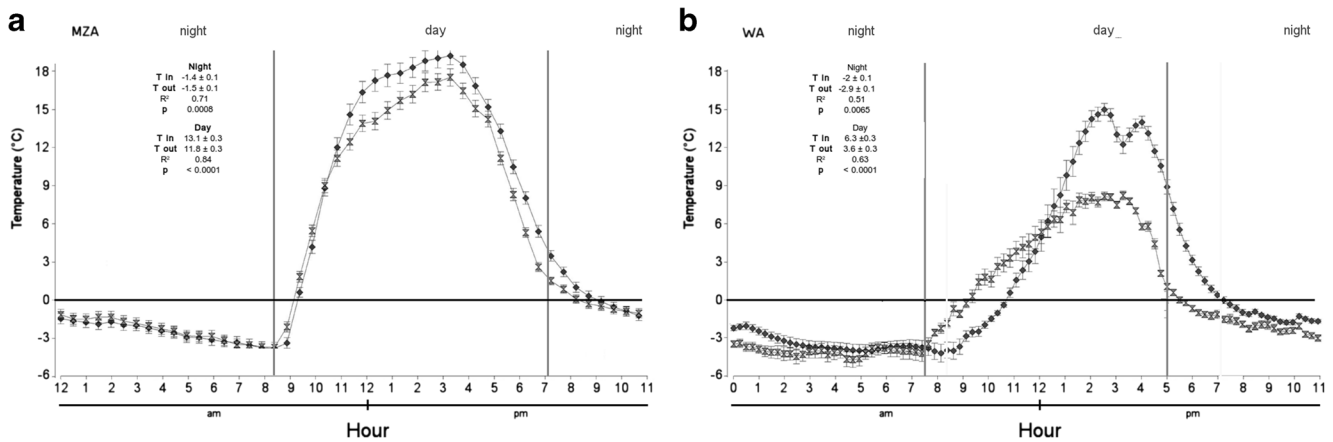
seasonal TA, precipitation, and continentality (Thompson et al. 1977). However, the magnitude of the effect caused by these barriers depends on the orographic characteristics in each case. In this sense, the Andes consists in a formidable obstacle to the Pacific Ocean winds, consisting in more than threefold of mean altitude and the double of width than the Cascade Range (Table 1). Moreover, whereas the Andes is an uninterrupted mountain system, the Cascade Range is broken up by the Columbia River gorge, showing a lower continentality index than MZA (Gedalof et al. 2005; WRCC 2013).

Both MZA and WA regions are affected by two semi-permanent high-pressure centers located above the South and North Pacific Oceans, respectively. In the coastal regions (windward side), these pressure centers are responsible for wet winters and dry summers (Mass and Dotson 2010; Ancapichun and Garcés-Vargas 2015). During DS, those anticyclones push winds toward the mountain ranges. As the wind climbs the slopes of the windward side of the range, the moist air loses water as rain or snow. However, the altitude of mountain ranges is able to modify the rainfall distribution on both sides of the mountains (Bianchi and Yáñez 1992). In

**Table 2** Plant microclimate characterization in MZA and WA during the DS

	MZA			WA		
	Day	Night	TA	Day	Night	TA
<b>(A) Vertical gradient in air</b>						
$T_{top}$	12.6 ± 0.3	3.5 ± 0.2	17.1 ± 0.4	6.0 ± 0.1	1.6 ± 0.1	10.7 ± 0.5
$T_{base}$	13.2 ± 0.3	1.5 ± 0.2	21.0 ± 0.4	6.6 ± 0.1	1.4 ± 0.1	11.9 ± 0.5
$r^2$	0.61	0.81	0.31	0.68	0.72	0.08
$p$	ns	<0.0001	<0.0001	ns	0.035	ns
<b>(B) Vertical gradient in soil</b>						
$T_{base}$	7.2 ± 0.24	4.8 ± 0.09	7.4 ± 0.63	7.4 ± 0.14	5.8 ± 0.27	6.5 ± 0.94
$T_{soil}$	12.1 ± 0.24	2.1 ± 0.9	19.2 ± 0.63	11.9 ± 0.14	2.7 ± 0.27	17.3 ± 0.94
$r^2$	0.65	0.84	0.79	0.73	0.68	0.65
$p$	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

(A) Day and night air thermal differences and TA between top ( $T_{top}$ ) and base ( $T_{base}$ ) of trunk. (B) Day and night air thermal differences and TA between above ground ( $T_{base}$ ) and below soil ( $T_{soil}$ ). *ns*: not significant



**Fig. 4** Hourly microclimate characterization inside and outside grapevine trunks during the dormant season. Diurnal temperature change of trunk ( $T_{in}$ ; filled diamond) and air near the plant ( $T_{base}$ ) in an average day during the dormant season in Mendoza, Argentina (MZA;

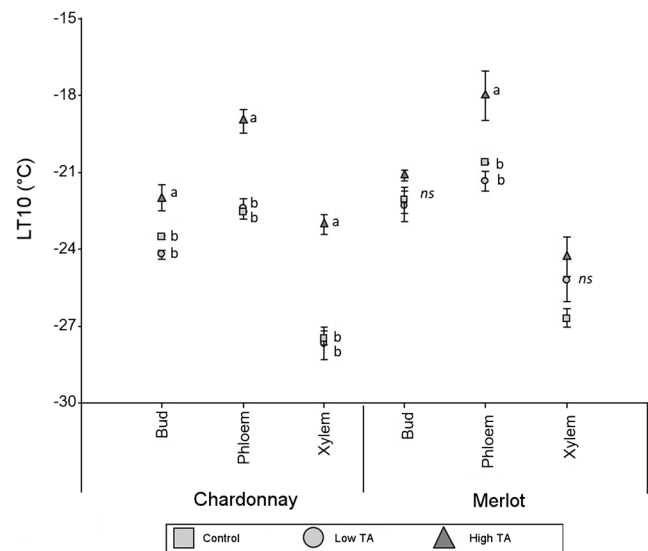
(a) and Washington, USA (WA; (b)). Symbols are means and bars are standard deviation ( $n =$  three plants) measured during 15 days in August 2013 in MZA and during 6 days in February 2012 in WA

the case of the Andes, its high altitude (~4000 m asl in average) favors rain and snow accumulation at the windward side, drying the air and preventing precipitation in the low plains of MZA (Bianchi and Yáñez 1992). However, the relative lower altitude of the Cascades (~1000 m asl on average) and their interruption by the Columbia river allow that clouds surpass the hills in their drift toward the west, favoring winter rains on both sides of the mountains (Mass and Dotson 2010). As a consequence of these characteristics, clear sky is frequent (~100%) in MZA during the middle DS, whereas in WA, the frequency of sunny days is significantly lower (~25%).

Another consequence of the passage of winds across the mountains is the “Föhn effect.” This phenomenon is caused by the adiabatic compression that winds suffer when they descend the lee slopes, causing a temperature increment and an unseasonal “little summer in winter” followed by a strong freezing event. As more pronounced is the altitude, as more strong, warm, and dry is the wind (Norte and Simonelli 2016). In the case of the Zonda winds, common in MZA, their occurrence is distributed from May to November, with nearly 50% of their incidence within the July–August months (late DS). Moreover, the probability of this wind would be severe (>10 °C) and duration (up to 3 days) is only high in late DS (Caretta et al. 2004). By contrast, the Chinook winds in WA rarely occur during the winter, and produce a relative small thermal effect due the low altitude of the Cascades (Burrows

1940). Therefore, the Andes are a much more important climatic barrier for MZA (Bianchi and Yáñez 1992), than are the Cascades for WA, where their effects are less marked and show more influences by the Pacific Ocean (Mass and Dotson 2010).

Temperature and RH are both variables closely related; consequently and depending on the degree of humidity of the air is the amount of energy that is required to heat or cool it, needing a saturated air of moisture more energy to raise its temperature (specific heat saturated air 0.43 kcal/kg/°C) than a



**Fig. 5** Lethal temperatures ( $LT_{10}$ ) in grapevine bud and cane tissues following different thermal treatments prior to a freezing simulation in cv. Chardonnay and cv. Merlot. Buds and internode sections were submitted to two temperatures of low thermal amplitude (7 °C TA) (filled circle) and high thermal amplitude (30 °C TA) (filled triangle) for 24 h followed by controlled freezing. The controls (filled square) were left untreated before controlled freezing. Symbols are means and bars are standard deviations (internode:  $n = 8$ ; buds:  $n = 20$ ). Symbols with different letters within a column are significantly different (Duncan's test,  $p < 0.0001$ )

Tissue	Chardonnay	Merlot
Bud	-1.54	ns
Phloem	-3.55	-2.62
Xylem	-4.43	ns

ns: not significative

totally dry air (specific heat dry air 0.24 kcal/kg/°C). In MZA, HR is quite constant during the DS (~60%) and with low probability of precipitation as rain or snow, resulting in with a very high TA (Fig. 2b, c). Moreover, Zonda winds have a high desiccant power that reduces HR at levels lower than 30% (Norte and Simonelli 2016) being probably the main cause of the high interannual variation in daily TA observed in this research (Fig. 2c). Conversely, in WA, the increase in HR shows a progressive increment reaching a highest value at the middle DS (~100%), a fact that explains the low TA observed there. In fact, on some days, the maximum and minimum temperatures are almost equal ( $TA \approx 0$ ). In this sense, our results suggest that the contrasting peak between climatic situations between MZA and WA coincides with the late DS, a period when grapevines are most susceptible to deacclimation.

Air temperature is recorded from different types of meteorological weather station and sensors. One of the most traditional and more employed worldwide are the Stevenson weather stations, created in the XIX century and composed by different analogical sensors protected by a white woody shelter. Nowadays, those stations are being replaced by modern digital automatic weather stations with white plastic shelter. However, temperature is a highly variable and sensible parameter and it depends on the sensors employed, constructive characteristics of shelter, closeness to buildings, etc. (Tarara and Hoheisel 2007; Fall et al. 2011; Menne et al. 2010). In fact, small differences such as the kind of employed paint affect the real temperature values of  $T_{max}$  in 1.66 °C (0.3 °F) and  $T_{min}$  in 2.78 °C (0.8 °F) (Fall et al. 2011).

The literature refers that thermal data recorded by different sensor technologies and shelter materials depend on the geographical scope, type of sensors, and researcher preferences. Meteorological research is commonly based on data generated by CWS (Giantomasi et al. 2009; Burgos 2010). However, in biological studies relating observations of living organisms with weather parameters, it is necessary to employ data obtained by nearby CWS and/or by data supplemented by sensors installed in situ (Hubackova 1996; Echarte et al. 2010).

The greater is the precision required for an inference, the more important is sensor density and proximity to the living organism under study. In this case, the comparison of temperature records from sensors installed in vineyard and those from the nearest CWS (<500 m) in MZA and WA revealed that despite field and CWS data being correlated, in both cases, we observed a significant bias for the CWS to overestimate  $T_{max}$  and underestimate  $T_{min}$ . Thus, the TA value is typically higher in the field than indicated by the CWS. Moreover, in particular cases, the values recorded by CWS differed markedly from the field measurement (Fig. 3). Similar results were also obtained by monitoring other plant species (Renaud and Rebetez 2009). Thus, although that temperature records from CWS are useful for describing

macroclimatic conditions of a particular region, our data suggest that to reach more accurate biological inferences, it is recommended to set up in situ sensors in order to understand real temperature conditions to which plants are exposed. Moreover, this control scheme has direct implications in active control, monitoring during freezing events, and evaluation of damage after it occurs.

At individual, the plant scale, there is spatial variability in freezing resistance and risk, from the root system to the top of the canopy (Charrier and Ameglio 2011, 2015). It is widely accepted that there is a vertical air temperature gradient, and the lowest temperature is close to soil (Leuning and Cremer 1988; Jordan and Smith 1995; Blennow 1998; Battany et al. 2012). However there is scarce information about the magnitude of this gradient and its implications on acclimation and deacclimation processes during the DS. Our results suggest that in MZA, the base is subject to high TA (Table 2(A)). This phenomenon could potentially lead to deacclimation, leaving the base of the trunk to more exposed conditions to cold injury during an unusually cold night. According to our observations, damage to the trunk base is the most common symptom of cold injury in grapevine checked in MZA; therefore, it could be related with a differential deacclimation between different parts of trunk of grapevine. Conversely, in WA, the near absence of this temperature gradient might be attributed to the higher RH during winter.

Trunk temperatures at the base level also had higher  $T_{max}$  and consequently higher TA than the surrounding air. The trunk's capacity to gain energy is related to its mass, which generates thermal inertia so that trunks start to warm up or cool down later than does the air (Fig. 4). A similar result has been observed by Paroschy et al. (1980). Although trunk temperatures are generally higher in MZA than in WA, the presence of snow in WA may produce an albedo effect, inducing a major thermal gain during daytime (Fig. 4).

Although the root is the most susceptible organ to freezing damage (Okamoto et al. 2000), the soil has thermal inertia that acts as a buffer (Table 2(B)). This capacity protects the root from excessive temperature decreases. Moreover, it allows shoot re-growth from latent buds on the below ground portion of the trunk, a characteristic symptom in freeze injured plants (Zabadal et al. 2007).

Few research estimates deacclimation rate by testing different combinations of temperature and time periods. These sources determine that it depends on species, varieties, and temperature to which the plant is subjected (Pagter and Williams 2011; Eagles 1989). Thus, in *Vitis* sp., it was demonstrated that thermal treatments of 14 °C during 3 weeks (cv. Thompson Seedless; Rubio et al. 2016), and 25/20 °C day/night during 4 days (cv. Chardonnay; Cragin et al. 2017), induced similar daily deacclimation rates of 2 °C. Our results indicate that high TA may lead to similar deacclimation rates in 1 day, but at higher TA values. Thus, a TA of 30 °C was

strong enough to change the CH status in all tissues and both cultivars evaluated, whereas a TA of 7 °C did not alter CH (Fig. 5). Although Chardonnay had higher initial CH, it was more susceptible to deacclimation than the seemingly more resilient Merlot.

*Vitis* sp. presents different rates of deacclimation according to the stage (early, middle, and late DS). In this way, it has been observed that during the late DS, it is faster than during the previous stages (Sakai and Larcher 1987; Levitt 1980; Steffen et al. 1989). Thus, temperature fluctuations (including high TA) during the late winter or extended periods of mild temperatures can lead to deacclimation, making plants vulnerable to injury from rapid temperature drops (Fennell 2004; Gu et al. 2008).

According to our results, plants subjected to high TA, such as the case of MZA, probably could not reach the maximum capability of acclimation status due small daily cycles of acclimation-deacclimation. Moreover, Haynes et al. (1992) demonstrated that there is more risk of damage if after a cold event, the temperature experiment a rapid increase. A situation like that is common in MZA in late DS when Zonda winds occur and where mean values of TA (16 °C) might experiment additional thermal jump of nearly 10 °C, and TA could reach up to 26 °C.

Finally, nowadays, it is common to study global climate change from the GS viewpoint. In fact, Friend et al. (2011) described that spring freeze event can result in substantial grapevine yield losses by damage in inflorescences and shoots. However, according current predictions, it is expected extreme and abrupt events of warming and freezing waves and Föhn winds during DS (Bernstein et al. 2008) and it could induce to a loss of CH (Farrell 2010; Easterling et al. 2000). This situation could leave the plants in a more vulnerable status. In addition, the process of deacclimation occurs at a faster pace than cold acclimation (Kalberera et al. 2006) and plants are in general more rapidly deacclimated during late winter. Thus, the determination of the rate of deacclimation of vine cultivars is necessary to identify more apt areas for crops.

## Conclusions

The comparison of climatic conditions between MZA and WA revealed contrasting thermal conditions, yet the conditions in both regions are able to induce freezing damage in vineyards. One of the most relevant climatic differences between MZA and WA is the much higher daily TA in MZA. In addition, the incidence of Zonda winds during the DS in MZA produces unseasonal warm spells followed by a freezing event that potentially increases the incidence of cold injury. The absence of temperature data recorded in the field represents a problem, since this study demonstrated that field TA tends to be higher

than that registered by CWS. Moreover, we demonstrate than the thermal environment around individual plants is also variable. The base of grapevine trunks proved to be the most vulnerable portion, because it is subjected to the highest temperature variation, whereas roots tend to be protected by the soil. We suggest that this is the main reason of the under soil re-growth observed in fields in plants affected by freezing damage. Finally, we demonstrated that it is possible for a cold deacclimation in a short period of time, and that it depends on the TA.

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