

1 **Research Article**

2 **Clone and Rootstock Interactions Influence the Cold**  
3 **Hardiness of *Vitis vinifera* cvs. Riesling and Sauvignon blanc**

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17  
18 **Abstract:** Cold damage is a threat to grapegrowers worldwide. Cold hardiness varies across *Vitis*  
19 *vinifera* cultivars, but the influence of clone and rootstock selection on this trait is unclear. Five  
20 clone x rootstock combinations of Riesling (clone 49 x Riparia Gloire (RG); 49 x SO4 Teleki;  
21 clone 239 x RG; 239 x SO4; 239 x Couderc 3309) and four clones of Sauvignon blanc (clone  
22 242, 297, 376, and 530 on SO4 Teleki rootstock) were evaluated over three dormant seasons  
23 (2016/17, 2017/18, and 2018/19). Bud cold hardiness was quantified every two to four weeks by  
24 differential thermal analysis (DTA) and reported as low temperature exotherms (LTE). Yield and  
25 pruning weights were recorded every year. Rootstock did not consistently influence the cold  
26 hardiness of Riesling buds, although clone 239 was generally hardier than clone 49. Significant  
27 clone\*rootstock interactions were observed more frequently in the first year of the study. No  
28 consistent differences were observed between Sauvignon blanc clones, although clone 242 and  
29

30 297 were often amongst the least hardy clones. Differences in hardiness were not consistently  
31 related to yield, pruning weight or crop load in the prior growing season. This study  
32 demonstrates the importance of both clone and rootstock selection in cool climate regions where  
33 freeze injury may occur. Future research should consider the clone identity and the possibility of  
34 a clone x rootstock interaction when comparing the cold hardiness of different cultivars.

35 **Key words:** clone, cold hardiness, cool climate, differential thermal analysis, rootstock, *Vitis*  
36 *vinifera*

## 37 Introduction

38 Freezing temperatures are the most significant abiotic threat to grape production in many  
39 cool climate regions (Fennell 2004). Grapevine cold hardiness depends on supercooling, which is  
40 the capacity to prevent freezing of intracellular water (Pierquet et al. 1977). This ability develops  
41 during endodormancy, that is first initiated by a shorter photoperiod and enhanced by decreasing  
42 temperatures (Schnabel and Wample 1987). Cold hardiness develops through acclimation which  
43 occurs concurrently to endodormancy and can continue into ecodormancy (Cragin et al. 2017).  
44 Early acclimation is accompanied by periderm formation around the canes and decreased water  
45 content in the buds and in the canes (Wolpert and Howell 1986). Within-vine cold hardiness is  
46 not always uniform. Cane differences in periderm development, sunlight exposure, and presence  
47 of lateral shoots lead to uneven acclimation, resulting in differences as much as 12°C in some  
48 cases (Howell and Shaulis 1980). The geographical location of a vineyard can also significantly  
49 affect cold hardiness (Stergios and Howell 1977).

50 Cold hardiness differences among cultivars are well known (Pool et al. 1990, Cindric and  
51 Korac 1990; Mills et al. 2006, Dami et al. 2016), but intra-cultivar differences are rarely studied.  
52 Rootstocks have long been thought to influence cold hardiness of the scions because of their  
53 ability to mitigate many plant stresses. The rootstock-scion communication is important; the root  
54 system facilitates several physiological processes that are related to cold hardiness such as water  
55 (Tramontini et al. 2013) and nutrient uptake (Fisarakis et al. 2004), carbohydrate availability to  
56 the buds (Cox et al. 2012), and production of hormones such as abscisic acid (Stoll et al. 2000).  
57 Vine size and crop load are impacted by the rootstock (Reynolds and Wardle 2001) and are  
58 thought to decrease cold hardiness by increasing the number of canes with poor acclimation  
59 through shading effects (Striegler and Howell 1991). Mixed results have been reported in the  
60 literature on the relationship between crop load and cold hardiness; both no relationship (Dami et  
61 al. 2013, Lefebvre et al. 2015) and a negative impact of overcropping on cold hardiness (Howell  
62 1978, Dami et al. 2015) have been reported. The only reported influence of rootstock on cold  
63 hardiness is a small reduction of shootless nodes on certain years when Riesling was grafted to  
64 3309 rootstock (Miller et al. 1988).

65 An effective way to maximize winter survival of specific cultivars could be identification  
66 of plant material, clones or biotypes, with superior cold hardiness. Clones are a potential source  
67 of genetic diversity but are not traditionally selected for their cold hardiness. Intra-cultivar  
68 phenotype differences in pruning weights, yield components, and fruit composition are common  
69 in several cultivars such as Chardonnay, Pinot noir, and Merlot (e.g. Wolpert et al. 1994, Benz et  
70 al. 2006, Castagnoli and Vasconcelos 2006, Anderson et al. 2008). To our knowledge, only Pinot  
71 noir clones have been studied in the context of cold hardiness, with clones Mariafeld,

72 Spätburgunder and Espinette being less tolerant and clone 29 and Pernand having generally the  
73 smallest percentage of shootless nodes (Pool et al. 1990).

74 Cold hardiness to acute cold exposure can be measured directly by differential thermal  
75 analysis which detects the low temperature exotherm (LTE) from the latent heat released when  
76 intracellular water freezes (Mills et al. 2006). Buds are placed in a programmable temperature  
77 chamber and exposed to steadily decreasing temperatures to simulate a naturally occurring cold  
78 event. As the buds reach their lethal temperature, intracellular water freezes causing bud death.  
79 This event releases latent heat detected by a thermomodule and converted to a change in voltage  
80 that can be quantified by a multimeter. It is a destructive test that kills the buds and is directly  
81 related to cold injury (Pierquet et al. 1977, Mills et al. 2006).

82 Growing grapes successfully in cool climates depends on the ability of grapevines to  
83 resist winter minimal temperatures. New ways to improve cold hardiness and reduce freeze  
84 injury must be identified to ensure sustainable grape growing of *V. vinifera* in cool climate  
85 viticultural areas such as Ontario, Canada where climate change may increase extreme weather  
86 events such as minimal winter temperatures or spring frost. Clone and rootstock both have the  
87 potential to improve cold hardiness through direct or indirect effects. It was therefore  
88 hypothesized that clone and rootstock selection might be a significant source of intra-cultivar  
89 cold hardiness variation. To test this hypothesis, five clone and rootstock combinations of  
90 Riesling and four clones of Sauvignon blanc were sampled at regular intervals in a commercial  
91 vineyard over three dormant seasons, from acclimation to deacclimation, and their cold hardiness  
92 was quantified by DTA.

93

## Materials and Methods

**Vineyard and experimental design.** This research was conducted in a commercial vineyard located in the Four Mile Creek VQA sub-appellation within the Niagara Peninsula (ON, Canada) on loam to sandy-loam soil with 1.0 m by x 2.5 m (vine x row) spacing. Vines were trained in double cordon with vertical shoot positioning and spur-pruned to an estimated 9 viable buds/cordon following dormant bud survival assessments. Vineyard maintenance and pest management were based on normal industry standards and were consistent across the experimental site. Three randomized, replicated experimental blocks (units) per clone x rootstock combination were selected; each block containing eight 7-vine panels. 44 vines per block were sampled for DTA measurements, and 12 vines were flagged at random in each block to measure vine balance and yield components. Clone x rootstock combinations selected for Riesling were ENTAV-INRA® clone 49 x Riparia Gloire (RG), 49 x SO4 Teleki (SO4), clone Geisenheim 239 x RG, 239 x SO4, and 239 x Couderc 3309 (3309). Vines were 10 years old at the onset of the study with the exception of 239 x 3309 which were 19 years old. The clones of Sauvignon blanc selected were ENTAV-INRA® clones 242, 297, 376, and 530 all grafted to the SO4 rootstock and were 15 years of age at the onset of the study. Weather data was collected from a weather station within 1 km of the vineyard maintained by Weather Innovations Consulting – Vine & Tree fruit Innovations (<http://www.vineinnovations.com>). Daily growing-degree days were calculated from daily mean temperatures with a base of 10°C, from 1 April to 31 October.

**Cold hardiness measurement and analysis.** Six cane-replicates per block, from six different vines, were collected every two to four weeks from October 2016 to April 2017 for

116 LTE measurements by differential thermal analysis (DTA). The collection was replicated during  
117 the 2017-2018 and 2018-2019 dormant seasons. Frequency of sampling was higher during the  
118 acclimation and deacclimation stages. DTA was performed according to Mills et al. (2006) with  
119 modifications. Buds from positions 3 to 7 were removed from canes in the lab and placed cut-  
120 side up on the thermo electric modules (TEM) covered with a Kimwipe™ (Kimberley-Clark  
121 Professional, Roswell, GA) moistened with distilled water. Up to five buds were placed on each  
122 TEM. Nine TEM were set up per tray, and six trays maximum were used in one freezer run. The  
123 temperature of the programmable temperature chamber (Tenney T2C, SPX Thermal Product  
124 Solutions, White Deer, PA) was decreased by 4°C/hr, from 4°C to -40°C, and the TEM signal  
125 was acquired throughout the run by a multimeter data acquisition system (Keithley 2700 Integra  
126 Series, Cleveland, OH). The TEM voltage output versus the temperature of the tray were plotted  
127 by the software BudProcessor for each TEM (2018 version 1.7.2, Brock University, St.  
128 Catharines, ON). Peaks representing LTE for primary buds were visually identified on the  
129 output. Because the cold hardiness of the buds from the same cane are related, raw bud LTEs are  
130 not independent data points. The average cane LTEs were calculated and used in subsequent cold  
131 hardiness statistical analysis.

132 **Phenological stage monitoring.** Phenological stages were monitored throughout the  
133 growing season to identify the dates of bud break, bloom and veraison, estimated by the date at  
134 which 50% of the vines are at the Eichorn-Lorentz stages 4, 23, and 35, respectively according to  
135 Coombe (1995). To identify these stages, each block was regularly monitored. For bud break, the  
136 number of buds beyond stage 4 was recorded for 7 vines/block. For bloom and veraison, 20

137 clusters/block were examined, and the number of clusters beyond stages 23 and 35, respectively,  
138 was recorded until more than 50% had reached the appropriate stage.

139 **Yield components and vine balance.** Yield component measurements were performed  
140 immediately prior to commercial harvest. Flagged vines were harvested individually to record  
141 cluster numbers and vine yield. Pruning was performed on the harvested vines during the  
142 dormant seasons following the decisions of the vineyard manager. The weight of the pruned  
143 canes was recorded in the field. The Ravaz index (RI) was used to measure vine balance and  
144 was calculated by dividing the yield/vine by the pruning weight/vine.

145 **Statistical analysis.** All statistical analyses were carried out using XLSTAT version  
146 2019.3.2 (Addinsoft, France). LTEs from Riesling clone x rootstock combinations for all dates as  
147 well as the yield components, pruning weights, and Ravaz indices were compared by two-way  
148 analysis of variance (ANOVA) to identify clone\*rootstock interactions and the contributions of  
149 the clone and rootstock factors individually. Additional one-way ANOVA were used to compare  
150 Riesling clone 239 on the RG, SO4 and 3309 rootstocks for the last two years of the study to  
151 identify rootstock effect. LTEs from Sauvignon blanc and the yield components, pruning  
152 weights and Ravaz indices were compared by one-way ANOVA. All significant ANOVA tests  
153 were followed by Tukey HSD post-hoc test.

## 154 **Results**

155 **Weather conditions/vintage variation.** The weather differed between the years studied.  
156 The 2016/17 dormant season was preceded by the lowest total rainfall of the three years studied,  
157 with only 207 mm received from May to October and long periods without rainfall in May, June

158 and July (Figure 1). The lack of rainfall was accompanied by 42 days with a maximal  
159 temperature above 30°C, the highest number in the three years studied. A total of 1626 GDD  
160 accumulated between 1 May 2016 and 31 October 2016. The 2017 growing season was wetter  
161 and colder than 2016, with more than twice as much rainfall, 444 mm, only 1454 GDD and 12  
162 days with maximal temperature above 30°C (Figure 1). In 2018, a total of 369 mm of rain was  
163 received, accompanied by 1662 GDD and 34 days with temperature above 30°C (Figure 1). The  
164 three dormant seasons had also different temperature patterns. The 2016/17 dormant season was  
165 relatively mild, with a minimum temperature of -13.1°C reached on 7 January 2017. Only 12  
166 days had minimum temperatures below -10°C, and important temperature fluctuations were  
167 measured (Figure 2). Some large fluctuations in temperature occurred during many periods of  
168 dormancy. The 2017/18 dormant period was colder than the preceding, with 12 days below -  
169 15°C and the lowest temperature, -19.8°C, was recorded on 7 January 2018. Severe temperature  
170 fluctuations were recorded again particularly from mid-December to the end of February (Figure  
171 2). Fewer fluctuations in temperature were observed in 2018/19, but the coldest temperatures of  
172 the study were recorded with two days below -20°C and a minimum of -20.3°C on 31 January  
173 2019. The temperatures were generally lower during the 2018/19 acclimation period than for the  
174 other two dormant seasons (Figure 2).

175 **Phenology.** Phenological stages were reached almost simultaneously for Riesling and  
176 Sauvignon blanc at the experimental vineyard site. Bud break was uniform amongst the  
177 Sauvignon blanc clones and was observed on 13 May 2016, 10 May 2017, and 16 May 2018.  
178 Bud break of the Riesling clone x rootstock combinations differed by one or two days, depending  
179 on the year. It was observed on 14 May for all combinations but 239 x RG (16 May) in 2016, on



180 9 May 2017 (for 49 x SO4, 239 x RG, and 239 x SO4) or on 10 May for the others, and on 15  
181 May 2018 for all combinations. Bloom was observed on 20 June 2016, 14 June 2017, and 21  
182 June 2018 for all the Sauvignon blanc and Riesling clone x rootstock combinations, and veraison  
183 was observed on 16 August 2016, 25 August 2017, and 23 August 2018. Any difference in  
184 timing of phenological stages at budbreak were no longer observed by bloom or veraison in  
185 either cultivars, regardless of the clone or rootstock.

186 **Riesling clones and rootstocks comparison.** *Cold hardiness.* Clones of Riesling differed  
187 with respect to their cold hardiness late in the acclimation phase for all years studied and during  
188 deacclimation, particularly in the first two years of the study (Table 1). Significant differences  
189 were also observed during the maximum hardiness phase of the first year (Table 1). In general,  
190 clone 239 was hardier than clone 49. Year one had the highest proportion of dates with  
191 significant differences, followed by year two. Rootstock differences were rarely observed and  
192 inexistent in year one (Table 1). They were mostly observed during acclimation on year two and  
193 only present during maximum hardiness in year three. No clear trend of superior hardiness  
194 emerged for the scions grafted to the RG or SO4 rootstocks comparable to that observed in the  
195 clones.

196 The clone\*rootstock interactions were significant for eight of the twelve sampling dates  
197 of the first year of the study, indicating hardiness differences between the clones depending on  
198 the rootstock it was grafted to (Table 1). Significant interactions were observed in year two  
199 during deacclimation and in year three at acclimation and at the start of deacclimation. Clone 239  
200 was hardier when grafted to RG, but clone 49 was generally less hardy when grafted to the same  
201 rootstock (Table 1; Supplemental Figure 1).

202 Since only clone 239 was evaluated on 3309 rootstock in two of the three dormant  
203 periods, the effect of three rootstocks on cold hardiness of clone 239 was analyzed separately. In  
204 the first dormant season, 239 x RG appeared hardier (Supplemental Figure 2) but was rarely  
205 statistically distinguishable from 239 x 3309 (Table 2). On the second year, 239 x RG appeared  
206 less hardy than the other combinations (Supplemental Figure 2), but the differences were only  
207 statistically significant on two sampling dates (Table 2).

208 *Yield and vine size.* Yields, pruning weights and RI were compared between the clone x  
209 rootstock combinations of Riesling. Clone 239 had significantly lower yield and pruning weights  
210 per vine, but overall similar crop load based on RI to clone 49 (Table 3). Similarly, RG-grafted  
211 vines had lower yields and pruning weights, but similar crop loads compared to SO4-grafted  
212 vines (Table 3). The clone\*rootstock interaction was significant for pruning weights and crop  
213 load, often due to the pruning weights of the 239/RG combination which were significantly  
214 lower than the other combinations in all years sampled (Table 3). When evaluating the impact of  
215 the three rootstocks on clone 239, crop load was not different between the three rootstocks  
216 although the 239 x 3309 combination had lower yield and pruning weights (Table 4).

217 **Sauvignon blanc clone comparison.** *Cold hardiness.* The cold hardiness of Sauvignon  
218 blanc was inconsistent from year to year and fluctuated between sampling dates (Supplemental  
219 Figure 3). Next to no significant differences were observed in the first year of the study. Some  
220 differences in hardiness during acclimation and deacclimation were observed in year two and  
221 three, but the clones did not behave consistently (Table 5). Clones 242 and 297 were more likely  
222 to be amongst the least hardy clones compared to clone 376 and 530.



245 was generally hardier than clone 49, regardless of the rootstock it was grafted to. Differences  
246 were observed in all three years during late acclimation and deacclimation, but maximum  
247 hardiness differences were vintage-dependent. The clones had different levels of vigor and yield  
248 regardless of the fact that they were in the same vineyard with consistent management practices  
249 across all combinations. Vine balance measured by the Ravaz index (RI) was significantly  
250 different only in 2016, but the average of all years indicated that clone 239 had generally a  
251 higher crop load than clone 49 mostly due to lower pruning weights. *V. vinifera* cultivars with a  
252 RI between 5 and 10 are considered in balance (Bravdo et al. 1984), and the Riesling clones were  
253 generally within these limits. The relationship between crop load and cold hardiness is variable  
254 and depends on the crop size, the vigor of the vines and environmental conditions that impact  
255 grapevine hardiness responses. Previous research involving crop load manipulation by cluster  
256 thinning have found no difference in cold hardiness between control and thinned *V. vinifera*  
257 vines that had RI in the desired range or below (Lefebvre et al. 2015), or between Vidal vines  
258 that were considered in balance and slightly overcropped (Dami et al. 2013). Reports of a  
259 negative correlation between cold hardiness and crop load have been explained by overcropping  
260 and incomplete cane lignification (Dami et al. 2005). It is therefore unlikely that the superior  
261 hardiness of clone 239 was caused by the higher RI or lower yield and pruning weights reported  
262 in this study.

263         The Riesling clones selected for this study come from different breeding programs. Clone  
264 49 is an ENTAV-INRA® clone that was certified in 1971 in Alsace, while clone 239 is a  
265 Geisenheim clone originating from Germany. Certain clones of Riesling show enough genetic  
266 variability to make them differentiable and identifiable using appropriate genetic markers

267 (Regner et al. 2000). Moreover, accessions grown in different geographical origins can be  
268 differentiated by a combination of various genetic tests (Meneghetti et al. 2012) demonstrating  
269 the impact of the origin of the clone on the genotype. Although genetic testing would be  
270 necessary to confirm this hypothesis, the clones in this study could have a high level of genetic  
271 diversity, particularly considering their diverse geographical origins. This diversity could lead to  
272 the cold hardiness phenotype differences observed. Differential gene expression during  
273 endodormancy or a higher production of cold-protecting metabolites could lead to the superior  
274 hardiness of clone 239 throughout the dormant season, but this hypothesis is yet to be confirmed.

275 **Influence of rootstock and clone x rootstock interactions in Riesling.** Rootstocks did  
276 not appear to contribute to the cold hardiness of the scions. The DTA differences between RG  
277 and SO4-grafted clones 239 and clone 49 were inconsistent across years and rarely significant.  
278 The same observations were made for clone 239 grafted to RG, SO4, and 3309. While minor  
279 differences were measured by DTA, the rootstocks expectedly influenced yield, pruning weights  
280 and RI in most years for both clones. Yields and pruning weights were equal or higher for the  
281 clones grafted to the SO4 rootstock, while crop load was higher on vines grafted on RG, as a  
282 result of smaller vines produced, when significant differences were reported. The RI indicated  
283 that all grafted combinations were in balance for all three years of study (Bravdo et al. 1984).  
284 Using primary bud survival of Riesling as a measure of cold hardiness, Miller et al. (1988) had  
285 identified 3309 as better suited than SO4 to the colder climate, but this conclusion could not be  
286 reached in this study.

287 Clone x rootstock interactions for cold hardiness were particularly important on the first  
288 year of the study, indicating that the clones performed differently on the SO4 and RG rootstocks.

289 The interaction between clone and rootstock was significant also for pruning weights in all years,  
290 but scion x rootstock combinations showed little to no difference in crop load and the vines were  
291 generally considered to be in balance. Clone 49 x RG was often significantly less hardy than 49 x  
292 SO4 and, conversely, 239 x SO4 rootstock was either less hardy or equally hardy when it was  
293 grafted to RG. The vigor of clone 49 x RG was also unexpectedly higher than when grafted to  
294 SO4 rootstock (Cousins 2005). There are important communications between rootstock and  
295 scion (Aloni et al, 2010), and some interactions resulting in reduced hardiness and overly  
296 vigorous vines might be occurring in this case. Considering that similar observations were not  
297 made for the combination of 239 x RG in the same vineyard, the observations on 49 x RG cannot  
298 be explained solely by soil and climate of the site.

299 The lack of consistent rootstock-based cold hardiness differences in this study could be  
300 explained by the fact that complex multi-genic traits that are under scion regulation such as cold  
301 hardiness (Wisniewski et al. 2014) are not significantly impacted by rootstock alone when  
302 growing conditions are appropriate (Sabbatini and Howell 2013). However, specific clone x  
303 rootstock combinations may impact cold hardiness responses in selected years, and these  
304 findings require further elucidation.

305 **Sauvignon blanc clones.** Conversely to Riesling, the Sauvignon blanc clones were not  
306 easily differentiated by their cold hardiness. Clone 242 and 297 were more often amongst the  
307 least hardy clones, but the differences were inconsistent throughout the dormant seasons and  
308 across the years. In general, all Sauvignon blanc clones were considered overcropped (Bravdo et  
309 al. 1984) in 2016/17. This was related to small pruning weights rather than high yields. The  
310 clones were also considered to be overcropped in 2017, and undercropped in 2018. Clone 242

311 had higher yields and lower pruning weights than the other clones, but clone 297 was not  
312 differentiable from clone 376 and 530 for these traits. While this could have led to a reduction in  
313 hardiness for clone 242, crop level, pruning weights and crop load did not consistently affect  
314 overall cold hardiness and clone 242, at times, was the hardiest clone. The inconsistent  
315 differences in hardiness could be caused by a larger range of bud hardiness along the canes  
316 created by uneven acclimation following unfavorable growing seasons and important  
317 overcropping practices. Bud survival can vary greatly from vine-to-vine and within vine  
318 following improper acclimation (Howell and Shaulis 1980) and practices leading to a  
319 maintenance of vine size lead to superior hardiness (Howell et al. 1978). Studies typically focus  
320 on overly vigorous vines, but this study demonstrates that unbalanced very small vines also do  
321 not acclimate optimally and that crop load alone cannot be used to predict how relatively cold  
322 tolerant a grapevine may be.

323         The Sauvignon blanc clones arose from the same breeding program; they are ENTAV-  
324 INRA® clones from Alsace (<http://plantgrape.plantnet-project.org/en/>). All but clone 530  
325 originated from the Loir-et-Cher vineyard between 1973 and 1975; clone 530 came from the  
326 Cher vineyard in 1976. The Sauvignon blanc clones varied from their trait descriptions at times  
327 (<http://plantgrape.plantnet-project.org/en/>). For example, clone 242 has previously been  
328 documented by ENTAV-INRA® to be the most vigorous clone, however it was the least  
329 vigorous in this study. This indicates a regional effect, or a potential clone x rootstock interaction  
330 between this clone and the SO4 rootstock to which it was grafted.

331         **Cultivar differences.** All clone x rootstock combinations of Riesling were hardier than  
332 the Sauvignon blanc clones for every date sampled, regardless of the dormant season, with the

333 exception of the final sampling date in the first year of the study. The differences between the  
334 two cultivars appeared with the first DTA measurements during acclimation and were  
335 maintained during maximum hardiness and deacclimation. The superior cold hardiness of  
336 Riesling was also demonstrated when the LTE from the clone x rootstock combinations of both  
337 cultivars were pooled. Low temperature exotherms were more tightly grouped around the median  
338 for Riesling than Sauvignon blanc, indicating that the acclimation and cold hardiness of Riesling  
339 was more uniform, even with the significant clone differences. This difference was particularly  
340 large in 2016/17 and 2017/18. In the year with the most optimal acclimation weather, 2018/19,  
341 the Sauvignon blanc buds acclimated more uniformly than in the previous two years, and the  
342 clustering around the median was similar to that of Riesling. The Sauvignon blanc buds during  
343 that dormant season were sometimes as hardy as the Riesling buds and the hardest 25% often  
344 partially overlapped with that of Riesling. This indicates that, particularly on favorable years,  
345 Sauvignon blanc buds have the capacity to develop a more uniform and superior cold hardiness  
346 even if the vines were previously overcropped. The data set suggests that individual buds of  
347 Sauvignon blanc can be as cold tolerant as Riesling, but hardiness is not as uniform across the  
348 vine. These varying behaviors between the cultivars could possibly be explained by different  
349 response to the molecular pathways leading into dormancy, but more work needs to be done to  
350 verify this hypothesis. Riesling is considered hardier than Sauvignon blanc, but this study  
351 demonstrates that the magnitude of this difference can be influenced by clone and rootstock  
352 selection, and by the vintage.

353 **Weather impacts.** Hardiness differences between the clone and rootstock combinations,  
354 for both Riesling and Sauvignon blanc, varied from year to year. The 2016 growing season, with



355 its periods without rainfall, lead to important Riesling clone x rootstock interactions in the  
356 2016/17 dormant season. The abnormal rainfall events and the low GDD accumulation during  
357 the 2017 growing season preceded the dormant season with the largest clonal differences for  
358 Sauvignon blanc. Both cultivars require at least 1390 GDD to reach acceptable fruit maturity  
359 (Winkler 1962), and the important GDD differences between the years studied could have  
360 contributed to the yearly differences in cold hardiness. Optimal hardiness was observed in the  
361 2018/19 dormant period which had lower temperatures during acclimation compared to the other  
362 years studied. Considering that exposure to cold temperatures during endodormancy leads to  
363 better cold hardiness (Cragin et al. 2017), it is possible that the warmer weather during  
364 acclimation in October and November of 2016 and 2017 might have reduced the maximum  
365 hardiness, particularly of Sauvignon blanc. Riesling 49 x RG and Sauvignon blanc clone 297  
366 were particularly sensitive to the varying weather, as demonstrated by their lower cold hardiness  
367 during the 2016/17 and 2017/18 dormant seasons, respectively. This indicates that weather  
368 events during the growing season and cold acclimation influence vines maximum hardiness with  
369 varying impact on clone x rootstock combinations. This is an important finding as some grafted  
370 combinations may be more resilient in variable weather conditions during dormancy, which is  
371 likely going to be exacerbated with climate change.

372 Deacclimation initiated between February and March in all years. Commercial operations  
373 in 2017/18 and 2018/19 limited the observation of the full deacclimation dynamics of both  
374 Riesling and Sauvignon blanc. Vines appeared to have deacclimated more rapidly in 2016/17  
375 than any other year as a result of warmer temperatures in February. Seasonal variations were also  
376 expected since the temperatures in the days preceding sampling dates impact cold hardiness

377 during maximum hardiness (Proebsting et al. 1980). Partial deacclimation and reacclimation  
378 have been observed in every year of this study and have been noted previously (Keller et al.  
379 2014). These patterns were more frequent in Sauvignon blanc, particularly in the 2016/17 and  
380 2017/18 dormant seasons, putting this cultivar at a greater risk of cold damage following the  
381 brief deacclimation periods. The favorable growing season and acclimation weather of the  
382 2018/19 dormant season also led to a more uniform deacclimation amongst the Sauvignon blanc  
383 clones.

384 Site difference in hardiness for the same cultivar have been reported (Dami et al. 2015),  
385 and the impact of the varying growing seasons on cold acclimation, maximum hardiness and  
386 deacclimation is akin to that phenomenon. Cold tender cultivars could be more susceptible to the  
387 factors impacting their cold hardiness and are more affected in unfavorable years. This could  
388 explain why the LTE differences were larger on the first two years between Riesling and  
389 Sauvignon blanc. During the rainier seasons, the soils remained quite saturated at times even  
390 with vineyard tiling drainage installed in the Sauvignon blanc vineyard. Water-logged soils are  
391 known to negatively impact grapevine development (Brown et al. 2001) and health (Fisher 1997)  
392 which could result in poor cold acclimation of grapevines. This drainage issue may also explain  
393 the important lack of clonal uniformity in cold hardiness throughout the dormant season for  
394 Sauvignon blanc following the growing season with the highest precipitation. Soil moisture  
395 likely influences cold hardiness both directly and indirectly through encouraging late season  
396 growth and impacting soil temperatures in the root zone. This may be one reason for differences  
397 in performance for a given cultivar in an arid region (i.e. Pacific Northwest) versus a more humid  
398 and wet climate such as Eastern North America (Bowen et al. 2016). Our study outlines the

399 necessity to reproduce cold hardiness experiments over multiple growing seasons before drawing  
400 conclusions on the hardiness of specific cultivars or clones.

## 401 **Conclusion**

402 The purpose of this study was to characterize the differences in cold hardiness between  
403 clones of Riesling and Sauvignon blanc, and to understand the impact of the rootstock on  
404 specific Riesling clones. Different clones of *Vitis vinifera* have different levels of cold hardiness,  
405 and an interaction between clone and rootstock can influence this trait. A consistent, direct  
406 influence of rootstock on cold hardiness was not observed, and none of the cold hardiness  
407 differences could be directly explained by yield components, vigor and crop load. This study also  
408 described the impact of weather on cold acclimation and uniformity of cold hardiness and  
409 demonstrated that Sauvignon blanc was more susceptible to uneven cold acclimation and climate  
410 variability compared to a more cold hardy cultivar such as Riesling. Future cold hardiness  
411 studies should consider clone and rootstock interactions when comparing cultivars, should  
412 include multiple sampling dates, and should be repeated over multiple vintages. The  
413 identification of clones with superior cold hardiness could potentially be accelerated by first  
414 identifying different biotype of cultivars originating from colder climates where natural selection  
415 might have favored the development of more cold hardy clones.

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**Table 1** Comparison of low temperature exotherms by two-way ANOVA for Riesling clone x rootstock combinations (Clone 49 x Riparia Gloire; 49/RG; Clone 49 x SO4 Teleki; 49/SO4; Clone 239 x Riparia Gloire, 239/RG; Clone 239 x SO4 Teleki, 239/SO4) for all dormant seasons (2016/17, 2017/18, 2018/19).

	2016/2017	6 Oct	18 Oct	2 Nov	16 Nov	30 Nov	14 Dec	4 Jan	26 Jan	14 Feb	1 Mar	19 Mar	1 Apr
Clone/Rootstock													
49/RG		-10.1	-10.4	-12.4	-15.1 a <sup>b</sup>	-18.6 a	-20.9 a	-20.7 a	-21.5 a	-20.9 a	-18.2	-18.9 a	-15.0 a
49/SO4		-9.9	-10.7	-13.2	-15.7 ab	-19.3 b	-22.4 b	-22.2 b	-22.8 bc	-22.5 b	-19.4	-19.9 ab	-16.7 b
239/RG		-10.4	-12.0	-13.0	-17.2 c	-20.5 c	-22.4 b	-23.6 c	-23.4 c	-24.0 c	-20.2	-20.6 b	-18.0 c
239/SO4		-10.2	-11.3	-13.3	-16.4 b	-19.2 ab	-20.9 a	-22.9 bc	-21.9 ab	-22.7 b	-19.8	-20.1 b	-17.7 bc
<b>Clone* Rootstock interaction</b>		ns <sup>a</sup>	ns	ns	*	****	***	***	***	****	ns	*	**
<b>Clone factor</b>		ns	**	ns	****	***	ns	****	ns	****	***	**	****
<b>Rootstock factor</b>		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	2017/2018	29 Oct	10 Nov	24 Nov	11 Dec	20 Dec	8 Jan	29 Jan	14 Feb	8 Mar	26 Mar		
49/RG		-13.5	-14.6	-18.8	-21.6	-22.1	-22.0	-22.1	-23.2	-21.2 a	-21.4 a		
49/SO4		-12.8	-16.4	-19.0	-20.8	-22.8	-20.2	-22.8	-22.8	-21.7 a	-22.1 a		
239/RG		-13.5	-17.7	-20.9	-22.8	-23.0	-22.3	-22.7	-24.4	-23.1 c	-22.1 a		
239/SO4		-12.4	-18.3	-20.9	-22.4	-23.0	-22.1	-22.7	-23.5	-22.3 b	-21.6 a		
<b>Clone* Rootstock interaction</b>		ns	ns	ns	ns	ns	ns	ns	ns	**	*		
<b>Clone factor</b>		ns	****	****	****	ns	ns	ns	***	****	ns		
<b>Rootstock factor</b>		**	**	ns	*	ns	ns	ns	**	ns	ns		
	2018/2019	3 Oct	19 Oct	30 Oct	12 Nov	27 Nov	18 Dec	11 Jan	1 Feb	19 Feb	5 Mar	18 Mar	
49/RG		-10.5 a	-12.7	-13.2	-17.5	-19.6	-21.8	-23.0	-22.9	-22.4 a	-22.3	-21.8	
49/SO4		-11.9 b	-13.1	-12.7	-16.6	-20.5	-21.7	-24.0	-23.8	-23.7 b	-23.3	-22.5	
239/RG		-11.0 ab	-12.8	-13.4	-18.6	-21.5	-23.2	-22.7	-23.9	-23.1 ab	-23.6	-22.5	
239/SO4		-10.6 a	-13.3	-13.7	-17.9	-21.6	-23.1	-24.0	-24.5	-23.1 ab	-22.9	-22.9	
<b>Clone*Rootstock interaction</b>		*	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	
<b>Clone factor</b>		ns	ns	ns	**	****	****	ns	ns	ns	ns	*	
<b>Rootstock factor</b>		ns	ns	ns	ns	ns	ns	**	ns	*	ns	ns	

<sup>a</sup>Significance level of the two-way ANOVA indicating difference at \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ , \*\*\*\*:  $p < 0.0001$ , or no significant difference: ns.

<sup>b</sup>Means followed by different letters are significantly different ( $p < 0.05$ ) for the date within a factor separated by Tukey HSD post-hoc test.

**Table 2** Comparison of low temperature exotherms by one-way ANOVA for Riesling 239 grafted to Riparia gloire (239/RG), SO4 Teleki (239/SO4), and Couderc 3309 (239/3309).

	2017/2018	29 Oct	10 Nov	24 Nov	11 Dec	20 Dec	8 Jan	29 Jan	14 Feb	8 Mar	26 Mar	
Clone/rootstock												
239/RG		-13.4 b <sup>b</sup>	-17.7	-20.9	-22.9	-23.0	-22.4	-22.8	-24.4 b	-23.1 b	-22.1	
239/SO4		-12.5 a	-18.0	-20.9	-22.4	-23.0	-22.0	-22.6	-23.4 a	-22.3 a	-21.7	
239/3309		-13.2 ab	-17.2	-20.7	-22.5	-23.1	-21.5	-23.2	-23.9 ab	-22.1 a	-21.6	
	<b>Significance<sup>a</sup></b>	*	ns	ns	ns	ns	ns	ns	*	***	ns	
	2018/2019	3 Oct	19 Oct	30 Oct	12 Nov	27 Nov	18 Dec	11 Jan	1 Feb	19 Feb	5 Mar	18 Mar
239/RG		-10.9	-13.1 a	-13.4	-18.6	-21.6	-23.2	-22.7 a	-23.8	-23.0	-23.6	-22.5
239/SO4		-10.6	-13.2 a	-13.6	-17.9	-21.6	-23.1	-24.0 b	-24.5	-23.1	-22.9	-22.9
239/3309		-11.3	-14.4 b	-13.8	-18.1	-21.4	-23.1	-23.7 b	-24.4	-24.0	-23.2	-22.6
	<b>Significance</b>	ns	*	ns	ns	ns	ns	***	ns	ns	ns	ns

<sup>a</sup>Significance level of the one-way ANOVA indicating difference at \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ , \*\*\*\*:  $p < 0.0001$ , or no significant difference: ns.

<sup>b</sup>Means followed by different letters are significantly different ( $p < 0.05$ ), separated by Tukey HSD post-hoc test.



**Table 3** Comparison of yield, pruning weights, and Ravaz index by two-way ANOVA for Riesling clones 49 and 239 grafted to Riparia gloire and SO4 Teleki (49/RG; 49/SO4, 239/RG; 239/SO4) for three years and the average of all year.

Factor	Yield (kg/vine)				Pruning weights (kg/vine)				Ravaz index			
	2016	2017	2018	all years	2016	2017	2018	all years	2016	2017	2018	all years
Clone												
49	3.3	4.7	3.3	3.8	0.46	0.57	0.55	0.53	7.8	9.3	6.2	7.8
239	3.1	3.9	2.7	3.2	0.38	0.42	0.50	0.44	9.8	10.3	5.7	8.6
Significance <sup>a</sup>	ns	****	***	****	**	****	*	****	**	ns	ns	*
Rootstock												
RG	3.0	4.1	2.9	3.3	0.41	0.50	0.47	0.46	8.9	9.4	6.6	8.3
SO4	3.4	4.5	3.0	3.6	0.43	0.49	0.59	0.50	8.6	10.2	5.3	8.0
Significance	**	*	ns	***	ns	ns	****	**	ns	ns	***	ns
Clone*Rootstock interaction												
49/RG	3.3	4.4	3.4 a <sup>b</sup>	3.7	0.50 a	0.62 a	0.54 a	0.55 a	7.3	8.1 b	6.6	7.3 b
49/SO4	3.4	5.0	3.2 a	3.8	0.43 ab	0.52 ab	0.57 a	0.51 a	8.3	10.5 ab	5.9	8.2 ab
239/RG	2.8	3.7	2.4 b	3.0	0.33 b	0.38 c	0.40 b	0.37 b	10.5	10.8 a	6.6	9.3 a
239/SO4	3.4	4.1	2.9 ab	3.5	0.43 ab	0.46 bc	0.61 a	0.50 a	9.0	9.8 ab	4.8	7.9 b
Significance	ns	ns	*	ns	**	**	***	****	ns	*	ns	***

<sup>a</sup>Significance level of the two-way ANOVA indicating difference at \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ , \*\*\*\*:  $p < 0.0001$ , or no significant difference: ns.

<sup>b</sup>Means followed by different letters are significantly different ( $p < 0.05$ ), separated by Tukey HSD post-hoc test.

**Table 4** Comparison of yield, pruning weights and Ravaz index by one-way ANOVA for Riesling 239 grafted to Riparia gloire (239/RG), SO4 Teleki (239/SO4), and Couderc 3309 (239/3309) for both years and the average of all years.

	Yield (kg/vine)			Pruning weights (kg/vine)			Ravaz index		
	2017	2018	all years	2017	2018	all years	2017	2018	all years
239/RG	3.7 a <sup>b</sup>	2.4 b	3.1 b	0.38	0.40 b	0.40 b	10.8	6.6 a	8.7
239/SO4	4.1 a	2.9 a	3.5 a	0.46	0.61 a	0.53 a	9.8	4.8 b	7.3
239/3309	2.9 b	1.8 c	2.4 c	0.37	0.35 b	0.36 b	10.0	6.6 a	8.3
Significance <sup>a</sup>	****	****	****	ns	****	****	ns	**	ns

<sup>a</sup>Significance level of the one-way ANOVA indicating difference at \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ , \*\*\*\*:  $p < 0.0001$ , or no significant difference: ns.

<sup>b</sup>Means followed by different letters are significantly different ( $p < 0.05$ ), separated by Tukey HSD post-hoc test.

**Table 5** Comparison of low temperature exotherms by one-way ANOVA for Sauvignon blanc clones grafted to SO4 Teleki rootstock.

	<b>2016/2017</b>	<b>6 Oct</b>	<b>18 Oct</b>	<b>2 Nov</b>	<b>16 Nov</b>	<b>30 Nov</b>	<b>14 Dec</b>	<b>4 Jan</b>	<b>26 Jan</b>	<b>14 Feb</b>	<b>1 Mar</b>	<b>19 Mar</b>	<b>1 Apr</b>
Clone													
242		-7.2 ab <sup>b</sup>	-10.0	-10.1	-12.3	-14.9	-17.4	-15.1	-16.9	-17.9	-17.0	-15.7	-17.0
297		-7.8 b	-9.7	-9.3	-13.1	-13.7	-16.6	-14.9	-17.7	-16.2	-17.2	-15.6	-14.7
376		-7.8 b	-8.9	-10.2	-12.7	-14.3	-15.7	-16.2	-17.4	-15.8	-16.5	-17.0	-16.8
530		-6.4 a	-9.4	-9.7	-12.0	-15.0	-17.3	-16.9	-18.5	-16.6	-16.2	-16.9	-16.1
<b>Significance<sup>a</sup></b>		*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	<b>2017/2018</b>	<b>29 Oct</b>	<b>10 Nov</b>	<b>24 Nov</b>	<b>11 Dec</b>	<b>20 Dec</b>	<b>8 Jan</b>	<b>29 Jan</b>	<b>14 Feb</b>	<b>8 Mar</b>	<b>26 Mar</b>	<b>13 Apr</b>	
242		-10.3	-12.6 b	-14.2 a	-14.9 a	-16.6	-18.0	-19.9	-20.3 b	-20.6	-19.8	-15.6	
297		-9.4	-10.9 a	-14.2 a	-18.0 b	-16.7	-16.9	-20.0	-18.1 a	-16.8	-20.0	-16.3	
376		-9.3	-13.1 b	-16.9 b	-16.9 ab	-15.7	-19.6	-19.0	-17.2 a	-18.1	-18.5	-16.3	
530		-10.1	-14.0 b	-15.5 ab	-16.7 ab	-17.5	-19.7	-19.5	-18.9 ab	-17.9	-20.2	-18.5	
<b>Significance</b>		ns	***	***	*	ns	ns	ns	*	ns	ns	ns	
	<b>2018/2019</b>	<b>3 Oct</b>	<b>19 Oct</b>	<b>30 Oct</b>	<b>12 Nov</b>	<b>27 Nov</b>	<b>18 Dec</b>	<b>11 Jan</b>	<b>1 Feb</b>	<b>19 Feb</b>	<b>5 Mar</b>	<b>18 Mar</b>	<b>13 Apr</b>
242		-8.5 a	-11.9 b	-11.6	-14.6 b	-16.6 a	-18.3 a	-19.4	-21.2	-19.7	-19.2	-16.4 a	-10.2 a
297		-8.8 a	-10.7 a	-11.3	-13.6 a	-17.0 a	-20.3 b	-20.5	-21.3	-19.2	-19.0	-17.7 ab	-11.9 b
376		-9.7 b	-10.7 a	-12.3	-15.3 b	-17.4 ab	-20.6 b	-19.8	-21.4	-19.2	-18.9	-18.4 b	-10.1 a
530		-9.6 b	-10.9 a	-11.7	-15.3 b	-18.7 b	-19.7 b	-20.2	-22.0	-18.7	-19.5	-17.1 ab	-9.4 a
<b>Significance</b>		**	**	ns	****	**	***	ns	ns	ns	ns	*	*

<sup>a</sup>Significance level of the one-way ANOVA indicating difference at \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ , \*\*\*\*:  $p < 0.0001$ , or no significant difference: ns.

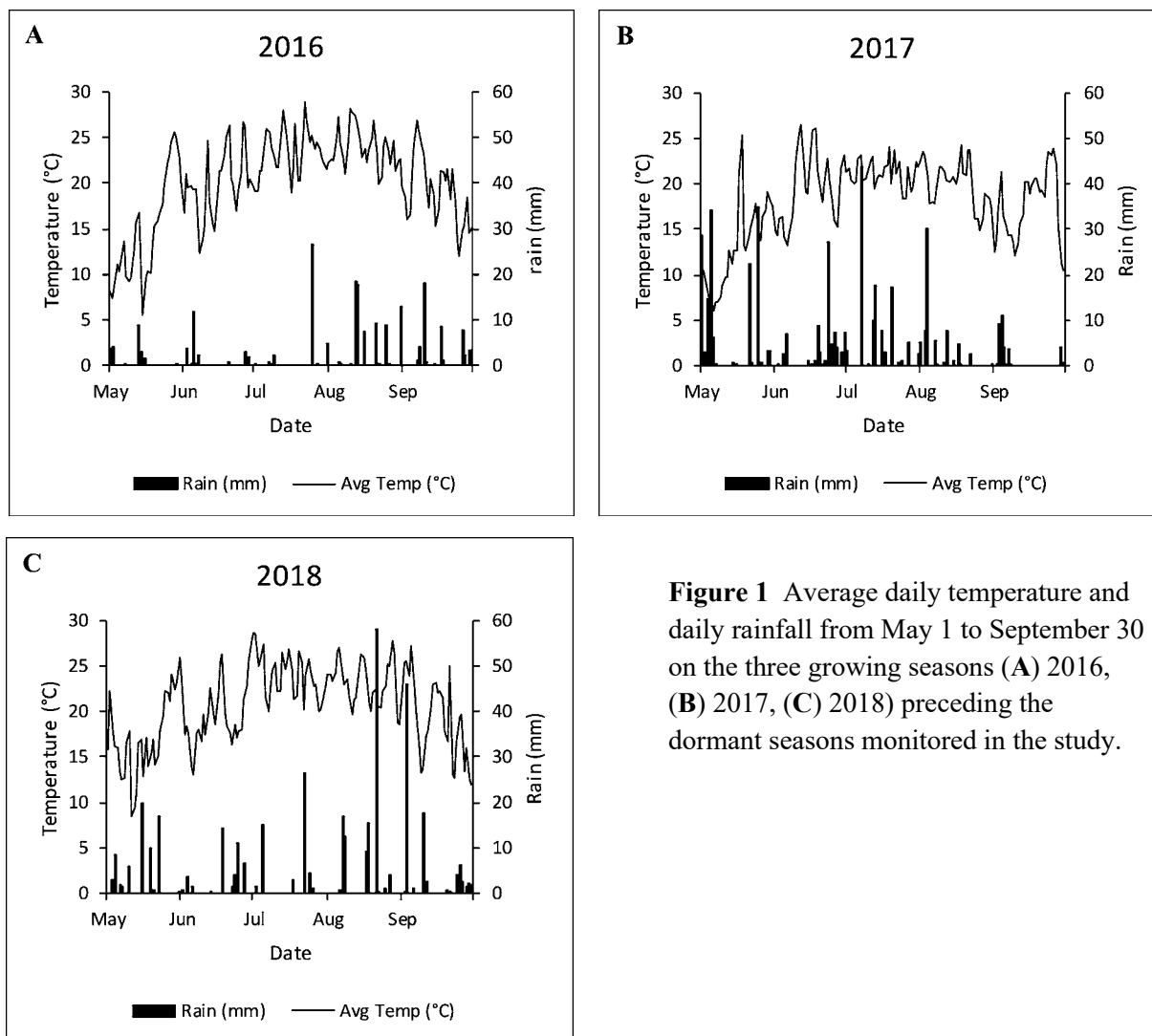
<sup>b</sup>Means followed by different letters are significantly different ( $p < 0.05$ ) for the date separated by Tukey HSD post-hoc test.

**Table 6** Comparison of yield, pruning weights and Ravaz index for the Sauvignon blanc clones grafted to SO4 Teleki rootstock for the three years and the average of all year.

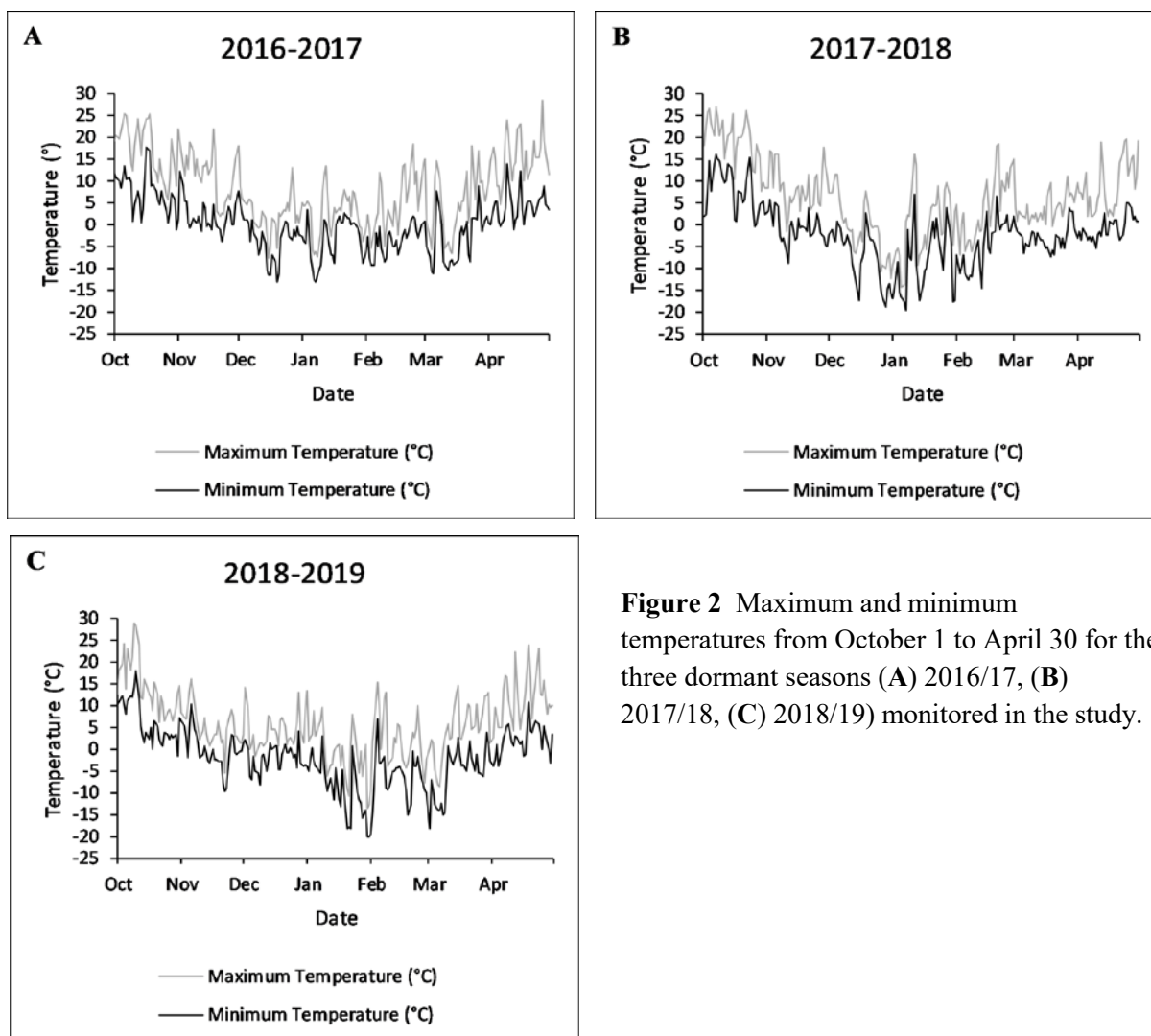
Clone	Yield (kg/vine)				Pruning weights (kg/vine)				Ravaz index			
	2016	2017	2018	all years	2016	2017	2018	all years	2016	2017	2018	all years
242	3.0	2.6	1.5 a <sup>b</sup>	2.4 a	0.08 c	0.15 c	0.30 b	0.16 c	53.1 a	20.1 a	5.2 a	25.9 a
297	3.0	2.1	1.0 ab	2.0 b	0.12 bc	0.17 bc	0.40 a	0.23 b	29.4 b	14.0 b	2.7 b	15.4 b
376	3.2	2.1	0.9 b	2.1 b	0.19 a	0.23 a	0.41 a	0.28 a	22.0 b	10.6 b	2.6 b	11.8 b
530	3.1	2.2	0.9 b	2.1 b	0.15 ab	0.21 ab	0.43 a	0.26 ab	27.7 b	13.3 b	2.2 b	14.4 b
Significance <sup>a</sup>	ns	ns	**	**	****	***	****	****	****	****	****	****

<sup>a</sup>Significance level of the one-way ANOVA indicating difference at \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ , \*\*\*\*:  $p < 0.0001$ , or no significant difference: ns.

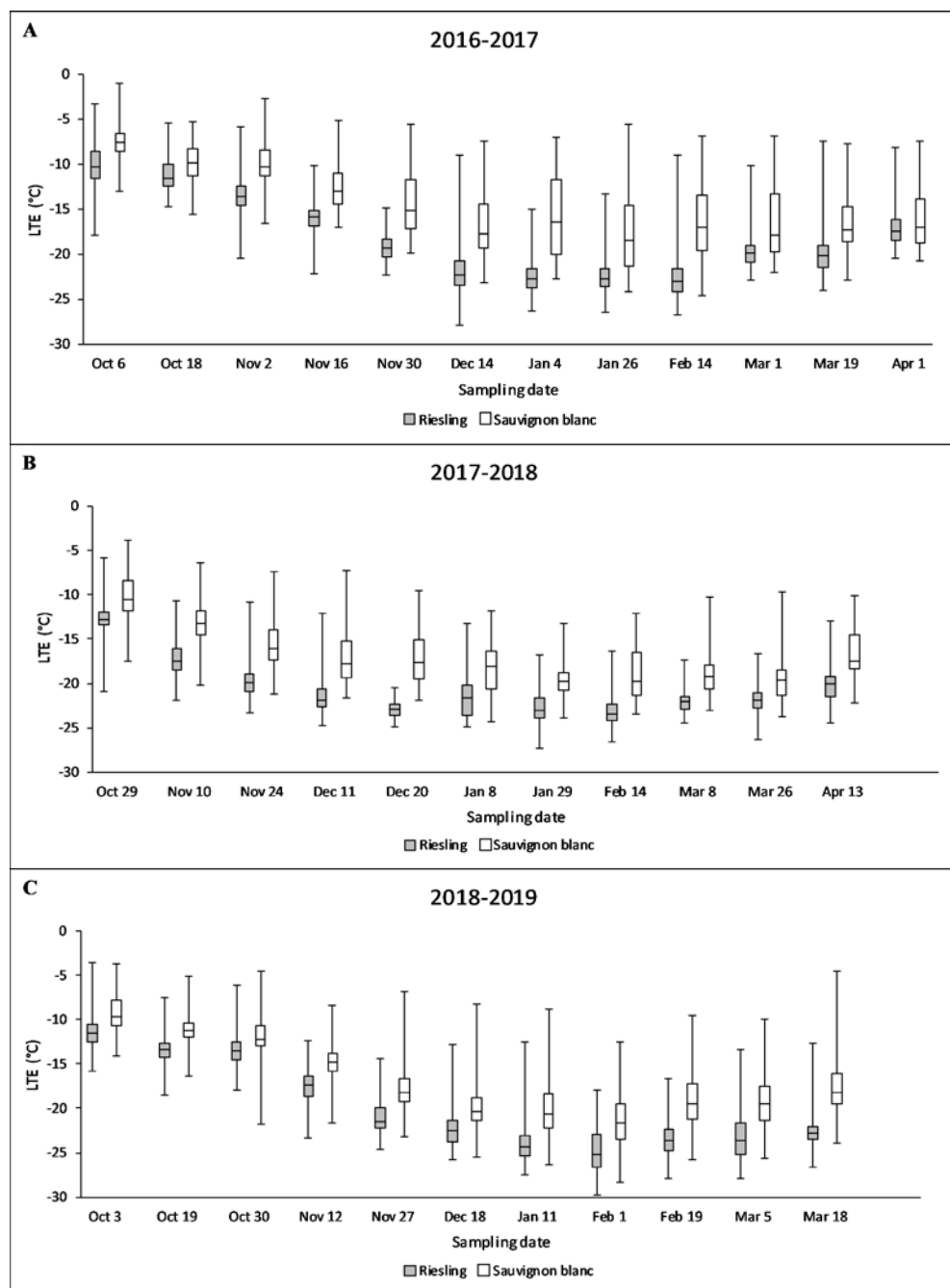
<sup>b</sup>Means followed by different letters are significantly different ( $p < 0.05$ ) for the date within a factor.



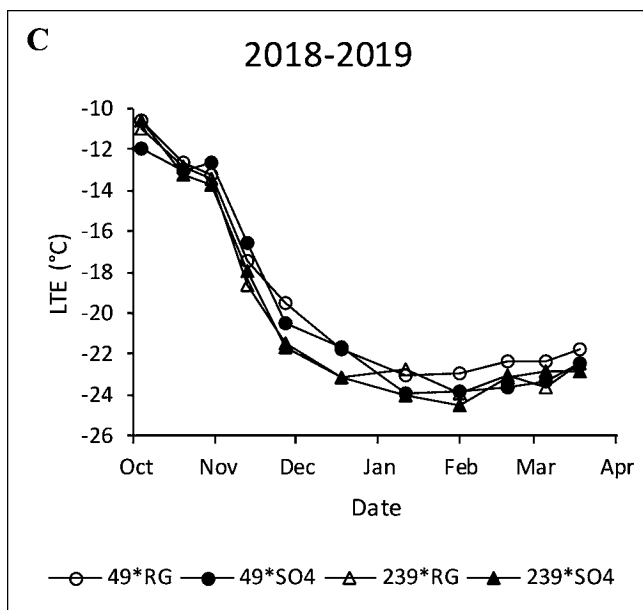
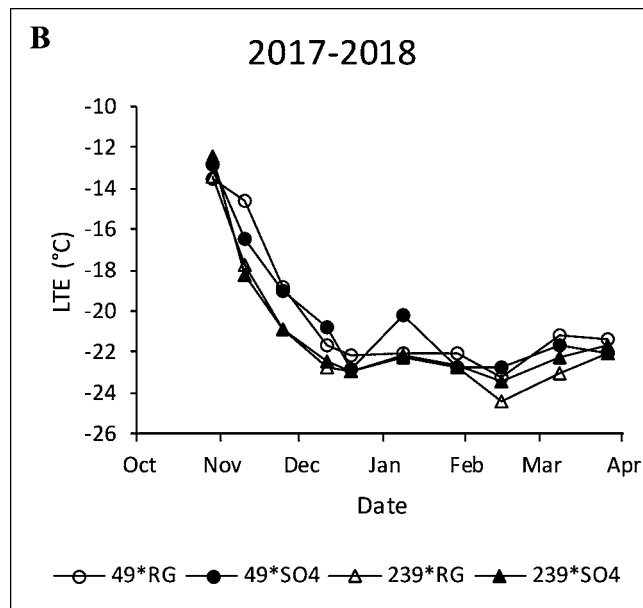
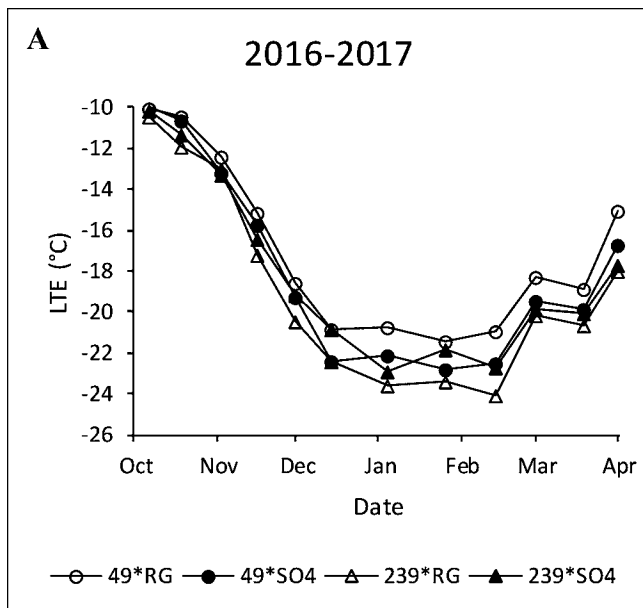
**Figure 1** Average daily temperature and daily rainfall from May 1 to September 30 on the three growing seasons (A) 2016, (B) 2017, (C) 2018) preceding the dormant seasons monitored in the study.



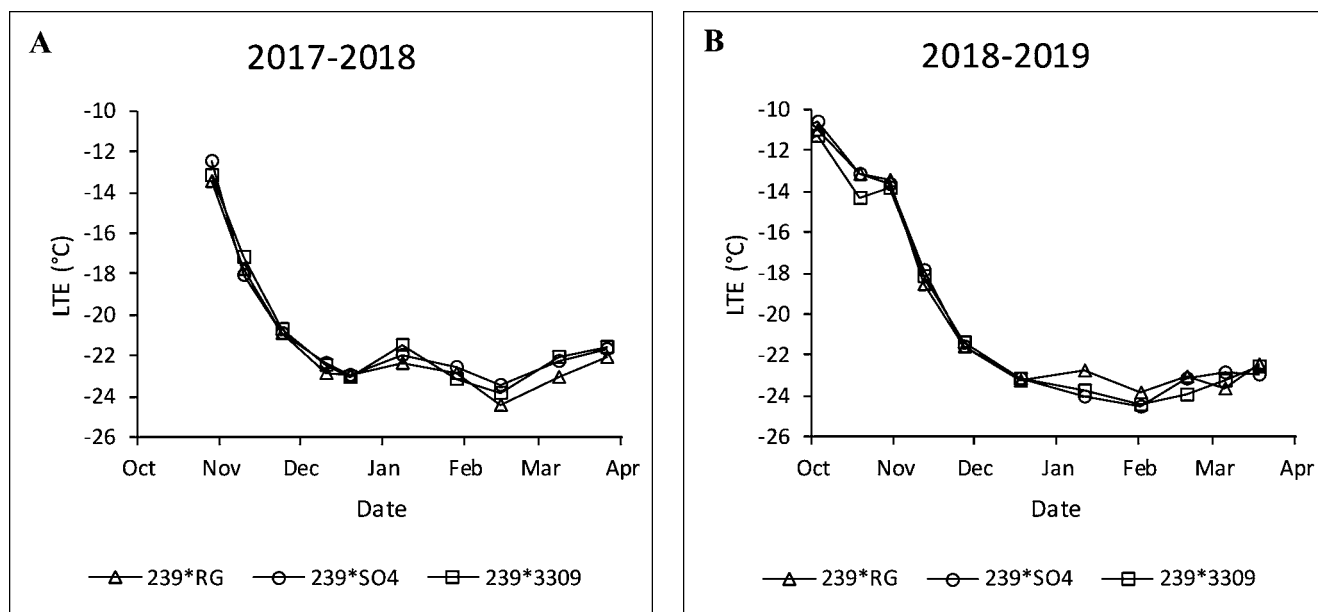
**Figure 2** Maximum and minimum temperatures from October 1 to April 30 for the three dormant seasons (A) 2016/17, (B) 2017/18, (C) 2018/19) monitored in the study.



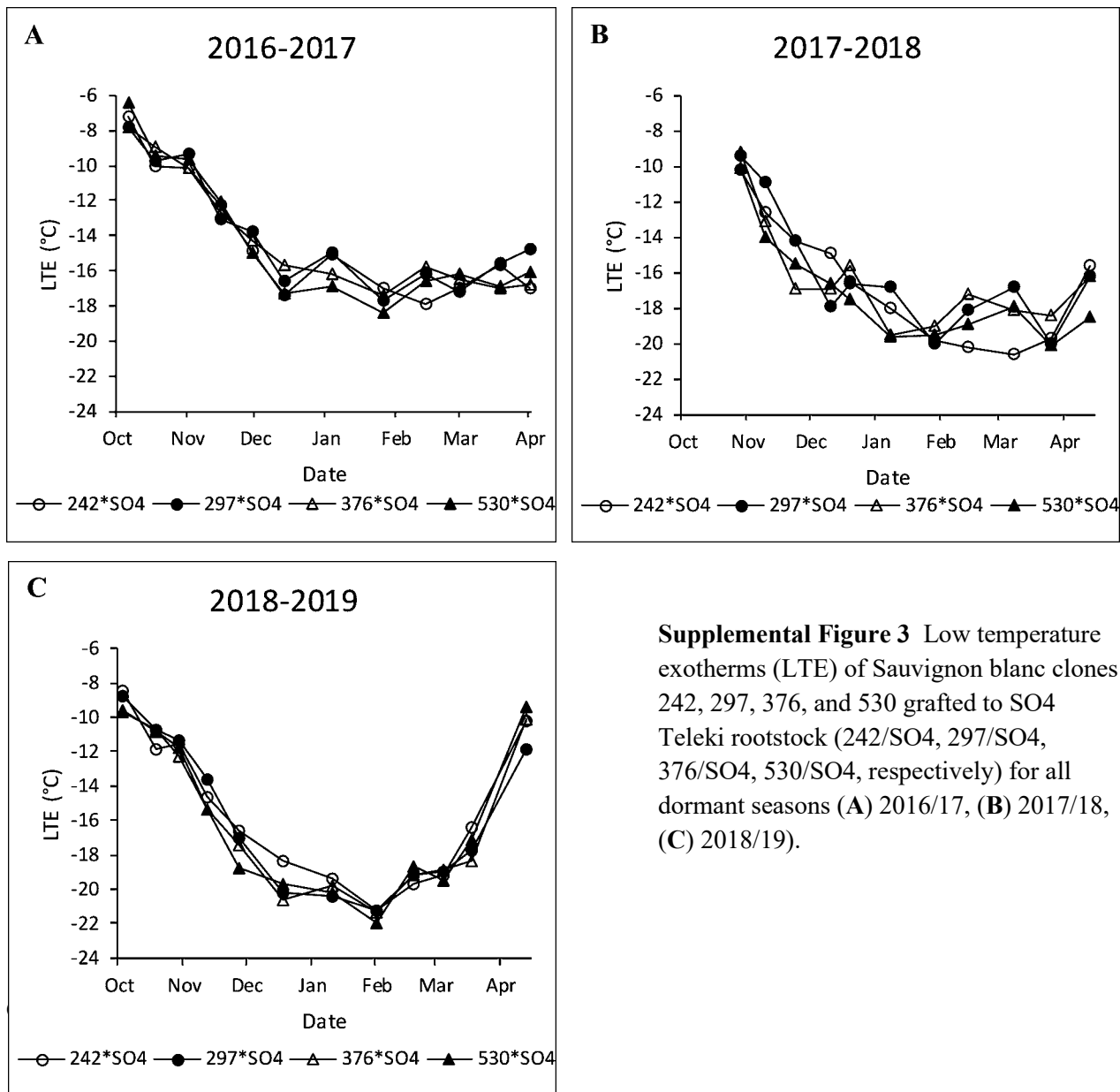
**Figure 3** Pooled low temperature exotherms (LTE) from all clone and rootstock combinations for Riesling (49/RG, 49/SO4, 239/RG, 239/SO4, 239/3309) and Sauvignon Blanc (242/SO4, 297/SO4, 376/SO4, 530/SO4) on the three years sampled (A) 2016/2017, (B) 2017/2018; (C) 2018/2019). The top and bottom whiskers represent the maximum and the minimum LTE, respectively. The top, median, and bottom edge of the box represents the 25<sup>th</sup>, median, and 75<sup>th</sup> quartile, respectively.



**Supplemental Figure 1** Low temperature exotherms (LTE) of Riesling clone x rootstock combinations (Clone 49 x Riparia Gloire; 49\*RG; Clone 49 x SO4 Teleki; 49\*SO4; Clone 239 x Riparia Gloire, 239\*RG; Clone 239 x SO4 Teleki, 239\*SO4) for all dormant seasons (A) 2016/17, (B) 2017/18, (C) 2018/19).



**Supplemental Figure 2** Low temperature exotherms (LTE) of Riesling clone 239 grafted to Riparia gloire (239\*RG), SO4 Teleki (239\*SO4), and Couderc 3309 (239\*3309) for two dormant seasons (A) 2017/18, (B) 2018/19).



**Supplemental Figure 3** Low temperature exotherms (LTE) of Sauvignon blanc clones 242, 297, 376, and 530 grafted to SO4 Teleki rootstock (242/SO4, 297/SO4, 376/SO4, 530/SO4, respectively) for all dormant seasons (A) 2016/17, (B) 2017/18, (C) 2018/19).