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Impact of spring freeze on yield, vine performance and fruit quality of *Vitis* interspecific hybrid Marquette

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ABSTRACT

In viticultural regions with spring freeze events, early budburst increases the risk of vine damages compromising industry long-term sustainability. Marquette is a cold hardy hybrid with *Vitis riparia* in its parentage, a source of cold hardiness and rapid budburst characteristics. In 2012 and 2013, the capacity of Marquette to rebound from significant bud mortality from a series of spring freeze events was quantified and the capacity of recovering the damages the following year, measuring vine yield and the effect on fruit quality. The compound bud on grapevine is actually three buds in one, with primary, secondary, and tertiary all present. Spring freeze events in 2012 killed over 80% of the shoots arising from primary buds (SPB) while secondary buds (SSB) were almost unaffected. By tracking the performance SPB and SSB, we were able to quantify vine response to spring freeze events. A comparative analysis of phenological and fruit quality characteristics of the SPB and SSB clusters showed different development and ripening of those of SBS. However, the differences disappeared at harvest, with no significant impact on yield or only partially on fruit composition. The results suggest that Marquette has the potential to generate significant yield from SSB with desirable fruit quality and could offer a solution to spring freeze losses. In 2013, year characterized by no spring freeze events, vines recovered full productivity, yielding 61% more fruit, due to an increased number of cluster per vine (+60%) with also better fruit quality at harvest.

1. Introduction

The development of a sustainable wine industry in a cool-cold climate is a challenge. An array of problematic environmental conditions confront growers, and climate change is expected to exacerbate them (Sabbatini and Howell, 2011; Schultze et al., 2014). In Michigan, where the nascent wine industry is committed to the cultivation of *Vitis vinifera*, losses from weather related events have been severe and that has driven the need to seek varieties to mitigate that risk. A key attribute guiding grower decisions is a cultivar's cold hardiness, as defined by its sensitivity to below freezing temperatures (Dami et al., 2016).While growing season phenomena, like low heat accumulation, excessive vigor, or high disease pressure can negatively affect the crop, once budburst occurs (Howell, 2001), spring frost poses the greatest threat (Trought et al., 1999; Schultze et al., 2016). Probable outcomes range from low levels of bud and shoot damage to 100% primary bud necrosis, depending on maximum low temperature, event duration, bud development stage, and vine health. Fortunately, the *Vitis* genus is characterized by a compound bud that includes a primary, secondary, and tertiary bud generating in the spring shoots arising from primary bud (SPB), from secondary bud (SSB) and rarely from tertiary bud (STB), with the potential for additional crop when these conditions appear (Mullins et al., 1992; Keller, 2010).

Spring's warming temperatures cause buds to lose their cold hardiness via two critical physiological processes: the rehydration of the meristematic tissues, which occurs with the movement of water into intercellular spaces, and concurrently, the degradation

Abbreviations: SPB, shoot originated from the primary bud of a compound bud; SSB, shoot originated from the secondary bud of a compound bud; STB, shoot originated from the tertiary bud of a compound bud; HTRC, Michigan State University Horticulture Teaching and Research Centre; T_{max}, maximum temperature; T_{min}, minimum temperature; T_{mean}, mean temperature; TA, titratable acidity.

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of sugars and protein complexes that bind water and act as cryoprotectants (Keller, 2010). Previous studies have documented this process and the relationships between bud water content, stage of development, and tissue temperature tolerance (Gardea, 1987; Trought et al., 1999). Under normal spring conditions, a new shoot begins its development arising from the primary bud of the compound bud (SPB), while the secondary (SSB) and tertiary buds (STB) typically remain undeveloped (Mullins et al., 1992). When spring primary bud shoot necrosis or injury is prevalent, grapevines react inducing the growth of new shoots from the secondary buds of the compound buds (Mullins et al., 1992; Keller, 2010; Friend et al., 2011). Friend et al. (2011) also observed that the number of shoots developing from these secondary buds after a spring freeze is positively related to the number of primary shoots injured or killed.

Marquette is a cold-hardy hybrid (Ravat 262 × MN1094) red wine grape released from the University of Minnesota in 2006 (Hemstad and Luby, 2008). Its parentage is complex and includes *V. riparia* and *V. vinifera*, with Pinot noir among others cultivars (Hemstad and Luby, 2008). Marquette inherits the deep winter cold hardiness, rich fruitfulness, rapid budburst, disease resistance and early ripening traits of *V. riparia*, but its enological properties more closely resemble those of *V. vinifera* (Hemstad and Luby, 2000; Manns et al., 2013; Pedneault et al., 2013; Slegers et al., 2015; Read and Gamet, 2016). Wines from Marquette have been judged tobe of superior quality when compared to the non-*V. vinifera* wines with high potential for excellent color, complex aromatics and no undesirable sensory attributes (Hemstad and Luby, 2008; Manns et al., 2013; Pedneault et al., 2013; Slegers et al., 2015). In growing regions wanting to compete more favorably with *V. vinifera* producers, Marquette is a unique and appealing cultivar, and it is for this reason that growers in the challenging climate regions of the Midwest and Eastern U.S. have shown great interest in it (Manns et al., 2013; Read and Gamet, 2016). However, reports of its early budburst and consequent spring frost susceptibility with potential crop loss (Reisch et al., 1993; Londo and Johnson, 2014; Schultze et al., 2016) are tempering the cultivar's popularity, but little is known about its ability to recover from spring freeze damage; investment in Marquette across the East of US would increase if the early season cold damage risk could be reduced.

When spring frost damages shoots derived from primary buds (SPB) of *V. vinifera*'s compound bud, shoots arising from secondary buds (SSB) have very low fruitfulness and yields are considered unacceptable and qualitatively unsatisfying (Trought et al., 1999; Keller, 2010; Friend et al., 2011). In contrast, *V. riparia* is characterized by higher fruitfulness on shoots from these secondary buds, which often carry clusters similar to those of primary shoots. They are even known to ripen fully under favorable environmental conditions (Gerrath and Posluszny, 1988a, 1988b; Mullins et al., 1992).

In March 2012, an anomalous and record-breaking warm weather system settled over Michigan presenting a set of conditions that allowed the field study of Marquette's ability to recover lost primary bud yield (due to freeze damage) from the production of shoots arising from the secondary buds (SSB) of the vines' compound buds. The premature warm temperatures, with highs staying above 20 °C for three weeks duration, triggered the rapid budburst of Marquette (28 Mar, approx. 50% of primary buds showing green leaf tissue), more than a month earlier than the historical average. When April low temperatures dropped well below 0 °C, most of the shoots were killed, so that in May secondary buds of the compound buds were induced to develop. These events provided the opportunity to systematically track, describe, and compare the vegetative and reproductive characteristics of shoots arising from primary buds (SPB) and from secondary buds (SSB) sourced in the compound bud of Marquette grapevines after the occurrence of several spring freeze events. Specifically, the objectives of this study included the measurement of canopy growth, berry development, and fruit quality from budburst through harvest in 2012. In 2013, the study focused on evaluating the recovery of the vines after the 2012 damaging events to understand the cultivar's potential to reliably produce an economically-viable yield with good fruit quality in a climate where spring freeze events routinely threaten damage to early budburst varieties.

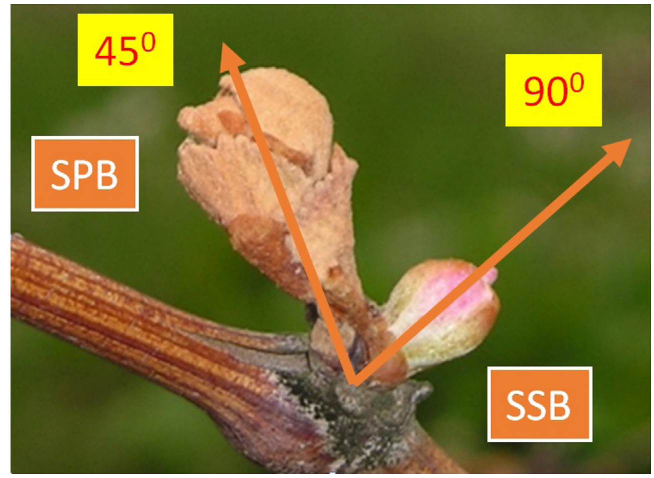

Fig. 1. Detail of a shoot from primary bud (SPB), grown with an angle of projection from the cane of 45° and killed by spring freeze events, and a shoot from secondary bud (SSB), growing with a angle of projection of from the cane of 90° and not damaged by spring freeze events.

2. Materials and methods

2.1. Site, plant material and experimental design

The experiment was conducted in 2012 and 2013 at the Michigan State University Horticulture Teaching and Research Center (HTRC) in Holt, Michigan (lat. 42°40′24″N; long. 84°29′13″W; elev. 264 m) on five year-old vines of the interspecific hybrid (MN 1094 × Ravat 262) cv. Marquette. The vines were own-rooted, planted in Marlette fine sandy loam soil (US Department of Agriculture, Soil Conservation Service 1957) at a spacing of 2.4 × 3.1 m (vine × row) in north-south rows and trained to a high-wire cordon system. The vineyard was not irrigated and standard cultural and pest control practices for hybrids grown commercially in the region were applied.

The experiment was a complete randomized block design of 27 vines organized in three blocks with two treatments: (1) shoots arising from primary buds (SPB), and (2) shoots arising from secondary buds (SSB) of a compound bud. During the first week of May 2012, after the threat of freeze events had ceased, all vines within the experimental plot were evaluated for bud damage. All surviving SPB were flagged. On 15 May 2012, each vine was again evaluated with any secondary buds developing new shoots counted and flagged. Shoot origin (SPB or SSB) was determined by the angle of projection from the cane. In detail, all the survived shoots with an angle of projection of about 45° were flagged as SPB and all the shoots growing with an angle of projection of 90°, in correspondence of a dead or alive SPB at 45°, were instead flagged as SSB (Fig. 1). STB were also present, but being small in number and not fruitful, they were identified, but not included in the study. All the shoots of each experimental vine were therefore flagged as SPB, SSB or STB (unflagged) and followed throughout the experiment. Three SPB and SSB per vine, fruitful and actively growing were randomly selected to serve as modal shoots and tagged with white laminated paper tags and numbered 1 through 3 for follow-up measurements. This resulted in three SPB and three SSB per vine (81 shoots per treatment; 162 total) randomized in the three blocks. In 2013, vines were evaluated for spring frost damages and vine performance measured throughout the season.

2.2. Weather conditions

Climate data was supplied from an MSU Enviro-Weather station located approximately 100 m to the east of the experiment site, at the same elevation. The daily maximum temperature (T_{max}) and minimum temperature (T_{min}) from 1 Mar 2002–31 Oct 2013 were retrieved and plotted. Mean temperature (T_{mean}) and growing-degree-day ([GDD] Base 10 °C) accumulation from 1 Mar to 31 Oct was calculated as described by Baskerville and Emin (1969) for 2012 as an average for the preceding ten-year period. Note that GDD for grape are usually calculated from 1 Apr per Winkler et al. (1974), however, the 2012 temperature anomalies required advancing the start to 1 Mar.

2.3. Canopy and shoot development

Weekly, from budburst to harvest, the phenological stage (modified E-L) for each tagged SPB and SSB was identified and recorded (Coombe, 1995). Vines were considered at flowering (E-L 23) when 50% of caps were off, at pea-size (E-L 31) when 50% of berries were approximately 7 mm in diameter, and at veraison (E-L 35) when 50% of the berries exhibited some red in color. The length of each tagged shoot was also measured during the growing season from the base to the end of the tip manually using a flexible tape and recorded. After harvest, the number of SPB and SSB per vine were counted and recorded. All tagged shoots were then removed, stored in a portable cooler with crushed ice, and immediately transported to the laboratory. Final shoot length was measured with a tape and leaf area calculated using a leaf area meter (Portable Area Meter LI-3000; LI-COR Environmental, Lincoln, NE, USA). Leaf area per vine and the leaf area-to-yield ratio were then calculated.

2.4. Vine yield and cluster parameters

In 2012 and 2013, when berries for SPB reached about 22 Brix with TA lower than 10 g/L, grape clusters were manually harvested from each of the tagged shoots and placed into pre-labelled poly zip bags. Clusters were immediately counted and bags individually weighed, then stored in a portable cooler with crushed ice and transported to the laboratory where they were frozen at -20 °C. Vine and total yields were calculated. The remaining fruit was harvested in the same manner with clusters separated by vine, shoot, and treatment. Average number of berries per cluster by treatment and the average berry weight by treatment were also recorded.

2.5. Basic fruit chemistry, color and phenolics

In 2012, once per week, for the four weeks from veraison through harvest, three groups of 90 berries per treatment were randomly sampled from different parts of the clusters of SPB and SSB. They were collected in pre-labelled poly zip bags, put immediately in a portable cooler with crushed ice, and transported to the laboratory where they were frozen at −20 °C to await analysis. The analyses of anthocyanins and total phenolics were performed as described by Iland et al. (2004). Samples were partially thawed then ground in a homogenizer (PT 10/35, Brinkmann Instruments, Luzerne, Switzerland). One g of each homogenate was weighed and transferred to a labelled 15 mL polypro tube with cap and centrifuged at 4000 rpm for 10 min (Legend X1R, Thermo Scientific, Waltham, MA). Measurements were taken using the total phenol assay at wavelengths of 280 nm, 520 nm, and 700 nm with a UV–vis spectrophotometer (UV-1800, Shimadzu Corporation,

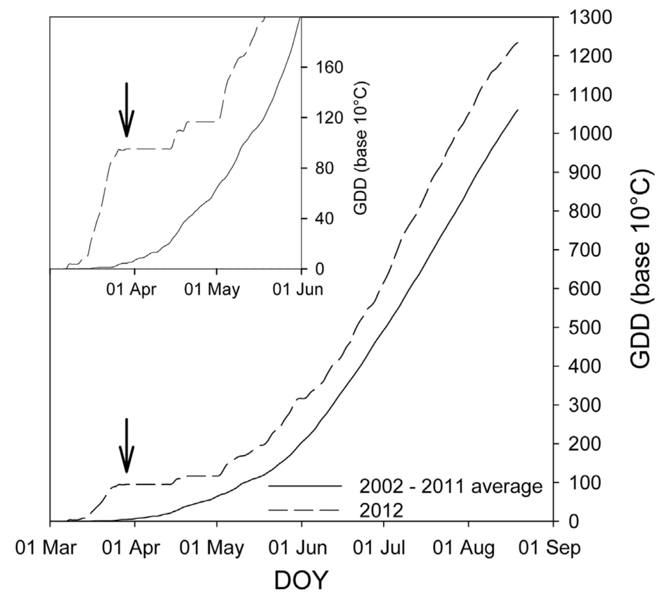

Fig. 2. Growing-degree-days (base 10 °C) accumulated in 2012 (broken line) in comparison with the average accumulation of the ten precedent years (entire line) for Michigan State HTRC. Considered period: 01 Mar to 18 Aug 2012. Insert graphic is a zoom on the period 01 Mar–01 Jun. Growing degree days were calculated as described by Baskerville and Emin (1969). Arrows indicate Marquette budburst on 2012 (28 Mar 2012). GDD = Growing-degree-days, DOY = days of the year.

Kyoto, Japan). The remaining supernatant was kept available for performing basic fruit chemistry analysis. In 2012 and 2013, TSS, pH, and TA were analyzed per Iland et al. (2004). The TSS (Brix) were measured once for each sample using a handheld refractometer (ATAGO 3810 PAL-1, Kirkland, WA, USA). The pH for each sample was measured using a digital pH meter (Orion 370, ThermoFisher Scientific, Beverly, MA, USA) that was calibrated daily. Titratable acidity was measured using an automated titrator paired with an auto-sampler and control unit (Titroline 96, Schott-Geräte, Mainz, Germany) and expressed as g/L of tartaric acid equivalent. For each TA sample, 10 mL of juice was diluted with 100 mL deionized water and titrated against a standardized 0.1 M NaOH solution to a pH of 8.2.

2.6. Statistical analysis

Results were tested for normality and homogeneity of variance (ANOVA) using SAS statistical analytics software and the PROC MIXED procedure(version 9.1.3; SAS Institute, Cary, N.C., USA). In 2012, results were analyzed with a reduced one-way factorial statistical model and differences between SPB and SSB were assessed using the Student's *t*-test ($P < 0.05$). Differences in vine performance and fruit quality between the two years (2012 and 2013) were assessed using the Student's *t*-test ($P < 0.05$).

3. Results

3.1. Weather conditions

In March 2012,T_{mean} were particularly high compared with the preceding 10-year average (Fig. 2). Thus, at the end of March, 94 GDD had already accumulated, while no temperatures above the 10 °C should have been recorded considering the 2002–2011 average for the same period (Fig. 2). In April 2012, when temperatures returned to near average for mid-Michigan, GDD accumulation stopped for a prolonged period. However, on eight occasions T_{min} lower than −1 °C were recorded with the lowest T_{min} (-4.6 °C)

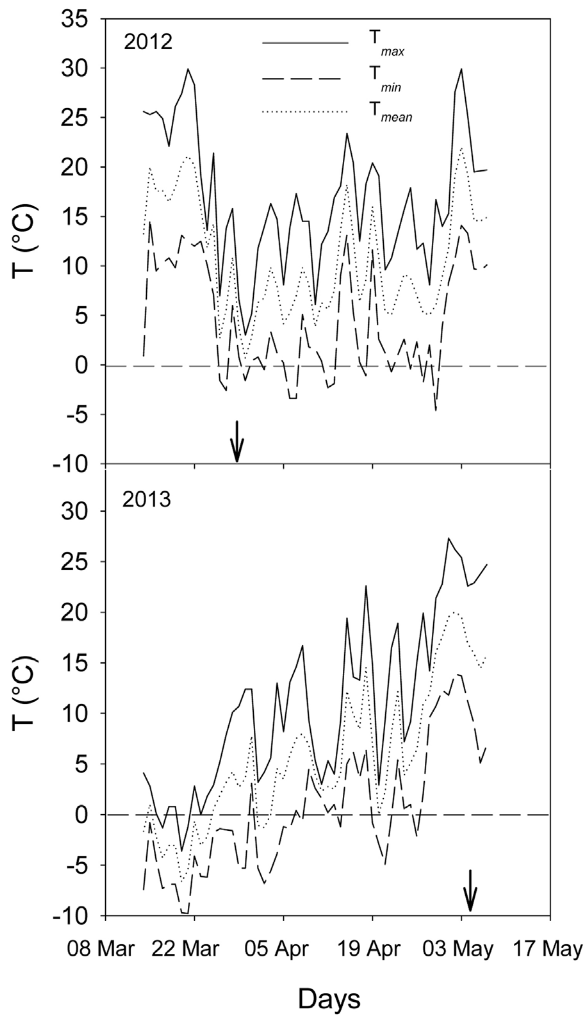

Fig. 3. Maximum (entire line), minimum (segmented line) and mean (dotted line) temperatures recorded daily at Michigan State HTRC from 13 Mar to 07 May in 2012 and 2013. Arrows indicates Marquette budburst (28 Mar 2012, 04 May 2013). No spring freeze events were recorded after 07 May in either 2012 and 2013. T = Temperatures, T_{max} = Maximum temperature, T_{mean} = Mean temperature, T_{min} = Minimum temperature.

occurring on 29 Apr (Fig. 3). The T_{mean} for the rest of the season were generally normal and the accumulation of GDD was similar to the 10-year historical average through July. In 2013, temperature were lower in March when compared to 2012 (Fig. 3). Temperature raised above 0 °C after the first week of April and there was a linear increase both in T_{min} and T_{max} with no freezing temperatures after 1 May 2013.

3.2. Spring freeze damage, phenological stages and canopy architecture

Due to the early warm temperatures, budburst occurred on 28 Mar in 2012 while occurred on 4 May in 2013, 37 days later than the previous year (Fig. 3). In 2012, full bloom on SPB was recorded on 31 May, while it occurred only six days later for SSB. The slow development of SSB increased slightly at pea-size stage compared to SPB (7 Jun 2012 and 21 Jun 2012, respectively). At veraison, the delay recorded for SSB had been reduced to 11 days (Table 1). The harvest date, fixed as the first sampling in which musts of SPB reached 22 Brix with TA lower than 10 g/L, was 18 Aug.

In 2012, after the spring freeze events passed, less than 20% of SPB survived producing only about three shoots per vine, while no dead SSB were found (Table 2). The average vine canopy at harvest was composed of approximately 40 shoots, 90% of which were SSB. SSB were shorter than SPB (-28%) and carried reduced leaf area (-0.11 m^2/shoot). Regardless, the SSB contributed more than 90% of total leaf area per vine by virtue of its greater numbers (Table 2) and poor performance of SPB with regard to shoot production. In 2013, no spring freeze events were recorded after budburst (Fig. 3)

3.3. Vine yield components

In 2012, harvest occurred on 18 Aug (Table 3) with the average total yield per vine at 3.30 kg or 4.44 ± 1.1 t/ha (≈1246 vines/ha), of which more than 90% was due to SSB productivity, reflective of the high percentage of SSB shoots. No difference were found on cluster weight, number of berries per cluster and average berry weight, comparing fruit on SPB and SSB. Lastly, no significant difference was observed in leaf area-to-yield ratio (Table 3) between SPB and SSB, even though the former carried more leaf area, due to their slightly greater bud fruitfulness. In 2013, harvest occurred on 11 Sep (Table 6), with an average yield per vine of 5.4 kg or 6.73 ± 0.74 t/ha.

When comparing the two years, we noticed that yield per vine increased of about 61% in 2013 in relation to 2012 (Table 6). The increase was due to the number of cluster per vine (+37 cluster), while other yield components parameters were not significantly different as well as the vine balance, indexed as leaf are to fruit ratio (Table 6). However, the grape juice grape chemical parameters were different between the frost year (2013) and no frost year (2013), with improved fruit quality, the year following the frost damages (Table 7). Higher total soluble solids couple with lower titratable acidity was reported in 2013 (Table 7)

3.4. Fruit chemistry and color

In 2012, immediately post-veraison, statistically significant differences were observed between clusters from SPB and SSB (Fig. 4). Those from SPB had higher TSS (+6.2 Brix), higher pH (+0.18) and lower TA (-9.3 g/L). The TSS remained higher (+1.5 Brix) in SPB clusters until 14 Aug, and finally, at harvest on 18 Aug, no difference was found (Table 4). In contrast, pH and TA remained significantly different right through harvest (+0.18 pH and −1.40 g/L TA for SPB). Total anthocyanins were significantly higher in berries of SPB clusters in the early stages of ripening (+0.25 mg/g on 24 Jul and +0.23 mg/g on 3 Aug), with later measurements showing the difference had disappeared by harvest (Fig. 5). Regarding total phenolics, the analyses showed no differences between SPB and SSB on any of the sampling dates (Fig. 5).

4. Discussion

In 2012 the anomalous temperatures occurred in Michigan caused a relevant accumulation of active temperatures for grapevines (daily $T_{mean>}$ 10 °C). In 2013, weather conditions were similar to the long-term average recorded in Michigan, with no frost events recorded after budburst (Fig. 3). Marquette is a cultivar that, due to its genetic parentage of *Vitis riparia*, uses to have early budburst, about one month earlier than most of *Vitis vinifera* cultivar. This attitude was exalted in 2012 by the March warm spell, so that budburst occurred approximately one month earlier than normal, while March 2013 was similar to the long-term average of the experimental site (Sabbatini et al., 2013). When in April 2012 temperature dropped repeatedly well below 0 °C most of SPB were irreversibly damaged and killed. The premature budburst and consequent freezing temperatures severely affected the normal phenology of the vines, inducing most compound buds to develop new shoots from the healthy secondary buds, at positions where

Table 1
Dates of major phenological stages on shoots from primary buds (SPB) and secondary buds (SSB) and related growing degree days (GDD) for Marquette in 2012.

	Budburst	Flowering (SPB[b])	Flowering (SSB[c])	Pea-size (SPB)	Pea-size (SSB)	Veraison (SPB)	Veraison (SSB)	Harvest
Date	28/03	31/05	06/06	07/06	21/06	14/07	25/07	18/08
Julian Date	87	151	157	158	172	195	206	230
GDD[a]	94.2	316.5	340.9	346.2	499.4	806.5	964.6	1228.8
Days after Budburst	0	64	70	71	85	108	119	143

[a] Growing-degree-days (10 °C) accumulation through the season calculated as described by Baskerville and Emin (1969) from 1 Mar through 31 Oct.
[b] SPB: Shoots arising from primary buds of the compound bud.
[c] SSB: Shoots arising from secondary buds of the compound bud.

Table 2
Canopy architecture post-spring freeze events in Marquette grapevines.

Treatment[a]	Bud survival rate [b] (%)	Shoots per vine [c] (n°)	Shoot length [c] (cm)	Leaf area [c] (m^2/shoot)	Leaf area [c] (m^2/vine)
SPB	19.9	3	143	0.39	1.2
SSB	100	37	103	0.28	10.5
Significance[d]	*	*	*	*	*

[a] SPB: Shoots arising from primary buds of the compound bud; SSB: Shoots arising from secondary buds of the compound bud.
[b] Measured on 15 May 2012.
[c] Measured on 18 Aug 2012.
[d] Means within the column by *t*-test.
[*] $P < 0.05$; ns, not significant.

Table 3
Yield, cluster morphology at harvest (18 Aug 2012), and relationship between leaf area and yield for Marquette. Each value is the mean of 27 vines. Cluster morphology has been determined on 54 clusters per thesis. Whole vine values are reported as mean (27 vines) ± SE.

Treatment[a]	Yield (kg/vine)	Clusters per vine (n°)	Cluster weight (g)	Berries per cluster (n°)	Berry weight (g)	Leaf area-to-yield ratio (m^2/kg)
SPB	0.31	5	59.2	55	1.08	3.77
SSB	2.99	51	63.2	62	1.02	3.46
Significance[b]	*	*	ns	ns	ns	ns

[a] SPB: Shoots arising from primary buds of compound buds; SSB: Shoots arising from secondary buds of compound buds.
[b] Means within the column by *t*-test.
[*] $P < 0.05$; ns, not significant.

Table 4
Basic fruit chemistry at harvest (18 Aug 2012) in Marquette grapevines.

Treatment[a]	Total soluble solids (Brix)	pH	Titratable acidity (g/L)	Total anthocyanins (mg/g berry wt)	Total phenolics (au/g berry wt)
SPB	22.8	3.50	8.59	1.219	0.805
SSB	22.1	3.32	9.99	1.230	0.854
Significance[b]	ns	*	*	ns	ns

[a] SPB: Shoots arising from primary buds of compound buds; SSB: Shoots arising from secondary buds of compound buds.
[b] Means within the column by *t*-test.
[*] $P < 0.05$; ns, not significant.

SPB were irreversibly damaged. Instead, in 2013, after budburst, the temperature continued to linearly increase without posing any frost threat to the growing vines (Fig. 3). In 2012, the composition of the post-freeze canopy of these Marquette vines was similar to that observed in *V. vinifera* cv. Chardonnay following SPB killed by spring frost and new SSB re-growth (Friend et al., 2011).

The main phenological stages recorded in 2013 on survived SPB and on new developed SSB tracked in parallel. The variability in the delay of SSB phenology during the season is probably due to different environmental conditions experienced during bloom and fruit set by each set of buds, as well as a possible difference in the dynamic of competition between vegetative and reproductive activity.

Considering the vines productivity, after the severe spring freeze events of 2012 Marquette produced about 4.4 t/ha. If compared to the amount of 8.9 t/ha listed in the Marquette patent registration, a yield one might expect from principally primary shoots of vines in same conditions as the pre-release trials (Hemstad and Luby, 2008), this is an estimated loss (or savings) of half of the expected crop. *V. vinifera* SSB are renowned for being less fruitful than SPB (Friend et al., 2011). Therefore, it is noteworthy then that the difference between SPB and SSB in average number of clusters per shoot was not large at 1.7 versus 1.4, respectively, with no significant difference observed in their average cluster weight, berry weight, or number of berries per cluster. In contrast, the SSB of New Zealand Chardonnay vines, tracked after a spring frost, were weaker performers producing only 0.5 clusters per shoot versus 1.5 clusters per shoots on SPB (Friend et al., 2011). However, from our data the lack of difference in cluster morphology and weight between SPB and SSB clusters can be attributed to freezing temperatures that partially damaged SPB clusters. In fact, when the last freeze event occurred (29 Apr 2012, $T_{min} = -4.6$ °C) SPB were already developing, meanwhile the secondary buds were still inhibited. Potential damages, not evaluated in this study, may have affected SPB, consequently producing clusters at the lower weight limit of the range (65.4–124.8 g) reported for the cultivar (Hemstad and Luby, 2008). The relationships between vegetative and reproductive parameters (leaf area-to-yield ratio) was not different between SPB and

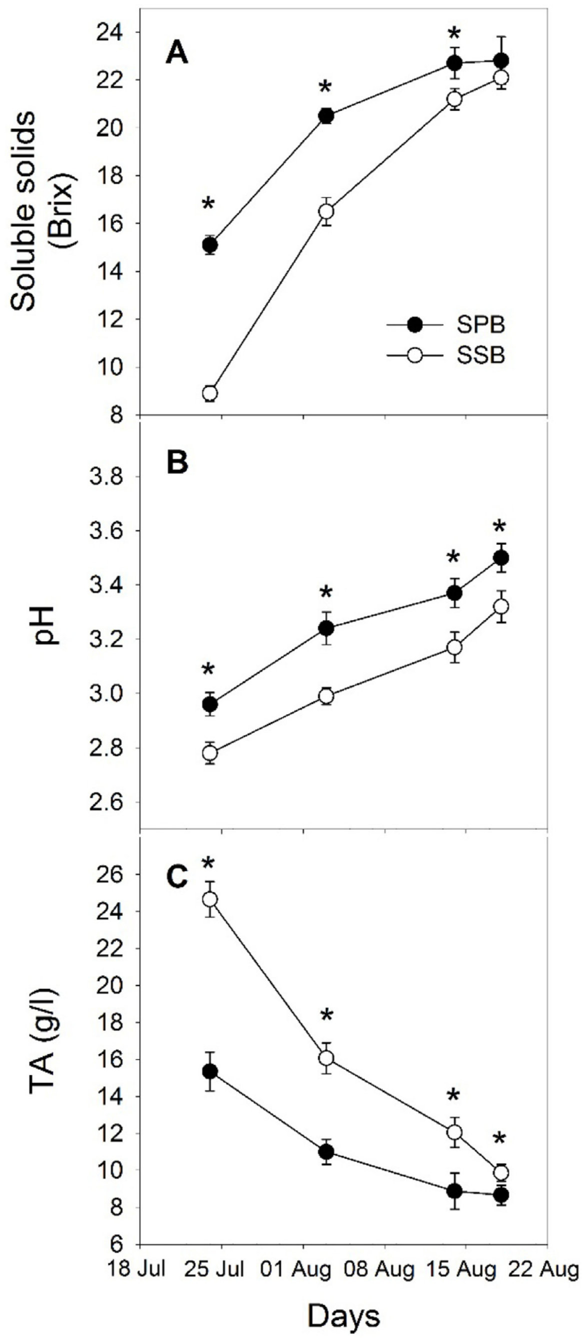

Fig. 4. Evolution of soluble solids (A). pH (B) and titratable acidity (C) during ripening in juice of berries sampled on shoots arising from primary buds (SPB) and berries sampled on shoots arising from secondary buds (SSB). Each point is the mean of readings on 9 juices obtained by 9 groups of 30 berries each ± SE. Points with asterisks are different per $P < 0.05$ (*t*-test).

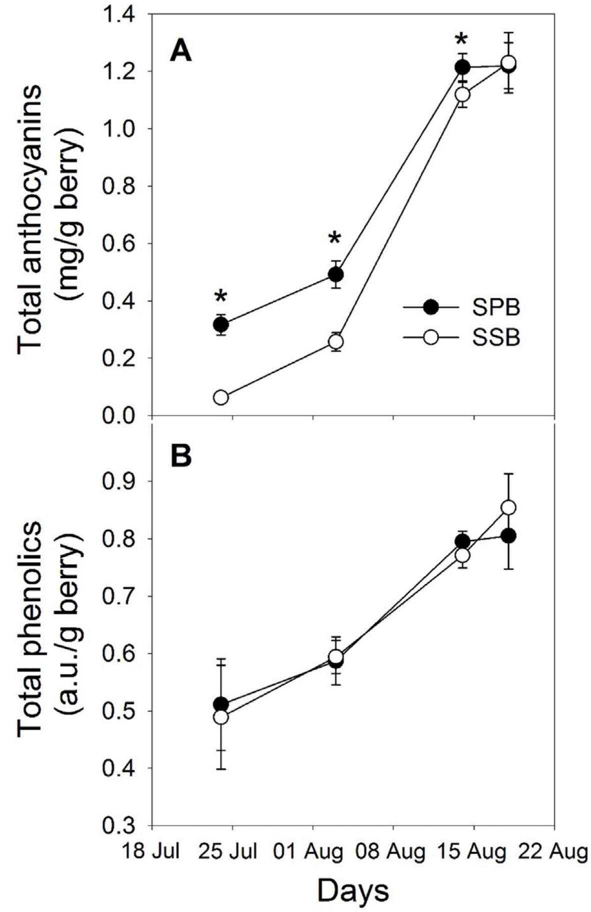

Fig. 5. Evolution of total anthocyanins (A) and total phenolics (B) during ripening in berries sampled on shoots arising from primary buds (SPB) and in berries sampled on shoots arising from secondary buds (SSB). Each point is the mean of readings on 9 groups of 30 berries each ± SE. Points with asterisks are different per $P < 0.05$ (*t*-test).

SSB, probably as a result of the balancing of a slightly lower number of clusters and of a lower leaf area on SSB. Interestingly, the ratio appears high in comparison with the ideal range reported for grapevine, which correspond to values comprised between 0.5 and 1.2 m^2/kg (Kliewer and Dokoozlian, 2005). This can be due to the genetic generous vigor described for Marquette (Hemstad and Luby, 2008), but it's not possible to exclude of the ratio from the cultivar's standard caused by spring freezing temperatures. As already mentioned, size of clusters from either SPB and SSB can be indeed reduced by eventual damages occurred during inflorescence differentiation. In 2013, vines recovered productivity, reaching 6.73 t/ha, very similar to values reported in the literature (Hemstad and Luby, 2008). The differences between 2012 and 2013 were due to a reduced numbers of cluster per vine in 2012 due to the repeated frost damages and the lower fruitfulness of the SSB (Tables 2, 3 and 5). In this year, when no freezing events were recorded, the leaf area-to-yield ratio was lower than 2012, but no statistical difference was found between the two years. This confirms that Marquette, having naturally a significant vigor (Hemstad and Luby, 2008), is characterized by a leaf area-to-yield higher than the ideal range reported for *V. vinifera* cultivars (Kliewer and Dokoozlian, 2005), independently by spring freezing events or the origin of the shoots composing of the canopy.

Clusters developed on SSB of *V. vinifera* are thought to be smaller and with a reduced number of berries (Keller, 2010), and yields arising from SSB are considered unacceptable (Trought et al., 1999; Friend et al., 2011). These differences observed between Chardonnay and other *V. vinifera* cultivars and the vines in this study are likely heavily influenced by *V. riparia*, which has demonstrated high fruitfulness on SSB (Gerrath and Posluszny, 1988a, 1988b; Mullins et al., 1992), and is included in the parentage of Marquette. Obviously, the uniform damage throughout the entirety of this study's plot prevents comparison to an undamaged control. Furthermore, due to Marquette's relatively recent commercial release, there is a lack of information about its biology and productivity, making it difficult to judge yield reduction and cluster morphology with confidence.

Table 5
Canopy architecture in Marquette grapevines on 2012 and 2013.

Year	Shoots per vine[a] (n°)	Shoot length [a] (cm)	Leaf area [a] (m^2/shoot)	Leaf area (m^2/vine)
2012	40	111	0.32	11.7
2013	43	132	0.35	15.1
Significance[b]	*	*	*	*

[a] Measured on 18 Aug 2012 and 11 Sep 2013.
[b] Means within the column by *t*-test.
* P < 0.05; ns, not significant.

Table 6
Yield, cluster morphology at harvest (18 Aug 2012, 11 Sep 2013), and relationship between leaf area and yield for Marquette grapevines in 2012 and 2013. Each value is the mean of 27 vines. Values are reported as mean (27 vines).

Year	Yield (kg/vine)[b]	Clusters per vine (n°)[b]	Cluster weight (g)[c]	Berries per cluster (n°)[c]	Berry weight (g)[c]	Leaf area-to-yield ratio (m^2/kg)
2012	3.30	56	59	57	1.04	3.55
2013	5.40	93	58	56	1.03	2.80
Significance[a]	*	*	ns	ns	ns	ns

[a] Means within the column by *t*-test.
* P < 0.05; ns, not significant.
[b] Yield and number of cluster per vine in 2012 were calculated as the sum of the values of SPB and SSB.
[c] Cluster weight, berries per cluster and berry weight were calculated in 2012 from a random sample at harvest.

Table 7
Basic juice chemistry at harvest (18 Aug 2012, 11 Sep 2013) in Marquette grapevines in 2012 and 2013.

Year	Total soluble solids (Brix)[b]	pH[b]	Titratable acidity (g/L)[b]
2012	22.2	3.39	9.77
2013	23.5	3.50	8.23
Significance[a]	*	*	*

[a] Means within the column by *t*-test.
* P < 0.05; ns, not significant.
[b] Total soluble solids, pH and titratable acidity in 2012 were calculated from a random sample at harvest.

A thorough search uncovered nothing in the literature addressing the fruit quality of vines simultaneously carrying two distinct populations of clusters from SPB and SSB. In this study, it's clear from the quality parameters that SSB had a delayed ripening evolution compared to SPB. Considering TSS, SSB berries reached 16.5 Brix on 3 Aug, a point similar to those of SPB recorded 10 days earlier on 24 Jul (15.1 Brix). The gap was narrowed through the next 10 days, with 21.2 Brix on 14 Aug for SSB and 20.5 Brix on 3 Aug for SPB. This confirms the phenological delay of 11–14 days found at pea-size and veraison. Moreover, a progression in ripening without alteration of the underlying dynamics can be supported by the lack of difference between the amounts of leaf area on which each of the two cluster populations could rely as sources of photosynthates (leaf area-to-yield ratio). Ultimately, by harvest on 18 Aug in 2012, the gap in TSS values had nearly closed for SSB compared to SPB at 22.1 Brix and 22.8 Brix, respectively. On the other hand, TA tracked similarly in SSB and SSB berries, consistently and significantly higher throughout the season including harvest when final values measured at 9.9 g/L and 8.7 g/L for SSB and SPB, respectively. Sugar accumulation may be related to potential interactions between leaf age and cluster microclimate in SPB and SSB. Younger leaves are known to have higher photosynthetic efficiency (Palliotti et al., 2000; Palliotti et al., 2009; Gatti et al., 2016). SSB had younger leaves than SPB and a potential for higher photosynthetic activity later in the season, granting higher sugar accumulation. In 2013, when no frost damages were recorded, vine recovered productivity while increasing also fruit quality at harvest also with similar canopy growth when expressed as leaf to yield ratio (Table 5–7).

5. Conclusions

Marquette cold-hardiness and early ripening, together with high quality of wines obtained, should continue to prove attractive to growers and wineries with only one apparent concern about early budburst. This study, however, reports how Marquette has the potential to avoid the yield loss that many *V. vinifera* cultivars face when severe spring freeze occurs, while also providing insurance against devastating mid-winter vine death. In April 2012, an exceptional year when multiple freeze events killed or injured nearly all developing primary shoots, yield per vine was lower than the average productivity expected for the cultivar, but a far better result than what any *V. vinifera* cultivar would deliver under the same conditions and without loss in fruit quality. This is due to the fruitfulness of shoots developed from secondary buds of the Marquette compound buds in response to a freeze event, a trait inherited from the *V. riparia* parent of the cultivar. The clusters carried on shoots from secondary buds were similar in size and compactness and only slightly delayed in ripening than those carried on the few surviving primary bud shoots. The early ripening of Marquette, and subsequent early harvest, definitely allows growers to achieve full technical maturity of clusters on shoots from secondary buds. In 2013, year characterized by no spring frost events, vines were able to fully recover the damages of the previous year with no significant carry over effects, producing higher yield than 2012 (+61%) with better fruit quality at harvest. Marquette is an interesting and promising alternative grape cultivar for many cool and cold climate viticulture regions, especially given the projected impacts of current climate trends.

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