Bud dormancy pattern, chilling requirement, and cold hardiness in Vitis vinifera L. ‘Chardonnay’ and ‘Riesling’1

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Source: Canadian Journal of Plant Science, 101(6) : 871-885

Published By: Canadian Science Publishing

URL: https://doi.org/10.1139/cjps-2020-0332

Canadian Science Publishing

ARTICLE

Bud dormancy pattern, chilling requirement, and cold hardiness in *Vitis vinifera* L. 'Chardonnay' and 'Riesling'[1]

Alireza Rahemi, Helen Fisher, Adam Dale, Toktam Taghavi, and John Kelly

Abstract: In recent years, new vineyards have been established in southwestern Ontario. The open water of Lake Erie provides some winter protection for *Vitis* hybrids and winter-hardy *Vitis vinifera* L. cultivars in this area. However, winter damage is possible when vines are grown distant from the open water or when lakes are frozen. To better understand the risks to winter survival, the dormancy and chilling phenology were studied over three winters from 2013–2016. Ten dormant canes of two *V. vinifera* cultivars, 'Chardonnay' and 'Riesling', were collected weekly from 1 September until 30 March from the mature vines in a commercial vineyard located at St. Williams (Ontario). The canes defoliated in early October, and the endodormancy was completed by the end of December. The cumulative chilling hours (0–7.2 °C) from defoliation until the completion of endodormancy were averaged 606 hours for 'Chardonnay' and 665 hours for 'Riesling'. 'Chardonnay' buds were slightly less hardy than 'Riesling' to cold temperatures, with a threshold of about −24 °C for 'Chardonnay' and −25 °C for 'Riesling'. Most primary buds of both cultivars died after 16 February 2015, and more than half died after 12 February 2014, due to severe low temperatures of −33.1 and −26 °C, respectively.

Key words: acclimation, bud sprout (budbreak), cold climate, de-acclimation, freeze injury.

Résumé : Depuis quelques années, on aménage de nouveaux vignobles dans le sud-ouest de l'Ontario. Libres, les eaux du lac Érié offrent en effet une certaine protection contre les conditions hivernales aux hybrides du genre *Vitis* ainsi qu'aux cultivars rustiques de *Vitis vinifera* L. dans la région. Cependant, des dommages restent possibles quand les vignes sont cultivées loin des eaux du lac ou lorsque celles-ci sont prises par la glace. Pour mieux comprendre ces risques, les auteurs ont étudié la phénologie de la dormance et du refroidissement trois hivers durant, de 2013 à 2016. À cette fin, chaque semaine du 1er septembre au 30 mars, ils ont prélevé dix sarments dormants sur les ceps matures de deux cultivars de *V. vinifera* (Chardonnay et Riesling), dans un vignoble commercial de St. Williams (Ontario). Les sarments ont perdu leurs feuilles au début d'octobre et l'endodormance était complète à la fin de décembre. De la défoliation à l'endodormance, les auteurs ont compté 606 heures de refroidissement (de 0 à 7,2 °C) en moyenne pour le cultivar Chardonnay et 665 pour le cultivar Riesling. Les bourgeons du premier résistent légèrement moins au froid que ceux du second, avec un seuil d'environ 24 °C sous zéro pour le cultivar Chardonnay et de moins 25 °C pour le cultivar Riesling. La majorité des bourgeons principaux sont morts après le 16 février 2015 et plus de la moitié avait été détruite après le 12 février 2014, consécutivement aux températures extrêmes de moins 33,1 et de moins 26 °C, respectivement, enregistrées à ces dates. [Traduit par la Rédaction]

Mots-clés : acclimatation, débourrement, climat froid, désacclimatation, dommages dus au gel.

Received 18 December 2020. Accepted 10 August 2021.

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*T. Taghavi served as an Associate Editor at the time of manuscript review and acceptance; peer review and editorial decisions regarding this manuscript were handled by K. Tanino.

[1] This article is part of a Special Issue entitled "Advancements in Canadian horticulture, in celebration of the 100th year of horticulture research at the University of Saskatchewan".

Can. J. Plant Sci. **101:** 871–885 (2021) dx.doi.org/10.1139/cjps-2020-0332

Introduction

Until the late 1970s, commercial wine grape vineyards in Ontario were established based on native (*Vitis labrusca* L.) and French-American hybrid cultivars and were replanted to *Vitis vinifera* L. cultivars (GGO 2018). However, most commercial *V. vinifera* are unable to withstand severe cold winters (<−20 °C) or fluctuating mid-winter temperatures that can lead to de-acclimation (Stafne 2007) and loss of hardiness (Zabadal et al. 2007). Winter injury is a constant challenge for most commercial vineyards in Canada (Schaer 2016). Several factors affect cold hardiness in fully acclimated grapes, including the stage of dormancy, cultivar/rootstock genetics, and environmental conditions leading up to a particular event (Fennell 2004; Stafne 2007).

Knowledge of the physiological traits affecting the hardiness of grape cultivars is a key component in the choice of adapted genotypes for a particular climate. Three physiological stages exist related to dormancy and cold hardiness in grapes. The first stage is acclimation (the ability to adjust to a more adverse environment). Acclimation in *V. vinifera* is triggered by decreasing day length and temperature and begins during late summer when the shoot growth declines (Jackson 1994; Willwerth 2014). The second stage is mid-winter hardiness (ability to survive specific environmental challenges, especially potentially lethal freezing temperatures during dormancy). The third stage is de-acclimation (loss of hardiness due to sustained warmer temperature conditions; Stafne 2007).

Dormancy is an adaptation process, which allows buds to survive under severe conditions during the winter (Arora et al. 2003). In fruit trees, recognizing the dormancy mechanism is very important for crop yields (Viti et al. 2013). Dormancy is categorized into para, endo, and ecodormancy according to the mechanism of growth restriction (Lang et al. 1987). Paradormancy is the inhibition of bud growth by physiological factors, which is influenced by another organ of the plant, such as lateral bud dormancy due to apical dominance of the terminal bud (Anzanello et al. 2018). Endodormancy in buds occurs when bud development is inhibited by physiological and biochemical factors inside the buds. During the endodormant stage, buds enter deep sleep and can only resume normal growth if exposed to a specific period of cumulative sustained chilling. The chilling requirement is satisfied by the presence of a specific number of hours of temperatures between 0–7.2 °C (Weinberger 1950; Horvath et al. 2003; Bielenberg 2015).

Ecodormancy is caused by a temporary environmental restriction, such as a lack of suitable growing temperatures (Anzanello et al. 2018). Ecodormant buds can resume growth under favourable environmental conditions (Fennell 2004). To determine the chilling period required to satisfy endodormancy in temperate fruit trees, many researchers have used cuttings excised from the orchard at various times during the winter (physiological method), and forced them at constant temperature (21–23 °C) to induce bud sprout (this term is considered as a synonym of budbreak in this manuscript), and observed either the time required for the buds to sprout (in peach, Weinberger 1950; and hazelnut, Mehlenbacher 1991) or the number of buds that sprout (in grapes, Andreini et al. 2009; and almonds, Prudencio et al. 2018). However, some researchers have used single node buds (Carvalho and Silva 2010 in apples, Londo and Johnson 2014 in grapes). Plancher (1983) warned against using one node cuttings and isolated buds for dormancy investigations in black currant (*Ribes nigrurm*), as the results may be modified by the method of sampling.

The chilling requirement can also be determined by calculated methods, which are less time-consuming than the physiological methods (Dale and Rahemi 2017). Hutchins (1932) proposed a calculated method for peaches that totaled chilling hours accumulated at temperatures ≤7.2 °C. Bennett (1949) described a cumulative chilling hours model on deciduous plants, and later Weinberger (1950) developed a comparative scale to rate the effects of cold temperatures on peach buds (initially presented by Hutchins 1932) using temperatures between 0 and 7.2 °C. Hadj-Hassan and Ferguson (2004) reported the most efficient temperature at which pistachio accumulates chilling time is 7.2 °C. The chilling response acts slowly, and it usually takes several weeks to several months before it is completed (Liu et al. 2015). The time-release of dormancy is genetically controlled and regulated by complex factors (Faust et al. 1997). Many cellular activities lead to morphological, physiological, and biochemical changes happened during the transition period from endodormancy to active bud growth. Environmental factors are directly related to biochemical changes involved in bud dormancy release (Viti et al. 2013). Different models were developed later to improve the measurement of the accumulation of chilling hours and determine when endodormancy is satisfied (Ben Mohammad et al. 2010; Luedeling 2012; Powell et al. 2019).

At present, the major chilling models are the Weinberger 'hours of chilling' (Weinberger 1950), the Utah weighted 'chill units' (Richardson et al. 1974 for peach; Anderson et al. 1986 for sour cherry), and the Dynamic Model 'chilling portions' (Fishman et al. 1987; Erez et al. 1990; Erez and Fishman 1997; Okie and Blackburn 2011 all four for peaches).

Endodormancy and cold hardiness are two processes of buds for surviving in winter. Rubio et al. (2016) believed that each phenomenon is stimulated by different environmental cues, endodormancy by short-day photoperiod, and cold hardiness by low temperatures. However, Schnabel and Wample (1987) reported that 'Riesling' acclimates little in response to short-day photoperiod, but short-day photoperiod combined with low temperatures increases acclimation or cold hardiness. Cold hardiness occurs mainly via the supercooling of intracellular water, the freezing of which is associated with a low-temperature exotherm (LTE, Pierquet and Stushnoff 1980; Rubio et al. 2016). Winter bud hardiness in grapes is calculated using the LTE50 (Pierquet and Stushnoff 1980), the temperature at which 50% of the buds are killed (Andrews et al. 1984; Zabadal et al. 2007; Mussell et al. 2011; Ontario Tender Fruit 2017).

The threshold temperatures for winter hardiness in ‘Chardonnay’ and ‘Riesling’ are somewhat variable. Threshold temperatures of about −24 °C have been reported by Andrews et al. (1984), Mussell et al. (2011) and Willwerth, (2014). However, ‘Riesling’ can withstand short exposures to −26 °C (Jackson 1994). ‘Chardonnay’ usually tolerates −22 °C and ‘Riesling’ about −25 °C (Hamman et al. 1996). Therefore, ‘Chardonnay’ often has more bud and cane damage than ‘Riesling’ (Miller et al. 1988).

The dormancy requirements of grape cultivars have not been determined in Ontario. Therefore, in this study, the onset of dormancy, the transition from paradormancy to endodormancy, chilling requirement, dormancy termination, and their effects on bud sprouting and freezing injury rate were determined in excised canes of two grape cultivars (‘Chardonnay’, and ‘Riesling’) grown in southwestern Ontario.

Materials and Methods

Two cultivars, ‘Chardonnay’ and ‘Riesling’, were used because they represent 25% of the vineyards in Ontario. Vines of the cultivars were considered in a commercial vineyard (Burning Kiln Winery) on St. Williams, Ontario, Canada. Vines were trained on a high trellis system with a single curtain and double cordons, and their spurs were annually pruned before new growth. Canes, branches and cordons were placed between lowest and highest cables of trellis system from 0.3 m to 1.8 m. Ten mature one-year-old canes (60 cm length located in 120–180 cm height of trellis) were collected from each cultivar to evaluate chilling requirement. Sampling was done weekly from September until March for three consecutive years (2013–14, 2014–15, and 2015–16). One 180 cm cane was also collected from the trunk close to base (above the graft union) once a week from January until March to evaluate bud winter damage. Each 60 cm and 180 cm canes had on average 10 and 30 nodes, respectively.

Defoliated 60 cm excised canes after re-cut (about 10 mm) were placed upright in 5-gallons buckets of water and 7 L of tap water was added and forced in a growth chamber at the University of Guelph, Simcoe Research Station, Simcoe, Ontario. Water was replaced with fresh tap water every week and the canes were re-cut weekly and kept under continuous white light (photon flux density = 100 mmol m^{-2} s^{-1}) at 21–23 °C, based on the methods of Antcliff and May (1961); Jacobs et al. (2002); Campoy et al. (2010), and Ben Mohammad et al. (2012).

All 60 cm canes were monitored every other day for bud sprouting. Bud sprouting was tracked on all buds, and the proportion of buds sprouted was calculated. Buds were considered sprouted when they showed a green tip (growth stage #4, E-L system; Coombe 1995). Newly sprouted buds were recorded and marked to ensure they were only counted once. The percent of 60 cm canes that showed bud sprouting and the percent of sprouted buds on 60 cm canes were calculated. Usually, the distal bud at the top of the cane showed apical dominance and sprouted earlier than other buds. The percent of total buds (10 canes × 10 buds) sprouted was also calculated at the end of the experiment.

The 180 cm canes of ‘Chardonnay’ and ‘Riesling’ were assessed weekly for three years by using a razor blade to cut cross-sections (dissection) of all the buds (kept for 24 hours after collecting at 21–23 °C in a growth chamber as a warm-up period to thaw) to evaluate actual winter damage and record the bud survival (Martinson 2011; Ker 2013; Dami et al. 2014). Primary and secondary bud survival was also observed and evaluated under 5× magnification.

Hourly temperature data were collected from Burning Kiln Winery in St. Williams from 1 September 1 to 31 March each year by a temperature data logger (HOBO U23-Pro V2 2X External). The temperature data logger had two sensors attached on the trellis, at 0.3 m and 1.8 m height from the ground. The number of days and hours below −20 °C was determined for each year.

A public database (Cool Climate Oenology and Viticulture Institute; CCOVI) provided threshold temperatures for bud hardiness for ‘Chardonnay’ and ‘Riesling’ from Vinemount Ridge, Niagara Peninsula. LTE 50, low temp exotherm 50, is the temperature at which 50% of the primary buds will be killed. The data are the only “official” set of bud injury data that would be remotely comparable to the experimental site (St. Williams, Lake Erie). This site is the closest monitored vineyard with climate similarities and was considered as a control site. The field temperatures at the vineyard in St. Williams were compared with LTE50 reported by CCOVI to identify lethal freezing temperatures.

Chilling hours accumulated during 2011–17 were calculated by seven methods: Dynamic model chilling portion (Erez and Fishman 1997); Cumulative chilling hours method (Weinberger 1950); North Carolina method (Shaltout and Unrath 1983); University of California, Davis model (http://fruitsandnuts.ucdavis.edu/Weather_Services/chilling_accumulation_models/); Utah Model (Richardson et al. 1974); Iowa State University method (http://agron-www.agron.iastate.edu/courses/Agron541/classes/541/lesson04a/4a.7.html); and a Raspberry model (Dale et al. 2003). Based on the results, we have chosen the cumulative chilling hours method to use in this manuscript for grapes in southern Ontario, Canada. This method was selected because it is the oldest and most straightforward method used widely globally (Luedeling 2013) yet still calculates the chilling hours accurately. Chilling hours were calculated based upon the hourly field temperature data, a number of hours between 0–7.22 °C from two starting dates: (a) 1 October and (b) after the date of defoliation (Dokoozlian 1999; Powell et al. 2019; Londo and Johnson 2014). If hourly temperature is less or equal than 0 °C, no chilling accumulation happen. If hourly temperature is more than zero and less than 7.22 °C, it is considered as one chilling unit, and if hourly temperature is equal or more than 7.22 °C, no chilling accumulation happen. The end of endodormancy was determined by the physiological method (using excised canes from vines; Dokoozlian 1999; Powell et al. 2019; Londo and Johnson 2014).

Time elapsed to bud sprouting was calculated as the time in days needed for bud sprouting to occur on 50% of the 10 canes. Also, average days until the first sprouting were evaluated for each 60 cm cane. The average number of days until the first sprouting of the 10 canes was considered an average day for first sprouting in the growth chamber (21–23 °C).

The chilling hours accumulated in the vineyard were tracked and when the collected weekly canes sprouted in the growth chamber, the corresponding chilling hours accumulated were recorded as the chilling hours needed to overcome the endodormancy.

Statistical analyses were conducted using SAS software [PROC GLM, SAS statistical software version 9.4, SAS Institute Inc., Cary, NC (Khattree and Naik 2000)].

Results

Hourly temperature data during the winter of three years (2013–14, 2014–15, and 2015–16) showed that 2014–15 had an extremely low minimum temperature (−33.1 °C Simcoe, ON), which was the second-lowest recorded temperature since 1884 (−37.8 °C Simcoe, ON). The historical weather data in the last 25 years (1991–16) showed that while the average daily temperature was −3.2 °C in the winters (21 December to 20 March), the average daily temperature in the winters of 2013–14, and 2014–15 were −6.7 °C and −6.5 °C, respectively. This ranked them as the second and third coldest winters in the last 25 years. Winter temperatures in 2015–16 were milder (the average daily temperature of −0.7 °C) with minimal winter damage. In all three years, freezing temperatures started from mid-October, which led to the leaf drop (Fig. 1, Table 1).

In 2013–14, 2014–15, and 2015–16, the absolute minimum temperatures were recorded at 1.8 m (−26, −33.1, and −23.6 °C, respectively), and at 0.3 m (−27.2, −33.9 and −24.6 °C, respectively). These minimums occurred in mid-February (Table 1 and Fig. 1). Minimum temperatures recorded at 0.3 m were generally 1 °C colder than 1.8 m due to the inversion.

To evaluate bud hardiness, the field temperatures were compared with the LTE50s (as the cold hardiness thresholds) from CCOVI. The number of days with temperatures less than −20 °C in 2013–14, 2014–15, and 2015–16 was 10, 17, and 1 days, respectively. However, the number of days in which the minimum temperature was lower than the LT50 (CCOVI data) and bud injury could be probable were 3, 7, and 1 day, respectively. These dates were as follows – 2013–14: 12, 17, and 28 February; 2014–15: 16, 17, 20, 23, 27, 28 February and 6 March; and 2015–16: 14 February (Fig. 1*a*–1*c*)

In the cumulative chilling hour method, temperature data of all three years showed that the temperature in August did not add to the chilling requirement since no hours had temperatures between 0 –7.2 °C (Table 2). In September, there were very few (2013–14 and 2014–15) or no (2015–16) days between these temperatures (Table 2). The chilling hours increased gradually in October, and by the end of December each year, the vines have received most of the chilling required (Table 3).

The biofix is a biological event or an indicator of a developmental event, which is the defoliation in this experiment. The difference of accumulated chilling hours between the biofix and 1 October was very small (12 hours on average over three years, Table 3). Since chilling hours did not accumulate before defoliation, we have chosen defoliation as the biofix for calculating the chilling hours in southern Ontario.

In 2015–16, the vines had more chilling hours accumulated than the previous two years (1140, 1153, 1557 hours from defoliation until 31 March, Table 3). The total chilling hours in fall 2015 were less than the previous two years. However, the chilling hours up to the end of March 2016 were 400 hours more than the previous two years (Fig. 1). In 2013–14, the budbreak was retracted (in the growth chamber) until 9 December, unlike the other two years, which started in November. Therefore, we concluded that early chilling hour satisfaction was negatively affected by warm temperatures in October (Fig. 1*a* and 2*a*).

In 2013–14, canes collected on 22 December for ‘Chardonnay’ and 29 December for ‘Riesling’ forced buds to push out and develop faster than those collected a week prior. While Perez et al. (2007) used 50% of bud sprouting as the end of endodormancy, we concluded endodormancy finished (chilling requirement was fulfilled) when forced buds developed faster than those collected a week prior. The cumulative chilling hours (calculated hours of temperatures between 0–7.22 °C, since defoliation to end of the endodormancy) were 592 and 653 for ‘Chardonnay’ and ‘Riesling’, respectively (Table 3).

Bud sprouting happened in at least 50% of ‘Chardonnay’ and ‘Riesling’ canes in the growth chamber when collected after mid-December 2013, mid-November in 2014, and early November in 2015.

Fig. 1. Hourly temperatures collected from the field (St. Williams, Norfolk County) with a polynomial trend line (order: 6), accumulated chilling hours (0–7.22 °C) from defoliation (St. Williams, Norfolk County), and bud hardiness data (LTE 50%) of *Vitis vinifera* L. 'Chardonnay' and 'Riesling' obtained from Vinemount Ridge, Niagara Peninsula, over three years, 2013–14 (*a*), 2014–15 (*b*) and 2015–16 (*c*), obtained from CCOVI website 2013–2016 (CCOVI 2018).

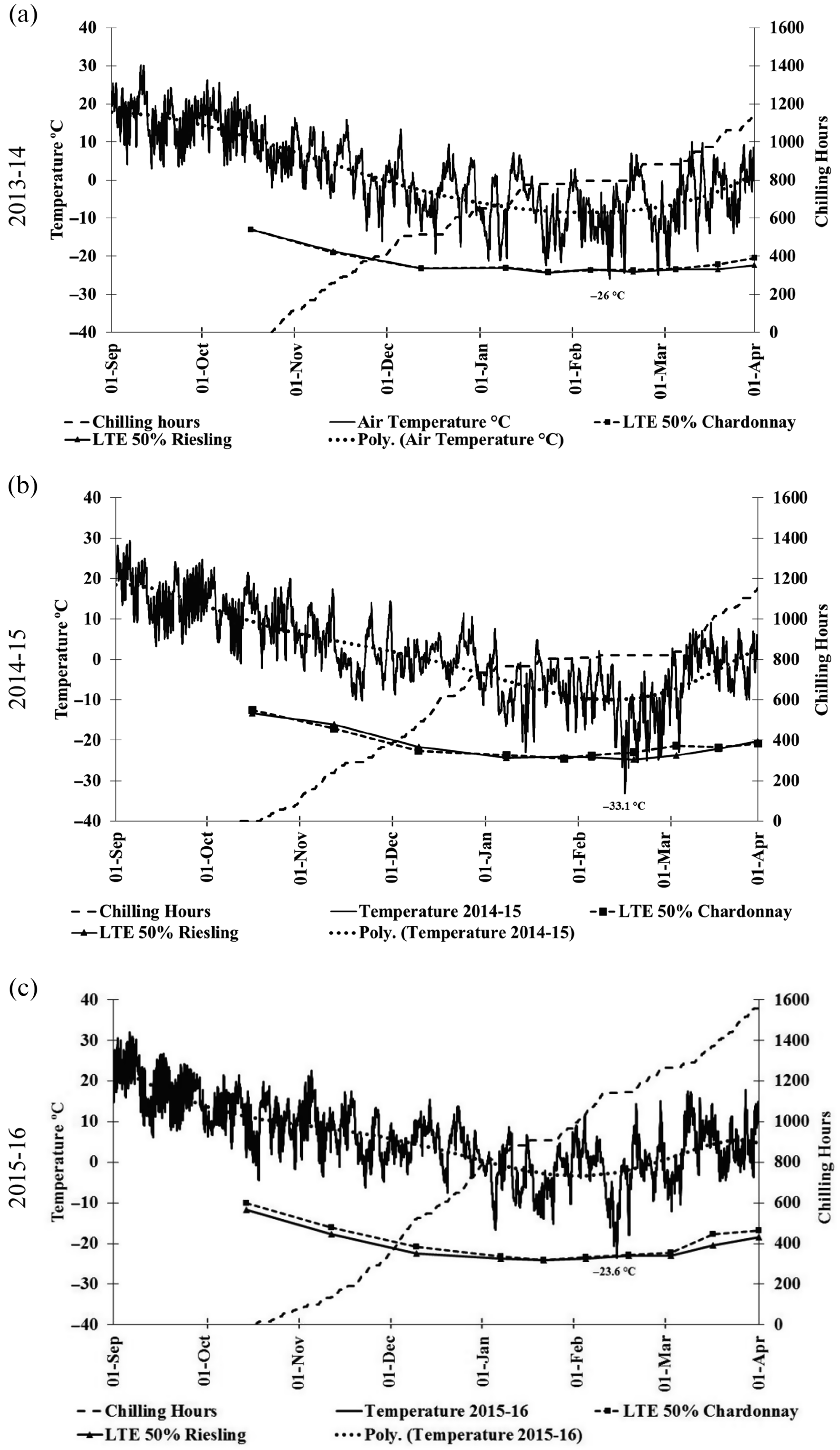


Table 1. Absolute minimum temperatures at 30 and 180 cm above the ground at St. Williams, Ontario, during 2013–2016; date of the minimum temperature recorded; number of days of each year that the temperature was below −20 °C; number of hours of each year that the temperature was below −20 °C; average temperature from September to end of the March; and early fall frost leading to the grapevine defoliation.

Crop year	Abs. min temp (°C) at 1.8 m	Abs. min temp (°C) at 1.3 m	Abs. min temp, date and time	# days below −20 °C	# hours below −20 °C	Ave. temp (°C), 1 Sep.–31 Mar.	First fall frost
2013–14	−26	−27.2	12 Feb., 7:30 AM	10	53	1.6	23 Oct. −0.2 °C
2014–15	−33.1	−33.9	16 Feb., 3:00 AM	17	99.5	1.5	12 Oct. −0.4 °C
2015–16	−23.6	−24.6	14 Feb., 5:45 AM	1	3.5	5.8	18 Oct −0.3 °C

Table 2. The minimum, maximum, and average temperatures and chilling units accumulated during August and September for three years (2013–16) of the experiment at St. Williams, Ontario.

	August				September			
Crop year	Min (°C)	Max (°C)	Average (°C)	Chilling units (h)	Min (°C)	Max (°C)	Average (°C)	Chilling units (h)
2013–14	9	26.8	19.2	0	3.8	29.9	18.6	38
2014–15	9.6	29.2	20.1	0	6.3	25.4	15.8	21
2015–16	10.1	29.5	20.1	0	9.7	17	13.4	0

Table 3. Chilling unit accumulation at Burning Kiln Winery (St. Williams, Ontario) for *Vitis vinifera* L. ‘Chardonnay’ and ‘Riesling’ using visualized method (physiological), and cumulative chilling hours method (0–7.22 °C) based on two different biofix times: (1) accumulated from first of October until end of March; and (2) accumulated from defoliation (first frost) until end of March.

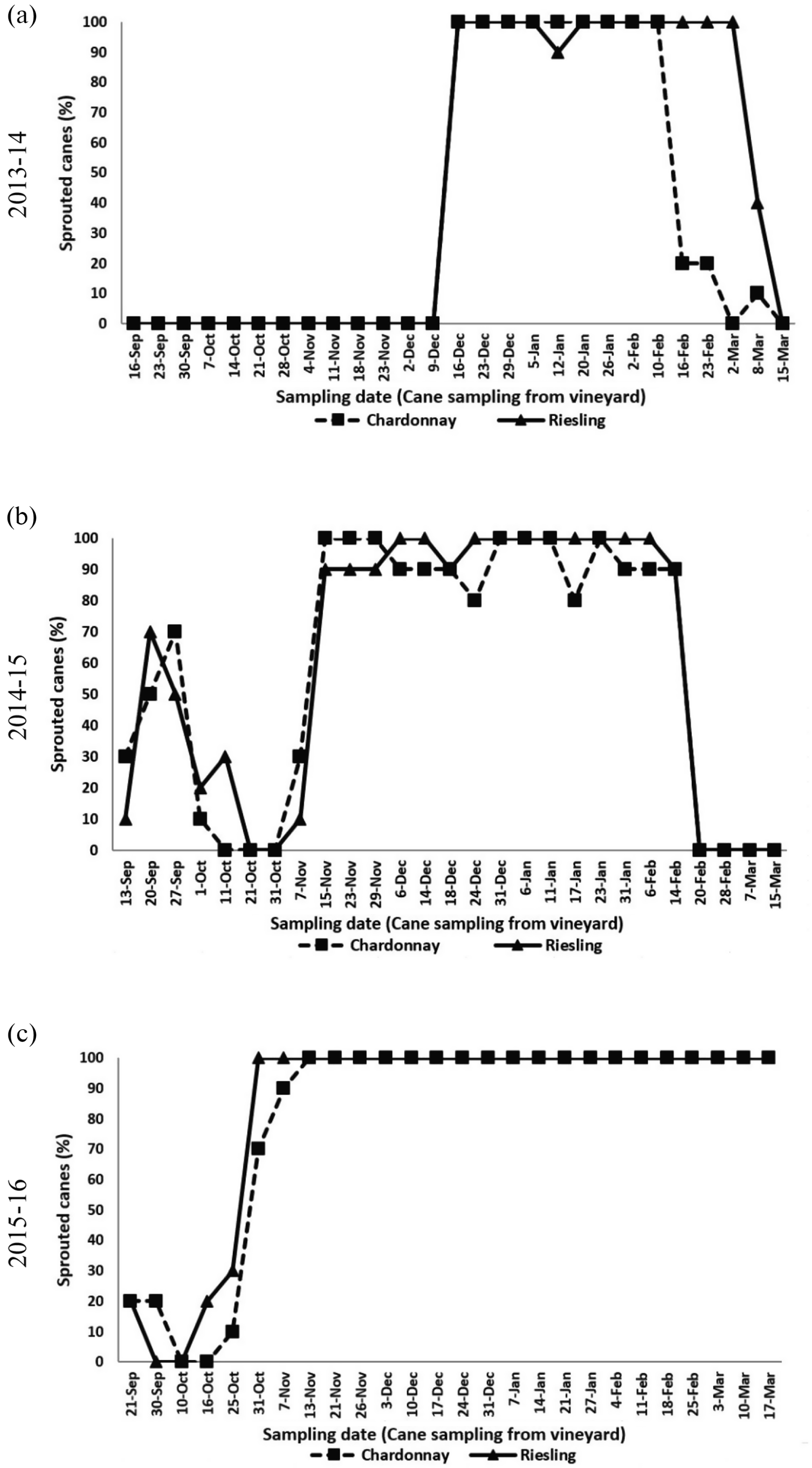
	2013–14		2014–15		2015–16		2013–16
Time	Date	Hours	Date	Hours	Date	Hours	Average hours
Accumulation of the chilling units from October	1 Oct. 2013	0	1 Oct. 2014	0	1 Oct. 2015	0	0
End of endodormancy in ‘Chardonnay’	22 Dec. 2013	675	17 Dec. 2014	661	18 Dec. 2015	760	699
End of endodormancy in ‘Riesling’	29 Dec. 2013	731	25 Dec. 2014	719	24 Dec. 2015	811	754
Chilling hours accumulated by March 31	31 Mar. 2014	1157	31 Mar. 2015	1142	31 Mar. 2016	1587	1295
Accumulation of the chilling units from defoliation	23 Oct. 2013	0	12 Oct. 2014	0	18 Oct. 2015	0	0
End of endodormancy in ‘Chardonnay’	22 Dec. 2013	592	17 Dec. 2014	618	18 Dec. 2015	607	606
End of endodormancy in ‘Riesling’	29 Dec. 2013	653	25 Dec. 2014	682	24 Dec. 2015	659	665
Chilling hours accumulated by March 31	31 Mar. 2014	1140	31 Mar. 2015	1153	31 Mar. 2016	1557	1283

Note: Endodormancy was concluded when forced buds developed faster than those collected a week prior. For example, in 2013–14, buds of ‘Chardonnay’ samples collected on 22 December sprouted faster than those collected a week prior (15 December). Therefore, accumulated chilling hours (675 h) were selected as the chilling hours required to fulfill endodormancy.

In 2013–14, ‘Riesling’ canes collected after 16 February were damaged due to an extreme low temperature of −26 °C on 12 February (Fig. 1). In 2014–15, lethal temperature of −33.1 °C was experienced by both cultivars on 20 February. (Fig. 2).

In 2013–14, canes collected on 9 December sprouted in 40 days. Canes collected later sprouted with fewer forcing days. In 2014–15, canes collected in September were not acclimated sufficiently and sprouted immediately in the growth chamber. The samples collected at

Fig. 2. The percentage of canes with sprouted buds in a temperature-controlled growth chamber at 21–23 °C for two cultivars (*Vitis vinifera* L. 'Chardonnay' and 'Riesling') on the *y* axis and the dates canes were collected from the vineyards on the *x* axis during three successive years, 2013–14 (*a*), 2014–15 (*b*) and 2015–16 (*c*).



the end of October did not sprout in the growth chamber, but samples from November sprouted after forty days. Samples collected from 14 February onward were dead. In 2015–16 the samples collected from early October did not sprout but the samples collected after mid-October sprouted after 90–100 days in the growth chamber. The forcing days decreased gradually until the end of winter, by which time the buds only needed 10 days to sprout (Fig. 3). In average over three years, as canes received more chilling, the number of days needed for sprouting decreased to 13 and 15 days for 'Chardonnay' and 'Riesling' respectively. Duncan's mean comparison for days to bud sprouting on 'Chardonnay' and 'Riesling' (2013–16) is summarized in Table 4.

The time needed for bud sprouting was significantly different between sampling dates, replicates, year, sampling × year in 'Chardonnay' and 'Riesling' in 2013–16 (Table 5). In 2013–14, the percentage of sprouted buds was 30% in 'Chardonnay', and 45% in 'Riesling', and neither sprouted after the lethal temperatures in February 2014. In 2014–15, the percentage of sprouted buds increased to 60% and then dropped to zero after 20 February 20 2015 in both cultivars due to the lethal temperature. In 2015–16, no winter damage was recorded, and the percentage of sprouted buds was ultimately 50% in 'Chardonnay' and 60% in 'Riesling' (Fig. 4).

The average bud survival percentage estimated by dissection on the 180 cm canes showed some primary bud damage over all three years in both cultivars. Primary buds survived better from 'Riesling' than 'Chardonnay' in all three years, and in general, the damage to the primary buds was greater than secondary buds (Fig. 5). The weekly dissection for bud survival evaluation showed bud damage in response to severe cold temperature. Bud survival in 2014–15 decreased gradually and, after 14 February, dropped to zero (Fig. 5).

Bud deaths in 2013–14 and 2014–15 in February were related to a severe cold temperature lower than the LT50 of the buds. In 2013–14, some 'Riesling' buds survived 11 hours at −26 °C (12 February) and 5 hours at −24.3 °C (17 February) but finally died after 8 hours at −24.9 °C (28 February). 'Chardonnay' buds died at −26 °C on 12 February. Although the minimum temperature in 2015–16 was −23.6 °C on 14 February, no damage was recorded for either 'Chardonnay' or 'Riesling'.

Discussion

The weather data graphs demonstrate the critical temperatures and growth stages and any damaging temperatures during endo and ecodormancy. When bud hardiness data of 'Chardonnay' and 'Riesling' from Vinemount Ridge, Niagara Peninsula (CCOVI 2018), were compared with the hourly temperatures collected in the field (St. Williams, ON) in all three years, several minimum temperatures were lower than the hardiness threshold (LT50s predicted by CCOVI 2018) and winter damage was expected.

Grapevines tolerate freezing temperatures by the induction of endodormancy (Shim et al. 2014). Maximum cold hardiness in buds is usually associated with full endodormancy (Zabadal et al. 2007). Bud cold hardiness is usually at its maximum in December, January, and February (Zabadal et al. 2007). This created the misconception that the grape buds would achieve chilling requirements and begin exiting dormancy by the end of January to mid-February in Ontario. However, the results showed that endodormancy ended in late December. Similarly, Colombo (1998) reported that the endodormancy (in white spruce) was fully satisfied by the end of December in Ontario. Cragin et al. (2017) also found that the maximum cold acclimation occurred in October during endodormancy and increased during ecodormancy to maximum cold hardiness in December for 'Chardonnay'. Identifying the end of December for fulfilling the chilling requirement is earlier than what we would have expected. It means any temperature fluctuations above 0 °C after late December could initiate de-acclimation, making vines more vulnerable to cold damage.

Zabadal et al. (2007) mentioned that buds were less resistant to colder temperatures when the severe cold temperatures happened later in February due to some de-acclimation. Ferguson et al. (2014) concluded that the bud sprouting occurred earlier in hardier genotypes, consistent with more rapid de-acclimation of genotypes originating from colder climates, making these genotypes more vulnerable to spring frost in warmer environments.

Results from this project showed that the grape buds entered the dormant phase during early October, which coincided with leaf drop, stayed fully dormant until late December, and then remained in ecodormancy until the warmer weather provided some physiological deacclimation of both cultivars.

The transition of the meristem in and out of dormancy affects bud survival and productivity (Shim et al. 2014). However, vines can re-acclimate and gain cold hardiness in response to return to low temperatures. Rongzhou et al. (2016) reported an increasing vulnerability of conifers to temperature fluctuations and freezing damage with the progress of chilling and dormancy release from fall to spring in Ontario.

Cultivated grapevine (*V. vinifera*) is considered a low-chill species and typically requires between 50 and 400 hours (≤ 7 °C) of chilling to satisfy dormancy (Magoon and Dix 1943; Nigond 1957; Weaver and Iwasaki 1977). Dokoozlian (1999) reported that while exposure to 200 hours (0–10 °C) resulted in commercially acceptable levels of bud sprout in 'Perlette', bud sprouting continued to improve as chilling duration increased up to 800 hours. After this, the chilling temperature had relatively little influence on cumulative bud sprouting. However, Londo and Johnson (2014) reported that the chilling requirement for some *V. vinifera* (such as

Fig. 3. Average days for the first bud sprouting in a growth chamber (21–23 °C) for *Vitis vinifera* L. 'Chardonnay' and 'Riesling' during 2013–14 (*a*), 2014–15 (*b*), and 2015–16 (*c*).

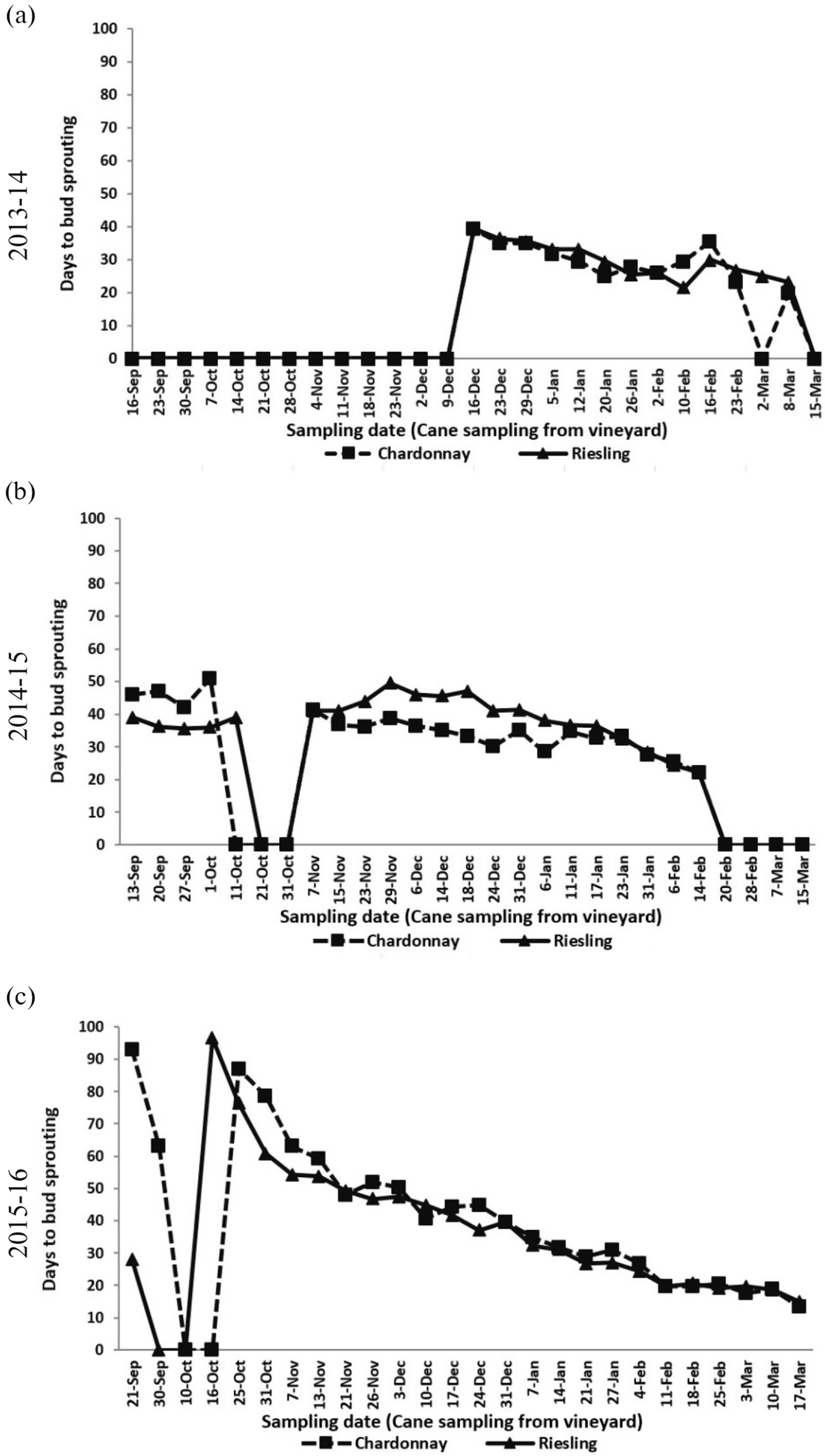


Table 4. Mean comparison of days to bud sprouting on *Vitis vinifera* L. 'Chardonnay' and 'Riesling' (2013–16) after a set period in a temperature-controlled growth chamber at 21–23 °C.

No	Sampling period (days)		Cumulative chilling hours fulfilled in the vineyard	'Chardonnay' (2013–16)	'Riesling' (2013–16)
1	September	13–19	0	46ef	39g
2		20–26	0	60c	34i
3		27–30	0	47ef	36i
4	October	1–10	28	51d	36hi
5		11–20	74	69	62b
6		21–27	138	87a	76a
7		28–31	173	79b	61b
8	November	1–10	274	58c	53c
9		11–17	331	48de	48de
10		18–22	395	42gh	47de
11		23–30	452	45efg	48d
12	December	1–8	566	44fg	47de
13		9–15	624	38i	45e
14		16–22	695	39hi	43f
15		23–28	771	37i	38gh
16		29–31	799	37i	39g
17	January	1–10	850	32j	35i
18		11–16	888	32j	34i
19		17–22	902	28jk	31j
20		23–31	937	31j	28k
21	February	1–5	963	27kd*	26kl
22		6–13	992	25lm	22mn
23		14–19	1002	22mn	24lm
24		20–28	1058	21no	23m
25	March	1–6	1072	18o	22m
26		7–14	1162	19no	20n
27		15–17	1196	13p	15o
LSD				3.3	2.3

Note: Means given different letters differ from each other in a column by Duncan's test at α = 0.05.

Table 5. Data analysis, days to bud sprouting on *Vitis vinifera* L. 'Chardonnay' and 'Riesling' (average 2013–16).

	'Chardonnay' (2013–16)		'Riesling' (2013–16)	
Source of Variation	df	Mean square	df	Mean square
Sampling dates (chill units)	25	2270.7**	26	2094.4**
Replicate (sampling)	197	57.2**	204	29.4**
Year	2	2889.1**	2	32.9**
Sampling × year	28	362.8**	29	255.5**

Note: **Significant differences at 1% probability levels.

'Cabernet Sauvignon') is up to 1250 chilling hours (0–7 °C).

Londo and Johnson (2014) reported 750 chilling hours are required for 'Chardonnay', and Grape Growers of Ontario reported 650 chilling hours (0–7 °C) for 'Riesling' (Grape Growers of Ontario 2018). These reports are very close to our results (average for three years). We showed the chilling requirements for 'Chardonnay,' and 'Riesling' are on average 606 (ranging 592–618) and 665 (ranging 653–682) hours (0–7 °C), respectively, in Norfolk County, Ontario.

While Londo and Johnson (2014) reported that the chilling hour could accumulate before vines are defoliated, our results showed before defoliation temperatures (August and September) do not provide proper chilling fulfillment in Simcoe, Ontario (Table 2).

Fig. 4. The average percentage of buds sprouted on 60 cm canes in the growth chamber (21–23 °C) for *Vitis vinifera* L. ‘Chardonnay’ and ‘Riesling’ over 2013–14 (*a*), 2014–15 (*b*) and 2015–16 (*c*).

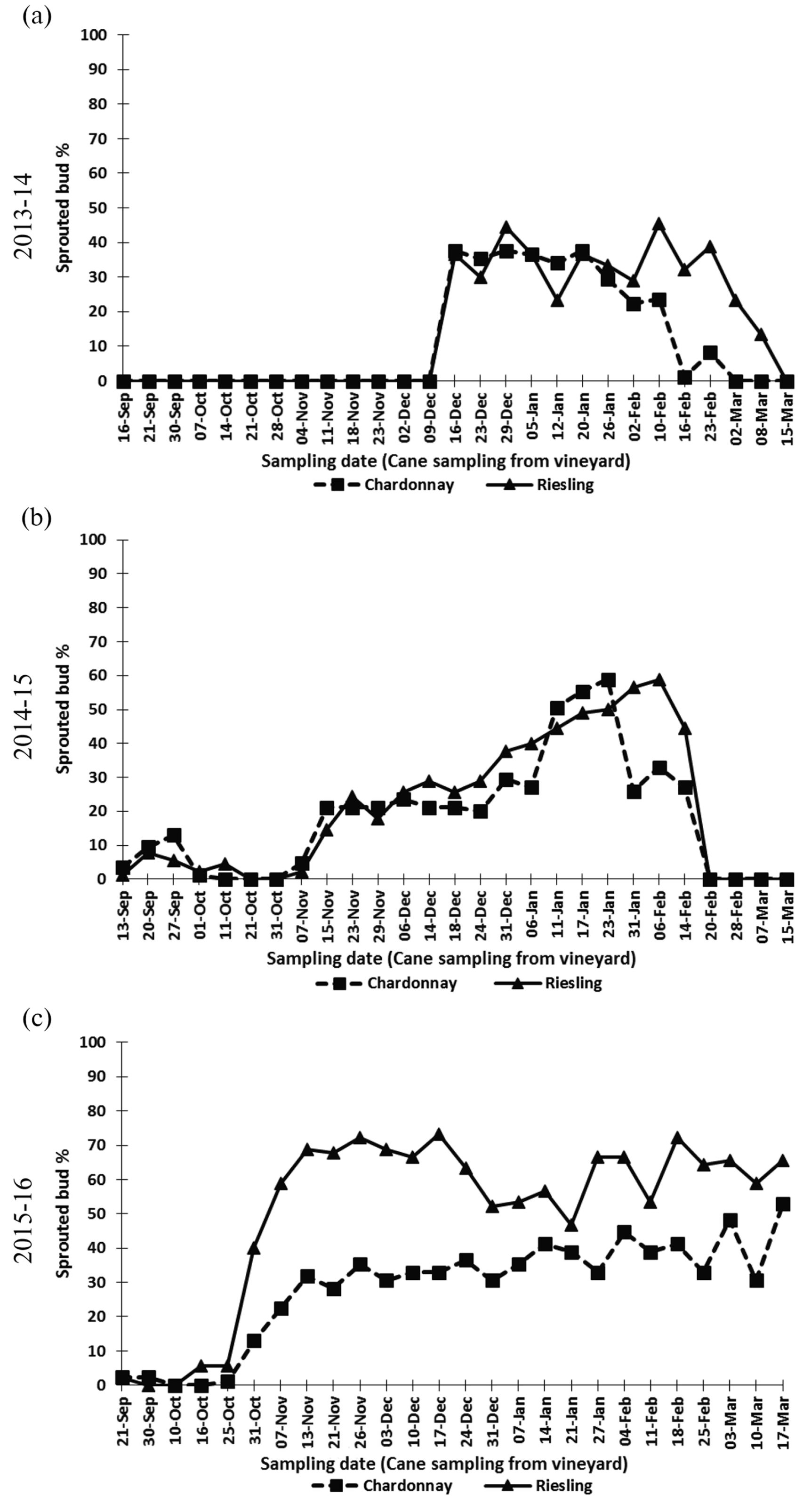
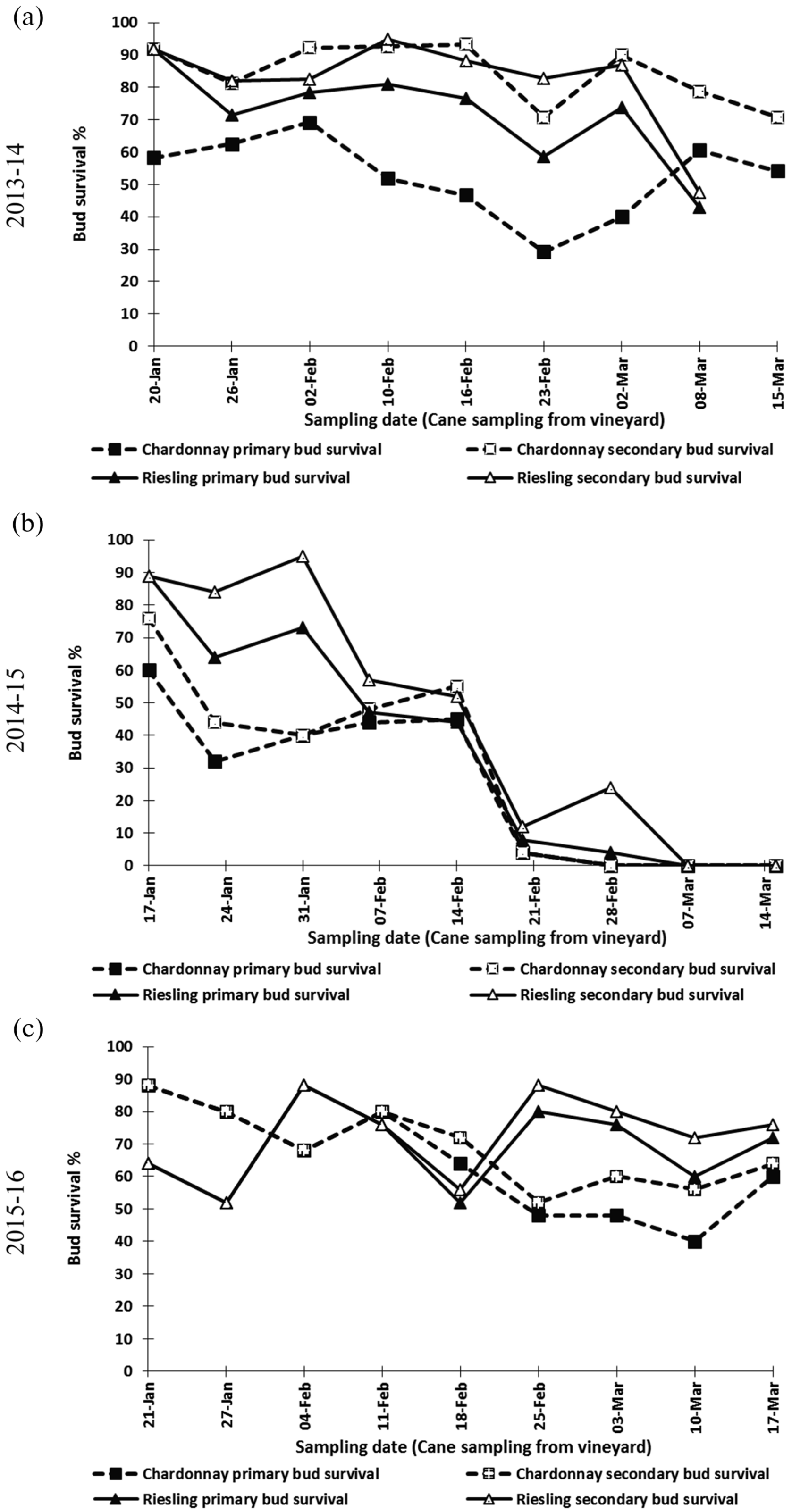


Fig. 5. Average bud survival percentage on primary and secondary buds on 180 cm canes for *Vitis vinifera* L. 'Chardonnay' and 'Riesling' in 2013–14 (*a*), 2014–15 (*b*), and 2015–16 (*c*).



The results showed that the percentage of canes that sprouted in the growth chamber differed in each of these three years. ‘Chardonnay’ and ‘Riesling’ buds died mid-February due to the winter damage at temperatures lower than −24 °C for ‘Chardonnay’ and −25 °C for ‘Riesling’. This confirms the results of Miller et al. (1988) that ‘Chardonnay’ buds were less hardy than ‘Riesling’.

Normal bud growth requires cessation of bud dormancy and sufficient chilling to overcome endo dormancy. Once fully dormant, on average 606 and 665 hours of cold temperatures (0–7 °C, calculated since defoliation) are required for ‘Chardonnay’ and ‘Riesling’, respectively, to ensure first bud spouting along the entire cane length.

The results showed that canes collected in September, before enough acclimation, sprouted in the growth chamber. However, the samples collected in October showed a transition phase to endodormancy. During this transition, buds of collected canes did not sprout at all at the forcing conditions in the growth chamber (21–23 °C). Cragin et al. (2017) reported the number of days to bud sprouting under forcing conditions increased consistently during endodormancy. In 2015–16, the samples started to sprout after mid-October, but they sprouted after 90–100 days forcing in the growth chamber, longer than 2014–15 samples. The forcing days dropped gradually until the end of March, when they needed just 10 days forcing for sprouting. In 2015–16, milder winter temperatures accumulated chilling hours faster than the previous two years (Fig. 1*c*), resulting in faster-sprouted buds (Fig. 2*c*). Increasing chill accumulation reduces the heat requirements for bud sprouting in fruit species, considering that loss of hardiness is required for bud sprouting to occur (Kovaleski et al. 2018). Okie and Blackburn (2011) reported that increasing chilling reduces heat requirement for flower bud sprouting in peaches and reduces the forcing days for bud sprouting. Kovaleski and Londo (2018) also reported increased chilling accumulation may lead to earlier bud sprouting in grapes in cooler regions.

The evaluation showed that about 40–50% of buds sprouted with the forcing method on 60 cm canes in the growth chamber. The percentage of the ‘Chardonnay’ that sprouted was less than the ‘Riesling’ in the growth chamber. During dissection, the primary and secondary buds were visible under magnification, but the tertiary buds were often not fully developed and not always visible. The bud dissection evaluation of winter damage showed that, although samples were assayed very accurately with magnifiers, the results do not always agree with the forcing buds in the growth chamber. This method estimated that 52% of ‘Chardonnay’ primary buds survived on 12 February 2014. However, when the canes were forced to sprout in favourable conditions, only 10% of the buds sprouted. Through dissection, it was also estimated that 76% of ‘Riesling’ primary buds survived in the same year, and yet 83% of buds sprouted in the forcing conditions (Figs. 4*a* and 5*a*). This may have happened because the secondary buds survived and sprouted in the growth chamber, whereas the primary buds had died. There are no reports to show separate data for primary and secondary bud mortality. Wolf and Cook (1994) reported that no attempt was made to estimate the cold hardiness of secondary buds or to tally secondary bud mortality in the field methodically. They suggested to cautiously estimate the cold hardiness of a population with a significant incidence of non-freeze injury and distinguish the LTEs of primary and secondary buds.

Conclusions

There has been a misconception that the grape buds would achieve chilling requirements and begin exiting endodormancy by the end of January to mid-February in Ontario. However, we have shown that the endodormancy was fully satisfied by the end of December. The chilling requirement was estimated at 606 hours (ranging 592–618) for ‘Chardonnay’ and 665 hours (ranging 653–682) for ‘Riesling’. The chilling hours can be calculated from the biofix of defoliations (around mid-October in Ontario) because chilling hours accumulated before defoliation are not significant. The results identified that in severe cold temperatures of February in southern Ontario, damage to the buds is inevitable, and midwinter minimum LT50s in January and February (at their lowest point) might be −24 °C for ‘Chardonnay’ and −25 °C for ‘Riesling’. Primary bud survival was higher in ‘Riesling’ compared with ‘Chardonnay’ in all three years. In 2015–16, chilling requirements were satisfied earlier; therefore, buds sprouted faster, confirming the results of other researchers that increasing chilling reduces heat requirement for bud sprouting. Also, the forcing method in a growth chamber is an appropriate method to identify the required chilling hours of grape varieties.

Acknowledgements

We are gratefully acknowledging the contribution of plant materials by Burning Kiln Winery. We also thank Cool Climate Oenology and Viticulture Institute. They provided the hardiness threshold temperature data for ‘Chardonnay’ and ‘Riesling’ for Vinemount Ridge, Niagara Peninsula, as the control for the project. This project was funded in part through Growing Forward 2 (GF2), a federal-provincial-territorial initiative. The Agricultural Adaptation Council assists in the delivery of GF2 in Ontario.

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