Implications of an exceptional autumn bud flush on subsequent cold tolerance of Garry oak (*Quercus garryana* Douglas ex Hook)

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Implications of an exceptional autumn bud flush on subsequent cold tolerance of Garry oak (*Quercus garryana* Douglas ex Hook)

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Abstract

In the fall of 2016, an unusual phenological event occurred in *Quercus garryana* Douglas ex Hook. in Victoria, B.C. After normal autumn leaf drop, some trees burst bud, and leafed out prematurely in late October. This allowed a comparison of the cold hardiness of the prematurely flushed and non-flushed trees over the following year. Cold hardiness of five tree pairs (premature fall flush and non-flush) in three locations in Victoria was assessed bi-weekly over the dehardening period from January to March, and again over the hardening period from September to December, 2017. Cold hardiness of ten non-flushed trees from the most northerly population of *Quercus garryana* was also assessed twice in spring 2017. Between January and March, all trees dehardened, but cold hardiness was greatest in non-flushed trees on the first sampling date, and thereafter the non-flushed trees dehardened more rapidly than prematurely flushed trees. Index of injury was consistently 10% greater in Victoria than in northern trees. In fall 2017, trees that had flushed prematurely in fall 2016 had the same cold hardiness as non-flushed trees. Hardiness of all trees decreased from mid-September to the end of October, followed by rapid hardening in November and December of 2017.

Keywords: premature flush, Garry oak (*Quercus garryana*), cold hardiness, phenology, dehardening, index of injury
**Introduction**

Phenology describes the seasonal timing of growth processes in plants and trees. It refers to visible events such as bud flush, flowering, bud set and senescence, events that delimit the seasonal growth period (Perry 1971, Gusta et al. 2009) and are tied to the annual growth, reproductive success and competitive abilities of trees in temperate climates (Vitasse et al. 2014). Cold injury to new growth exerts a strong selection pressure on the timing of bud flush and bud set (Gusta et al. 2009), hence cold hardiness and phenology are strongly correlated (Morin et al. 2007).

Many environmental cues influence the timing of growth cessation and bud set - photoperiod, temperature, soil moisture, nutrients and light quality, whereas bud flush primarily is influenced by temperature, once chilling requirements have been satisfied (Howe et al. 2003, Hänninen 2016). Because temperature has a key role in initiating phenological events, there are serious concerns that climate warming may disrupt the adaptive phenological cycles of plants. Warmer autumn temperatures have been shown to disturb the normal synchronization of declining photoperiod and temperature, and to delay leaf senescence and the development of cold hardiness (Saxe et al. 2001, Gill et al. 2015). Higher temperatures in late winter and early spring may cause plants to lose cold tolerance as soon as their chilling requirements are met, thus increasing the risk of late frost damage (Repo et al. 1996, Hänninen 2016). Spring events at temperate latitudes have advanced by 2.5 days per decade since 1971 (Menzel et al. 2006), thus effects of changing climates on plant phenology already appear to be evident.
Soil moisture availability and its influence on plant water potential acts in concert with temperature to affect cold hardiness in plants. Many genes that are induced by cold are also induced by drought and vice versa, and the protein products of these genes protect the plant from the consequences of freezing stress, including dehydration (Knight and Knight 2001). The effects of changing climates on summer precipitation patterns could further affect phenological cycles in plants.

In the autumn of 2016, a phenological disruption of Garry oak trees (Quercus garryana Douglas ex Hook.) occurred on southern Vancouver Island. Premature flushing of approximately half of the trees in natural stands was observed in late October after a dry summer was followed by an unusually wet October with above-normal temperatures (Figure 1). The new leaves were subsequently frost killed at the beginning of December. This incident, to our knowledge, occurred only on southern Vancouver Island (C. Harrington, pers. comm.), and provided the opportunity to study the effects of an environmental disturbance (i.e. unusual temperature and precipitation patterns) on the processes of cold acclimation and deacclimation.

The genus Quercus belongs to the family Fagaceae and includes approximately 400 species of trees and shrubs (Sibley 2009) predominantly from the northern hemisphere (Hora 1981). Garry oak, also known as Oregon white oak, is the only oak species native to western Canada. The species ranges from the central region of southern California (~34°N) through Oregon and Washington into south-west coastal British Columbia, where the most northern populations are
found on Savary Island and in Courtenay on Vancouver Island (~ 50 °N). Garry oak is a keystone species in the Garry oak ecosystem, which is home to many endangered species in British Columbia. The Garry oak ecosystem ranges from shady woodlands to open meadows with scattered trees, but the species is shade intolerant and sensitive to competition from faster growing species (Gould et al. 2011). On Vancouver Island, Garry oak meadows were historically maintained by First Nations peoples, who facilitated controlled burnings that kept coniferous species at bay (Erickson 1993, Pellatt and Gedalof 2014). In recent times, Garry oak habitat has been significantly reduced due to urban and rural development. In the future, bioclimate envelope and other models predict, in general, a broader extent for Garry oak suitability in Oregon and Washington, with some declines in specific areas (Bodtker et al. 2009). In British Columbia, suitability of Garry oak habitat is projected to decline (Bodtker et al. 2009). The northward expansion of Garry oak is limited by the oak’s dispersal capabilities and by the limiting climatic conditions of the coast range of British Columbia (Bachelet et al. 2011, Pellatt et al. 2012).

The unusual phenological event of bud flush in the fall of 2016 offered the opportunity to investigate the difference in subsequent cold hardiness of prematurely flushed and non-flushed trees over the following year, and to document any effects of premature flushing on subsequent spring bud flush or hardening processes the following autumn. We hypothesized that prematurely flushed trees would be less cold hardy than non-flushed trees later that winter because we expected that fall flushing would interrupt the acclimation processes. We did not expect to see any effect of 2016 late fall flushing on acclimation or cold hardiness.
the following autumn, but cold hardiness was monitored in the fall of 2017 to see if a
similar event might occur. We also measured winter and spring cold hardiness of
Garry oaks on deep, meadow soils near Courtenay, B.C., at the northern limit of their
distribution range. These trees were expected to show greater cold hardiness than
trees from dry, rocky sites in Victoria.

**Methods and materials**

Terminal branch samples from mature Garry oak trees were collected for
analysis of cold hardiness at three sites near Victoria, B.C.: Uplands Park
(48°26’28”N 123°17’53”W, 20 m elevation), Mount Tolmie Park (48°27’28”N
123°19’26”W, 100 m elevation) and Mount Douglas Park (48°29’35”N
123°20’48”W, elevation 215 m) generally from dry, rocky sites with shallow soils.
Samples were collected six times from mid-January to the end of March 2017 on the
following dates: January 9 and 23, February 6 and 20 and March 13 and 27. In each
location, branch samples were taken from five pairs of trees, each pair consisting of
a tree that had prematurely flushed in the late autumn of 2016 and a tree not yet
flushed. The tree pairs were chosen to be as close as possible in distance, microsite
and size. The tips of lower branches produced in the past growing season were
sampled and all samples were collected from south-facing branches.

At the beginning and end of the test-period (January 14 and March 25, 2017),
samples were also collected from several sites in the city of Courtenay, B.C. (49°42’
22” N 124°59’36” W, 5 m elevation). Courtenay is at the northern limit of the range
of Garry oak. For this reason, the trees were less abundant and individual trees were
sampled at several locations through the city, generally on deep, meadow soils. In total, 11 trees had the tips of lower, south-facing branches collected for cold hardiness tests. In Courtenay, none of the trees had flushed in the autumn, thus “flush” was not a factor. All samples were stored in sealed plastic bags on ice or in a 4 °C refrigerator until testing.

Cold hardiness of the same 15 pairs of trees from Victoria was investigated in the fall of 2017 to follow the process of cold acclimation and to relate that to the 2016 autumn flush and the deacclimation process in spring 2017. Samples were collected at three-week intervals on the following dates in 2017: September 15, October 10, October 31, November 21 and December 12.

The electrolyte leakage method, a widely-used method to assess the extent of plant injury in response to temperature or drought stress (Colombo 1984, Burr et al. 1990, Baji et al. 2002), was used to measure the cold hardiness of all samples. This method gives a relative measure of cold damage based on conductivity measures of leakage from the damaged cells. It is a useful method to follow the relative progression of cold acclimation/de-acclimation as it provides a quantitative evaluation of damage and the same trees can be sampled repeatedly. Test temperatures used in freeze-induced electrolyte leakage tests are often colder than temperatures in the natural environment to ensure measurable damage, thus results cannot be used as a measure of the actual low temperature tolerance of living trees. Using similar methods, Huebert (2009), found that three sub-zero temperatures, -22 °C, -28 °C and -34 °C, together with an unfrozen control temperature of 5 °C, provided a good spread of test temperatures encompassing
50% index of injury by which to investigate relative cold hardiness of Garry oak trees. Based on Huebert’s (2009) results, the same temperatures were chosen for this study and were used consistently throughout the study to maximize statistical power. The aim was to select freezing temperatures that would result in a range of freezing damage consistently across sampling dates.

Internodes of the sampled branches from each tree were cut in 2 mm thick cross sections. Two randomly selected branch sections were put in each of 12 scintillation vials, together with 0.2 ml distilled water, with three replicate vials per tree per temperature. The control sample vials were kept in the refrigerator and the rest were frozen in a programmable freezer at a cooling rate of 5 °C per hour (Colombo 1984, Deans and Harvey 1996, Morin et al. 2007, Huebert 2009). After reaching -22 °C, the samples were held at this temperature for an hour, after which all sample vials for that test temperature were removed and placed in the refrigerator to thaw. The cooling and freezing cycle continued for -28 °C and -34 °C test temperatures.

After thawing overnight (day 2), 10 mL distilled water was added to every vial, including the control samples, and the vials were shaken for 24 h, then the first conductivity measures were made (day 3). After measurement, all samples were heat-killed in an oven at 100 °C for 2 h, shaken at room temperature for three days, then maximum electrolyte leakage was measured (day 6). To evaluate the amount of cold damage, the index of injury was calculated. For all test vials, the index of injury was calculated from the conductivity measurements of the test- and control-vials, after treatment and heat kill, respectively (Flint et al. 1967). LT50 values
(temperature resulting in 50% index of injury) for each date were interpolated from a regression of the mean index of injury at each of the three test temperatures for flushed and non-flushed trees.

The winter/spring 2017 index of injury data from the three sites in Victoria were analyzed using a GLM mixed model with repeated measurements (MANOVA - SAS Institute Inc., Cary NC). The main effects of the model were: the unique tree-pair at each location (15 levels), test-temperature (3 levels), replicate (3 replicates for all measurements) and whether or not the trees were prematurely flushed (2 levels). All trees were sampled repeatedly at six different dates with a total of 270 observations per date. The location effect (3 levels) showed no significance in preliminary analyses and was excluded from the model. The explanatory power of the model in terms of the R^2 value was unchanged by this exclusion. Pair, Flush, Test-temperature and the interaction of Flush x Test-temperature were tested for significance (p < 0.05). Least square means tests adjusted for multiple comparisons (Tukey) (SAS Institute Inc, Cary NC) were performed on the Test-temperature and Flush x Test-temperature interaction and a repeated measures analysis of variance (SAS Institute Inc, Cary NC) was performed to test for temporal autocorrelation. Fit Diagnostics were undertaken to confirm that residuals were normally distributed.

The above model was also used to analyze the fall dataset, except the analysis was conducted on data averaged by tree due to missing values in the dataset. The number of replicates for each tree and test-temperature ranged from 3 (most
common) to 2 (n = 43) to 1 (n = 2). All trees were sampled repeatedly on 5 different
dates, except for one pair that was missed on the first date of sampling.

The data from trees from Courtenay had three sources of variation: Test-
temperature (3 levels), date of sampling (2 levels) and the sampled tree (11 levels),
with three replicates of each combination. These data were compared to a subset of
the Victoria data, where only the non-flushed trees and the first and last sampling
date (early January and late March) were used. The combined data set to compare
the two locations had the following structure: test-temperature (3 levels), month of
sampling (2 levels – January and March), location (2 levels – Victoria and Courtenay)
and the unique tree (26 levels). This data set was unbalanced as it held 15 trees
from Victoria and 11 from Courtenay, therefore it was analysed with a General
Linear Model (GLM), where temperature, sampling month and location were main
effects and the tree effect was nested within location. Means for all effects and
interactions were computed together with their standard errors. As our results for
the Victoria spring and fall data showed that autocorrelation became insignificant
over three weeks, repeated measurements analysis was not performed on the data
from Courtenay, where the two samplings were more than eight weeks apart.

All Victoria trees were assessed for the stage of spring bud flush in the first
and last weeks of April, 2017. Buds on the branches surrounding the branches
sampled for cold hardiness were rated on the following scale 1 = buds tightly closed,
2 = buds swollen, 3 = leaves just emerging from buds, 4 = leaves one quarter
emerged, 5 = leaves half emerged, 6 = leaves fully emerged. Mean spring bud flush
of the 15 prematurely flushed and 15 non-flushed trees were compared for each sampling date using a Wilcoxon signed-rank test for ordinal data.

Environmental data, including climate normals (1971-2000) for mean monthly precipitation and temperature for Victoria and Courtenay, B.C., and daily mean temperature and precipitation in the autumn of 2016 for Victoria, were obtained from Environment and Climate Change Canada (2018) and from the Victoria School-Based Weather Station Network (2018), respectively.

**Results**

Test temperature, unsurprisingly, had a strong, significant effect on the level of injury for all six winter/spring test dates \( p < 0.0001 \). The model for the analysis of the winter/spring cold hardiness data from Victoria trees showed high levels of explanation, with \( R^2 \)-values ranging from 0.76 to 0.94 for the different dates of analysis (Table 1), and test temperature was the greatest contributor to the \( R^2 \) values. The three test temperatures caused significantly different degrees of damage on all dates, and the freezing temperatures caused greater cold injury (higher index of injury-values) as the spring progressed (Figure 2). The level of injury increased in a linear fashion from the first to the final sampling date, indicating dehardening, but the slope depended on test temperature (Figure 2). The greatest range in index of injury was observed at the warmest test temperature of -22 °C, and injury was greatest, but varied the least, at the lowest test temperature of -34 °C. This pattern, and the fact that the three test temperatures bracketed 50% index of injury on all
test dates, confirms that the range of test temperatures was well suited to
differentiate among individuals with differences in cold hardiness.

The effect of the tree-pair factor was significant for all models ($p < 0.0001$).
This indicates considerable variation in cold hardiness among the 15 tree pairs. The
inclusion of the tree pair as a factor in the model removed the associated variation
from the analysis of the Flush factor. The interaction between Flush and Test-
temperature showed no significant effects.

Measuring the effect of premature flush on subsequent winter/spring cold
hardiness was the primary objective of this study. The analysis showed the effect of
Flush to be strongly significant in January, insignificant by early February and then
strongly significant in late February and March (Table 1). Comparing the change in
LT$_{50}$ values over time for the prematurely flushed and non-flushed trees, showed
the prematurely flushed trees were more damaged than the non-flushed trees on
the first sampling date in January (Figure 3). The non-flushed trees dehardened at a
faster rate than the prematurely flushed trees, however, as indicated by the two
quadratic regression lines crossing each other between the first and second
sampling date (Figure 3) ($R^2$ for the quadratic regression = 0.996 for flushed and
0.997 for non-flushed).

The more rapid rate of dehardening in the non-flushed trees did not result in
earlier bud flush. On both sampling dates in April 2017, there was no significant
difference in stage of bud burst between trees that had prematurely flushed in
October and those that had not ($3.7 \pm 1.0$ vs. $3.6 \pm 1.6$, respectively (mean ± S.D.) in
early April and $5.7 \pm 1.3$ vs. $5.6 \pm 1.6$, respectively in late April). Although crown
density was not quantitatively evaluated, casual observation indicated that leaf
density in trees that flushed prematurely the previous autumn was less than in non-
flushed trees.

The repeated measures analysis of variance of the winter/spring index of
injury data showed that the level of freezing damage for a given tree was highly
correlated with damage on the subsequent two sampling dates (i.e. $p$-values for
autocorrelations of data one and two sampling dates apart were $<0.0001$) and this
correlation became greater as time progressed (partial correlation coefficients
increased from 0.26 to 0.50 for data one sampling date apart and from 0.26 to 0.37
for data two sampling dates apart as sampling progressed). By early March, cold
injury of samples was no longer correlated with injury data collected in January.

As observed for the winter/spring dataset, the test temperature in the fall of
2017 had a strongly significant effect on injury level ($p < 0.0001$). The repeated
measures analysis of variance of the fall 2017 Victoria index of injury data had $R^2$-
values ranging from 0.65 to 0.84 for the different dates (Table 2) and test
temperature was the biggest contributor to the model $R^2$ values. The three test
temperatures caused significantly different levels of damage on all dates. In the fall,
cold hardiness did not increase in a linear manner over time, as was observed for
dehardening in the winter/spring. Instead, freezing damage showed first a decrease
(corresponding to a hardening of the trees) from September to October, then an
increase (dehardening) in late October, followed by a decrease again in late
November and December (Figure 4). The interpolated LT50 values were -23.6°C on September 15, -25.8°C on October 10, -20.3°C on October 31, -29.7°C on November 21 and -28.5°C on December 12. As was observed in the winter/spring data, the effect of the tree-pair factor in the models was significant \( (p < 0.05) \). The interaction between Flush and Test-temperature was not significant. The Flush factor showed no significant effect on any of the five sampling dates, hence the trees that had prematurely flushed one year before no longer differed in hardiness from other trees.

The repeated measures analysis of variance of the fall 2017 index of injury data showed similar trends in temporal autocorrelation as were observed in the winter/spring dataset. Index of injury of trees was correlated for three out of four sequential sampling dates.

The comparison of cold hardiness data from Garry oaks in Victoria and Courtenay included only non-flushed trees, and most sources of variation included in the ANOVA model were significant (Table 3). The model showed a high level of explanation \( (R^2 = 0.98) \). The mean \( (+ S.E.) \) index of injury levels were 31.85 \( \pm 0.35\% \) and 41.72 \( \pm 0.30\% \) in January, and 57.93 \( \pm 0.35\% \) and 68.24 \( \pm 0.30\% \) in March for Courtenay and Victoria, respectively. A consistent difference of approximately 10% index of injury was observed between the two locations in the two different months. The model showed significant interactions between the three main effects, but our focus was to compare cold tolerance between the two locations, and the interaction between month of sampling and location was not significant.
Discussion

Studies of cold hardiness in oaks show similar patterns of seasonal cold hardening and dehardening to those observed in other temperate and boreal forest trees. Flint (1972) measured the cold tolerance of 38 populations of *Quercus rubra* L. on six dates over two years, and showed all populations to have maximum cold hardiness in mid-winter followed by a rapid loss of hardiness in early spring. Morin et al. (2007) studied the cold hardiness of three European oak species sampled in October, January and March, and trees in all populations hardened between October and January, and dehardened from January to March. Pedunculate oak (*Quercus robur* L.) was shown to have a cold hardiness of -36 °C in December in Finland (Repo et al. 2008). While we could only assess relative levels of cold hardiness in our study, in Victoria, a clear trend of cold deacclimation in Garry oak was observed from the freeze testing of twigs from early January to late March. In the fall, cold hardening did not follow the typical pattern, however, as hardiness initially increased from September to October, then decreased at the end of October, followed by rapid hardening in November and December.

While a number of studies have investigated lammas growth in trees and the effects on autumn cold acclimation, to our knowledge, ours is the first study to investigate the phenomenon of trees flushing in late autumn and the subsequent effects on late winter and spring cold hardiness. Because full acclimation cannot occur before growth cessation, our initial hypothesis was that prematurely flushed trees would show lower levels of cold hardiness later in the winter than non-flushed
trees as acclimation was delayed. Surprisingly, this was the case only on January 9 -
the first sampling date. By the second and third sampling dates, the hardiness of
flushed trees was not significantly different from that of non-flushed trees, and for
the rest of the sample dates, non-flushed trees were more damaged by freezing than
the prematurely flushed samples. Many trees reach their maximum levels of cold
hardiness in January (Bigras and Colombo 2001, Ritchie and Landis 2003, Morin et
al. 2007), and then are in an ecodormant state during the dehardening process
(Hänninen 2016). When dehardening began in Garry oak, the trees that had flushed
three months earlier were slower to deharden than non-flushed trees. This suggests
that the prematurely flushed trees had to pass through a stage of full hardiness
before dehardening.

An alternative explanation is that, after a premature autumn flush and the
subsequent frost kill of newly flushed leaves in December, the flushed trees had
fewer buds than non-flushed trees, thus levels of plant growth regulators such as
auxins and gibberellins may have been lower, resulting in slower dehardening. By
the end of the winter, there was no influence of premature flushing on the rate of
spring flush of remaining buds, but the canopies of prematurely flushed trees did
appear more sparse, due to the winter kill of prematurely flushed buds.

Trees from Courtenay at the northern range limit of Garry oak, showed a
higher level of cold hardiness than trees from Victoria. Interestingly, the difference
was very similar on the two sampling dates in January and March. Although a
difference in cold tolerance between the two sites was anticipated, the difference
was larger than expected. The latitudinal distance between the trees in Courtenay and Victoria is only 138 km, but the mean index of injury was approximately 10% greater in Victoria. If a linear change in the injury-levels between the two dates of sampling in Courtenay (timespan of 10 weeks) is assumed, then a linear interpolation between these points could estimate the lead the Victoria trees have in their dehardening process. This calculation shows that Victoria trees were 3-4 weeks ahead of the trees in Courtenay in their dehardening process. Although the annual minimum, maximum and mean temperatures in Victoria (5.6 °C, 14.4 °C and 10.0 °C, respectively) and Courtenay (5.1 °C, 14.2 °C and 9.7 °C, respectively) are similar (Environment and Climate Change Canada), the winter minimum temperature typically drops 1-2 °C lower in Courtenay than in Victoria (Environment and Climate Change Canada), and this difference may be responsible for the 10% difference in index of injury (e.g. Lenz et al. 2016). Specific weather events may also create selection pressures that challenge the trees in Courtenay, for example events of night frost in late spring or arctic outflows from facing mainland valleys. Jensen and Deans (2004) demonstrated that oak provenances adapt to different climatic conditions, and that specific ecotypes exist, even where geographical distances between them are small. Flint (1972) demonstrated that cold hardiness during the fall acclimation period in Quercus rubra was strongly related to latitude and the estimated minimum annual temperature of origin. Huebert’s (2009) Garry oak common garden study showed a significant genetic cline for cold hardiness in late October / early November, with a mean 9% spread in index of injury among 13 populations from across the range of Garry oak. Cold hardiness
was most strongly correlated with the differential between the mean warmest
month and mean coldest month temperatures, with greater cold hardiness found in
more continental populations (Huebert 2009). As ours was not a common garden
study, we cannot determine if differences in cold hardiness between the two Garry
oak populations were due to genetic differences or to differences in the
environment.

The 2016 incident of premature late autumn flushing in Victoria Garry oak
was unusual and restricted to southern Vancouver Island, to our knowledge. On
some sites, roughly half of the trees flushed buds in late October. Because the
phenomenon was observed in many individuals, the local environment likely played
a key role. Cold acclimation can be induced by low temperatures, but also by
drought. We suggest that in Garry oak, the initial stages of cold hardening, primed by
declining photoperiod, are induced by the prolonged summer drought characteristic
of the Mediterranean climate. In normal years, the end of the drought coincides with
declining temperatures, at which time cold acclimation due to low temperatures
proceeds. The summer of 2016 was much drier and warmer than normal, the late
fall slightly warmer, and the October precipitation was almost double the normal
amount (Figure 1). We propose that the warm autumn temperatures of 2016
allowed some trees to flush once the drought was relieved by higher than normal
rainfall in September and early October. As the season progressed into late
November, cool temperatures were the signal for continued acclimation. Thus, the
dry summer conditions could have led to early dormancy of Garry oak, which was
broken and growth resumed in some trees when high rainfall was accompanied by
warm autumn temperatures. Buds enter a state of endodormancy in the autumn when cell division and growth have ceased, bud development is complete and dormancy is imposed by factors within the plant (Lang et al. 1987). Thereafter, endodormancy is broken by exposure to chilling temperatures, but in some species long days can substitute for chilling, especially when the buds have received little chilling (Saxe et al. 2001). Little is known about chilling requirements in Garry oak. The only related study explored the synchrony of acorn production in Garry oak, and this work suggested that Garry oak does have a bud chilling requirement (Peter and Harrington 2009). The hypothesis of a switch from drought-induced hardiness to cold-induced hardiness is supported by the fall 2017 hardiness data that also showed a decrease in hardiness in late autumn once rains had begun, although temperatures were not warm enough in that year to induce bud flushing.

The premature flushing of buds could prove to be an unfortunate event for the trees, “wasting” their buds and delaying their hardening; however, our results showed that while cold tolerance was lower in prematurely flushed trees in January, the difference did not last through the dehardening period and did not affect the rate of spring flush. Downscaling of global climate model predictions for future climates in British Columbia indicates that summers will be warmer and drier than in the recent past, and winters will be warmer and wetter, particularly on the coast (Rodenhuis et al. 2009). The unusual bud flush of Garry oak in 2016 and the subsequent effects on branch cold hardiness and deacclimation, indicate that the combination of changing temperature and rainfall patterns due to global change may lead to effects on tree phenology that we cannot predict.
Acknowledgements

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School-Based Weather Station Network, Campus View Elementary.


Table 1 Results from the winter/spring MANOVA models showing the difference in the least squares (LS) mean index of injury for flushed and non-flushed Garry oak twigs sampled in Victoria on six dates, the $R^2$ of each model and the p-value for the significance of the Flush factor

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</tbody>
</table>

Note: The variables in the model are the unique tree-pair at each location (15 levels), test temperature (3 levels), replication number (3 replicates for all measurements) and whether or not the trees were prematurely flushed (2 levels). (n = 270 per date)

Table 2 Results from the fall MANOVA models showing the, least squares (LS) mean index of injury for flushed and non-flushed Garry oak twigs sampled in Victoria on five dates, the $R^2$ of each model and the p-value for the significance of the Flush factor

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>LS mean index of injury</th>
<th>$R^2$ of model</th>
<th>Flush p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flushed</td>
<td>Non-flushed</td>
<td></td>
</tr>
<tr>
<td>15.09.2017</td>
<td>58.9</td>
<td>60.1</td>
<td>0.84</td>
</tr>
<tr>
<td>10.10.2017</td>
<td>53.7</td>
<td>53.8</td>
<td>0.82</td>
</tr>
<tr>
<td>31.10.2017</td>
<td>64.0</td>
<td>60.7</td>
<td>0.67</td>
</tr>
<tr>
<td>21.11.2017</td>
<td>50.4</td>
<td>51.6</td>
<td>0.65</td>
</tr>
<tr>
<td>12.12.2017</td>
<td>48.3</td>
<td>46.5</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Note: The variables in the model are the unique tree-pair at each location (15 levels), test temperature (3 levels) and whether or not the trees had prematurely flushed in fall 2016 (2 levels). (n = 84 per date)

Table 3 Results of the ANOVA model used to analyze the index of injury data from Garry oak twigs sampled in Victoria and Courtenay on two dates

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>2</td>
<td>28284</td>
<td>2274.3</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Month</td>
<td>1</td>
<td>81092</td>
<td>6520.3</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>
Month *Temperature  2   6563   263.9  <.0001  
Location          1    11639  935.9  <.0001  
Temperature*Location  2   42     1.7    0.19  
Month *Location     1    5      0.4    0.51  
Month *Temperature*Location  2  2229   89.6  <.0001  
Tree(Location)      24   6110   20.5  <.0001  
Temperature*Tree(Location)  48  2326    3.9  <.0001  
Month *Tree(Location) 24   2871   9.6    <.0001  
Month *Temperature*Tree(Location)  48  2289   3.8  <.0001  
Error              312   12.4    -     -      

Note: The variables in the model are temperature (test temperature), month (month of sampling), location and the 26 individual trees nested within location (Tree(Location)) and all their interactions. (n = 936)
Figures

Fig. 1 Mean monthly precipitation (mm) (bars) and temperature (°C) (lines) in Victoria, B.C. from climate normals (1971-2000) (black) (Environment and Climate Change Canada, 2018) and from 2016 (grey) (School-Based Weather Station Network, 2018)

Fig. 2 Mean index of injury of Garry oak twig sections frozen at three temperatures on six test dates from mid-winter into early spring (January 9 – March 27, 2017). Standard errors vary from 0.42-0.94 and are hidden by the symbols. R² values of the regression are 0.96 at -22°C, 0.98 at -28°C and 0.84 at -34°C.

Fig. 3 LT₅₀-values (the interpolated temperature resulting in 50% index of injury) on six sampling dates (January 9 - March 27, 2017) for Victoria Garry oak twigs from trees that had, or had not, experienced a late bud flush the previous autumn.

Fig. 4 The daily mean temperature (°C) (solid line), daily precipitation (mm) (bars) (School-Based Weather Station Network, 2018) and mean index of injury (%) (dashed line) for Garry oak twigs frozen to three temperatures in the autumn and early winter of 2017 in Victoria, B.C.
Fig 1.
Fig 2.
Fig 3.
Fig 4.