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Biomass, carbohydrate, and leakage conductance in buds of six ornamental tree species subjected to a "false spring" in Northeast China

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Abstract Information is highly scarce about the possible effect of a late spring frost on physiological response of buds in ornamental trees. In this study, spring temperature of Changchun at Northeast China was recorded to identify the characteristics of a false spring by detecting extraordinary warming and sudden freeze in early April of 2017. Buds of six local ornamental tree species were investigated for their dynamics in biomass, non-structural carbohydrates, frost resistance on days of 7, 14, 21, and 28 April 2017. According to a comparison with spring temperature records historically from 2007 to 2016, a false spring was determined. Black pine (*Pinus tabuliformis* var. *mukdensis*) had greater bud biomass than apricot (*Prunus sibirica* L.). Peach (*Prunus persica* L. var. *persica* f. *rubro-plena* Schneid.) reserved greater non-structural carbohydrate content in post-chilling buds than black pine, and apricot and willow (*Salix babylonica* L.) had greater soluble sugars and starch contents in buds, respectively. Cumulative number of days with temperature below 12°C had a negative relationship with relative conductance in sorbus (*Sorbus pohuashanensis* [Hance] Hedl.). Chokecherry (*Padus virginiana* 'Canada Red') had greatest bud starch content on 21 April. Overall, a late spring frost imposed interruption on carbohydrate metabolism rather than direct damage on buds of ornamental trees before late April. Advanced warming induced more pronounced negative impact of a false spring than the sudden decline of minimum temperature.

Keywords: global warming; late-spring frost; post-winter chill; budburst; tree physiology

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Introduction

A false spring is the springtime with harmful effect of late spring frost events on budburst in temperate tree and shrub species (Principe et al. 2017, Chamberlain et al. 2020). Under climate change, growing season has been prolonged in temperate forests across several biomes in the northern hemisphere (Bennie et al. 2010, Muffler et al. 2016, Schieber et al. 2017). A prolonged growing season starts with an advanced forest phenology driven by a unnatural warming (Chmielewski et al. 2018). An earlier phenology characterized as advanced sprouting and flowering increases the risk of frost damage on newly growing organs, which included sprouted buds, dehardened twigs, unfolded leaves, blooming flowers, and young fruits (Rodrigo 2000, Park 2016, Chmielewski et al. 2018, Vitasse et al. 2018). The loss of carbon (C) in a false spring was unlikely to be offset by post-spring continuous growth under a warming climate (Awaya et al. 2009, Hufkens et al. 2012, Chamberlain et al. 2020). The yearly productivity, however, may have been impaired in forests since the establishment of a late spring frost (Bascietto et al. 2018). Projected models indicated that the false spring would continue occurring in accordance with more frequency of earlier budbursts in temperate forests for the coming five decades (Olsson et al. 2017). Possible resolution to cope with false spring damage may be adapted by delaying the planting time for juvenile stocks (Kreyling et al. 2012, Malmqvist et al. 2017) or enhancing resistance of mature trees (Muffler et al. 2016). Both strategies, however, largely depend on awareness of the mechanism of frost damage on tree buds.

A false spring has brought damage to temperate forest trees in a wide range of species (Lenz et al. 2016, Muffler et al. 2016, Vitasse et al. 2019). Coniferous trees were usually reported to obtain a high frequency of occurrence as "victims". For example, Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) (Malmqvist et al. 2017), Norway spruce (*Picea abies* [L.] Karst. (Olsson et al. 2017, Vitasse et al. 2018), and Scots pine (*Pinus sylvestris* L.) (Muilu-Makela et al. 2017) were all reported to have suffered severe damages from a spring frost. In a broadened geographical range across North America, Europe, and Asia, broad-leaved trees and shrubs were found to be more reactive than conifers to winter-spring thermal time (Zohner et al. 2020). Documented cases of false spring frosts damaged budburst in broad-leaved trees that showed visual damage included but not limited to European beech (*Fagus sylvatica* L.) (Kreyling et al. 2012, Principe et al. 2017, Schieber et al. 2017, Bascietto et al. 2018, Vitasse et al. 2018), pedunculate oak (*Quercus robur* L.) (Puchalka et al. 2016), apple (*Malus domestica* Borkh.), and cherry (*Prunus persica avium* L. and *Prunus persica cerasus* L.) (Chmielewski et al. 2018, Vitasse et al. 2018). Most of current observations on phenological responses were derived from studies on remotely montane fields where forests were mostly dominated by tree species used for timber and fruiting harvests. Information is still scarce about the responses of ornamental tree species dominated urban forests subjected to late spring frosts.

Air temperature is one of the most critical cues that are responsible for timing of budburst for temperate woody plants (Heide & Prestrud 2005, Polgar & Primack 2011). To determine characteristics of a false spring depends on two conditions of dynamic change of temperature. The first condition is achieved with a significant thermal time that advances budburst and flower onset (Hufkens et al. 2012). A certain amount of days with thermal ranges between 0 and 5°C is required for bud burst and leaf unfolding closely following chilling (Cannell & Smith 1986, Heide 1993). For some tree and shrub species in temperate forests, the temperature of 12°C can be a threshold above which dormancy was broken in the time of late winter or up to early spring (Heide & Prestrud 2005). A false spring was mostly found to start with a sign of advanced extremely high temperature around 20°C in late March or early April (Scheifinger et al. 2003, Gu et al. 2008, Augspurger 2009). Thereafter, a sudden decline of minimum temperature down to the freezing point accounts for the other half condition that causes frost damage. Factors of a false spring can be concluded to a range of temperature fluctuation, days of warm spell, days of sudden freeze persistence, and cumulative days across temperature reverse, which together contribute to the perception of buds exposed to a late spring frost (Scheifinger et al. 2003, Augspurger 2013, Man et al. 2017, Man et al. 2020). Records of these temperature characteristics were scattered in different regions and varied species.

Current understanding about the late spring frost effects on temperate forest trees are mainly derived from assessments of canopy morphology using remotely sensed data (Bascietto et al. 2018, Gu et al. 2008, Marino et al. 2011). Main findings were obtained on inter- and intra-species variations in bud-break timing and sensitivity to air temperature fluctuation (Lenz et al. 2016, Muffler et al. 2016, Marquis et al. 2020). Stand investigation is a main approach to detect the relationship between spring temperature records and tree bud sensitivity (Bennie et al. 2010, Principe et al. 2017, Schieber et al. 2017, Zohner et al. 2020). An observation on just bud phenology is not sufficient to reveal freezing effects on physiological change during budburst. When a frost happens, internal cells may have been chilled although no surface symptom is visible. The sudden frost event in late spring unlikely brings significant influence on tree rings and trunk cell parameters in a short time although chilling has been imposed to newly growing organs (Puchalka et al. 2016). Therefore, detection on bud physiology is needed to figure out the mechanism for substantial responses of tree buds to an essential frost in a false spring.

Evidence is accumulating to reveal the substantial response of tree physiology to late spring frost. A spring freeze can suddenly disturb the expression of genes regulating the phenylpropanoid pathway, which makes trees do not have enough time to react and generate resistance (Muilu-Makela et al. 2017). The current-year organ growth would be reduced accordingly with a series of physiological changes (Vitasse et al. 2019). As a most abundant compound that respond to frost, soluble sugars are directly involved in the primary metabolism with mobilization, accounting for the tradeoff between C-store and assimilate (D'Andrea et al. 2019). This also results in a way that trees can maintain cold hardiness by reversing the consumption of soluble sugars prepared for respiration (Ögren et al. 1997). The metabolism of non-structural carbohydrates (NSCs) is a process to adjust a tradeoff between consuming remained soluble sugars and accumulating synthesized starch. All these changes in NSCs account for the resistance to frost which can be assessed by the magnitude of freezing harm on cells through quantifying freeze-induced electrolyte leakage in current-year leaves (Strimbeck & DeHayes 2000, L'Hirondelle et al. 2006). To our knowledge, rare has been known about the synthesis of all above mentioned physiological processes as an intact spectrum of mechanisms accounting for bud biomass in a false spring frost.

The occurrence of false spring is currently increasing in Europe and Asia because the "opportunistic" tree species are widely harbored with quick reacting to warming air temperature in these two continents (Zohner et al. 2020). The occurrence of false spring days was happening with lower deviation and higher frequency in Northeast China from 1961 to 2013 (Wang et al. 2017). The first flowering date in Northeast China was also found to be advanced at a rate of -1.52 days per decade from 1963 to 2009 (Dai et al. 2013). In this study, a city from Northeast China was chosen as the study site where historical spring temperature was analyzed to summarize the typical characters that can impose freeze. In 2017, six ornamental tree species in a local garden were employed as objects whose buds were monitored for NSC and leakage to reveal their relationships with springer temperature. Based on above-mentioned literature synthesis, we assume that: (i) temperature fluctuation in 2017 had typical characters as a false spring, (ii) broadleaved trees would show less resistance to freeze as high consumption of NSC and more leakage from cells in buds compared to coniferous species, and (iii) all physiological changes in buds can be explained by relationships with spring temperature characters.

Materials and Methods

Study site and weather condition

This study was conducted in a garden (44°06'N, 125°24' E) of Changchun (43°05'–45°15'N, 124°18'–127°05' E), Northeast China. Changchun had a total population of 7.67 million at the prefecture level (City population 2020).

Locally it is a semi-humid monsoon climate with temperature ranging between -15.1°C (January) and 23.1°C (July) (Weather of China 2020). Average temperature in March is mostly below freezing point and usually rises above 0°C in April with high wind velocity. Yearly average precipitation is about 580 mm and rainfall can reach 350–400 mm within the period from June to August (Weather of China 2020). Accumulatively, a total of 2,617 hours of sunshine would be seen in an ordinary year, whose frost-free period is 140 to 150 days.

Tree species and bud selections

Trees of black pine (*Pinus tabuliformis* var. *mukdensis*), sorbus (*Sorbus pohuashanensis* [Hance] Hedl.), peach (*Amygdalus persica* L. var. *persica* f. *rubro-plena* Schneid.), willow (*Salix babylonic*a L.), apricot (*Prunus persica sibirica* L.), and chokecherry (*Padus virginiana* 'Canada Red') were chosen as the research objects in this study. These trees are typically ornamental species used in local urban greening (Zhang et al. 2016, Bureau of Forestry and Landscaping of Changchun 2018). As an unique of conifer species, black pine was taken as the reference to test the difference of bud responses in comparison with broadleaf species. All trees had an average age of 11-15 years up to the year of 2017 in correspondence to 3-5 years after transplant to the garden from nursery. Density and initial growth for chosen tree species are shown in Table 1. Three trees were randomly chosen for one species as sampling objects and they were averaged for height and diameter 10 cm above ground. Four branches towards eastern, western, northern, and southern orientations were labeled for one objective tree where all buds exposed to full sunlight were sampled. Thus, overlap of canopies may generate shade on buds which was avoided by branches selection.

Table 1 Density and initial growth of black pine (*Pinus tabuliformis* var. *mukdensis*), sorbus (*Sorbus pohuashanensis* [Hance] Hedl.), peach (*Prunus persica* L. var. *persica* f. *rubro-plena* Schneid.), willow (*Salix babylonica* L.), apricot (*Prunus sibirica* L.), and chokecherry (*Padus virginiana* 'Canada Red') trees for buds sampling.

Species	Density (i ha^{-1})	Height (m)	A D10 (cm)
Black pine	968	3.20±0.46[1] d[2]	8.47±0.80a
Sorbus	656	5.40±0.75ab	5.73±0.45b
Peach	570	2.63±0.25cd	5.93±0.71b
Willow	274	7.53±1.35a	8.47±1.16a
Apricot	610	2.47±0.31cd	4.57±0.71b
Chokecherry	210	4.40±0.66bc	6.13±0.67b
F value	-	21.46	12.36
Pr>F	-	<0.0001	0.0002

Note:[1] results are represented as mean ± standard deviation; 2 different letters indicate significant difference according to Tukey test at 0.05 level. i=individuals; D10: Diameter 10 cm above ground

Temperature records, monitoring, and characters

Historical temperature records were obtained for recent 10 years from 2007 to 2016 (National Meteorological Information Centre 2020). A chronicle of ten years is long enough for analyzing and identifying temperature characters for a late spring frost event (Principe et al. 2017). Current literature indicates a gross period of late spring frost usually happens from a start on 1 March (61 days of year) to 30 April (121 days of years), when it is a time with frequent temperature fluctuations of alternative warming and freeze (Gu et al. 2008, Augspurger 2009, Hufkens et al. 2012, Weather of China 2020). Daily highest and lowest temperature points were recorded to describe the dynamics of extreme temperature in historical spring times as it was employed in former studies (Hufkens et al. 2012, Vitasse et al. 2018). Temperature data were monitored by a local meteorological service (Changchun City Meteorological Service 2020). Temperature dynamic during the fluctuation in spring of 2017 was compared to the corresponding period in historical records to determine the temperature characters of a false spring. These characters can be described by variables as follows:

(i)Cumulative temperature

Cumulative degree of temperatures: cumulative highest (ChT), averaged (CaT), lowest (ClT) daily temperature up to the investigating day. These three variables represent accumulation of daytime thermal effect (Thum et al. 2009), balance of heat between day and night, and cold air at night (Chung et al. 2006) as pre-conditions to determine spring phenology and potential frost, respectively.

(ii) Cumulative days with specific temperature

We focused on days cumulative number of days with lowest daily temperature over 0°C (CDo0), 12°C (CDo12), and 20°C (CDo20), which quantified the number of days accumulation for bud burst and leaf unfolding by the suggestions from different studies (Cannell & Smith 1986, Heide 1993, Scheifinger et al. 2003, Heide & Prestrud 2005, Gu et al. 2008, Augspurger 2009).

(iii) Cumulative days for advanced warming and sudden freeze

That is days of recent rising-up temperature prior to the next investigating date (DrTup), cumulative days of recent rising-up temperature prior to the investigating date (CDrTup), recent highest temperature prior to the investigating date (RhT), days of recent declining-down temperature prior to the investigating date (DrTd), cumulative days of recent declining-down temperature prior to the investigating date (CDrTd), and recent lowest temperature prior to the investigating date (Rlt). All these variables were summarized from the characters of a late spring frost event in Eastern USA (Gu et al. 2008, Augspurger 2009).

Tree bud sampling

We chose to sample tree buds in April of 2017 to meet a high frequency of false spring frost as it was repeatedly reported in former studies (Gu et al. 2008, Augspurger 2009, Awaya et al. 2009, Augspurger 2013). We set the start of the sampling date since 7 April in reference to the assumption of false spring occurrence that was put forward according to a late spring frost event on Eastern USA in 2007 (Gu et al. 2008, Augspurger 2009). Another case of false spring of the year 2011 in Japan was employed to support the assumption that late spring frost may keep the possible occurrence on 21 April (Awaya et al. 2009). We did not consider the possibility of spring frost occurrence in May because the frequency to show minimum temperature below 0°C was very low in local climate (Weather of China 2020). In addition, the documented records about late spring frost in May mostly happened in higher latitudes (Hufkens et al. 2012, Menzel et al. 2015). However, we extended the SFD up to 28 April 2017 to cover the possibility of late spring frost at the end of April. Therefore, we designed the sampling dates of 7, 14, 21, and 28 April 2017. On investigating day, two buds were randomly removed from the furthest tip of one labeled branch. Eight buds were collected in total from

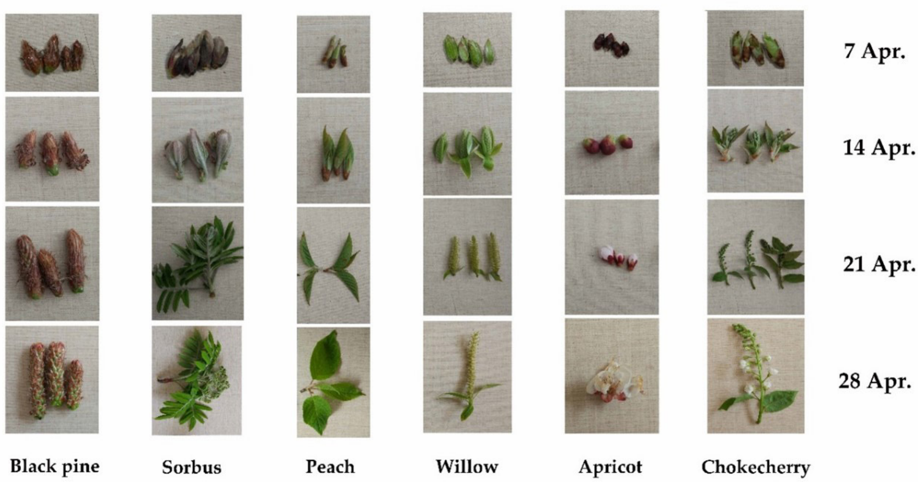

Figure 1 Typical performances of buds in black pine (*Pinus tabuliformis* var. *mukdensis*), sorbus (*Sorbus pohuashanensis* [Hance] Hedl.), peach (*Prunus persica* L. var. *persica* f. *rubro-plena* Schneid.), willow (*Salix babylonica* L.), apricot (*Prunus sibirica* L.), and chokecherry (*Padus virginiana* 'Canada Red') trees photographed on 7th, 14th, 21st, and 28th April of 2017 at Changchun city, Northeast China.

four orientations for an objective tree. Buds were bulked for a tree and pooled to average for a tree. Measures on three trees were assigned as biological replicates for a species. Typical growth performances of six tree buds from four investigating dates are shown in Figure 1.

Biomass measurement and chemical analysis

Half of pooled buds were oven-dried at 70°C for three days and measured for dry weight biomass. Dried buds were ground to pass 1-mm sieve and used to determine NSC concentrations by a method adapted from Wei et al. (2014). Briefly, a dried sample of 0.5g was dissolved in 80% ethanol (v/v), diluted, centrifuged, and determined for soluble sugar concentration using supernatant. The residual was added to by sulfuric acid and phenol and used for starch concentration. The spectrophotometer was used for determination at 490-nm wavelength.

Relative conductance (RC) of electric leakage was conducted by a method that was adapted from L'Hirondelle et al. (2006). The other half of pooled buds were reserved at fresh status on ice (0 to 4 degree centigrade) and took to the laboratory. Buds were cut into small pieces in length of about 5 mm and placed in capped 10-mL tubes with 5 mL distilled water (C_1). Blanks were those tubes filled by distilled water (B_1). Tubes were maintained in an opaque bag at room temperature for 24 h, then subjected to oven-heating at 90°C for 2h. Heated samples were maintained in the dark again for 24 h both for both treated samples (C_2) and blanks (B_2). Therefore, LC can be calculated by the equation as:

$$\mathrm{LC} = \frac{\mathrm{C}_1 - \mathrm{B}_1}{\mathrm{C}_2 - \mathrm{B}_2} \times 100\% \quad (1)$$

Statistical analysis

All statistics were analyzed using SAS software (Ver. 9.4, SAS Institute, Cary, NC, USA). Data were tested for normal distribution and logarithm transformation was employed when necessary. A mixed-model analysis of variance (ANOVA) was used to detect the difference among six tree species from repeated comparisons on sampling trees on four investigating dates. When significant difference was detectable, means of six tree species were arranged and compared according to Tukey's honest significant difference test at the 0.0125 level from Bonferroni adjustment on α=0.05. Pearson correlation was analyzed to detect the relationship between any pairs of bud variables. Correlations were also detected between bud variables and temperature characters of a false spring for each of tree species. Principal component analysis (PCA) was employed to depict the grouped variation of data about bub and temperature.

Results

Determination of a false spring in 2017

Daily highest temperature in 2017 showed an increasing trend with time in accordance with that in historical records (Figure 2A). On 93, 94, and 95 days after year-start, the highest daily temperature in 2017 was 18, 20, and 23 °C, which were higher than the historical records by 1.7, 5.6, and 8.0 °C, respectively (Figure 2A). During 91 to 96 days after year-start, the lowest daily temperature in 2017 ranged between -4.1 and 10.0 °C, which was higher than historical records by 2.80 and 5.60 °C, respectively (Figure 2B). On 103 days of year, daily lowest temperature in 2017 was -3.6 °C which was lower than historical record by -0.6 °C (Figure 2B). Therefore, the spring of 2017 was predicted to have a warm spell during 91–96 days of years that can promote sprouting, followed by an unordinary freeze on 103 day of year (Figure 3).

As a result, our first investigating date on 98 days of year immediately followed the warm spell, then the second date on 105 days of year followed the sudden decline of daily lowest temperature (Figure 3). The third and fourth investigating dates fell in the period when buds had experienced a freeze.

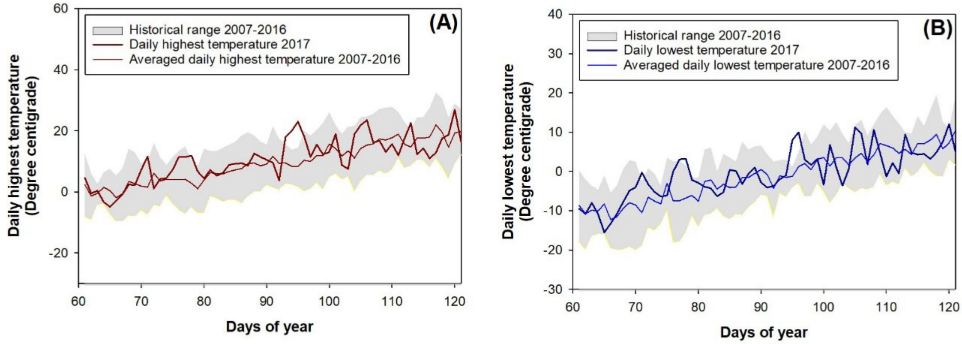

Figure 2 Dynamics of daily highest (A) and lowest (B) records of temperature in March and April at Changchun, Northeast China. Lines in dark red and dark blue colors indicate dynamics of highest and lowest records of daily temperature in 2017, respectively. The shaded polygons stand for the range between maximum and minimum historical records for the period 2007-2016.

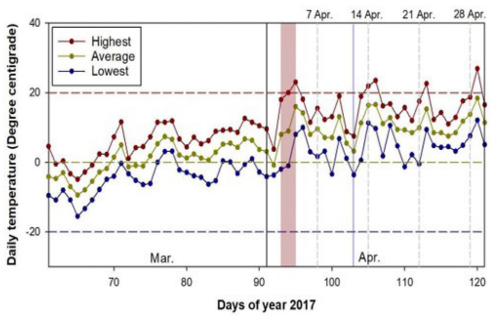

Figure 3 Dynamic of daily highest, averaged, and lowest records of temperature in March and April of 2017 at Changchun, Northeast China, with four investigating dates of 7th, 14th, 21st, and 28th April in 2017. The shaded bar with transparent red color indicates the days when 2017 records of highest temperature is higher than the historical records and that with blue color was lower than the historical minimum records.

False spring temperature characters in 2017

Cumulative degree of temperature increased with time with different increasing patterns for different parameters. ChT was lower than the freezing point on 7 April and ranged from 58.9°C on 14 April to 283.1°C on 28 April (Figure 4A). CaT increased from -503.1°C on 7 April to 287.9°C on 28 April (Figure 4B). ClT increased from -979.6°C on 7 April to -897.7°C on 28 April (Figure 4C).

Cumulative degree of temperature increased with time to different extents of increment for different parameters. Compared to cumulative degree of temperature on 7 April, that on 28 April increased by 667%, 360%, 550%, and 200% for CDo0, CDo12, CDo20, and CDb0, respectively (Figure 4D–G).

DrTup declined with time from 4°C on 7 April to 2°C on 28 April (Figure 4H). In contrast, CDrTup increased from 4°C on 7 April to 11°C on 28 April (Figure 4I). RhT fluctuated between 19°C on 14 April and 23.5°C on 21 April (Figure 4J). DrTd increased since 2°C on 7 April to 5°C and declined to 2°C again on 28 April (Figure 4K). CDrTd in-creased with time from 2°C on 7 April to 14°C on 28 April (Figure 4L). RlT declined from -2°C to -3.6°C since 7 to 14 April, then increased up to -0.5°C on 28 April (Figure 4M).

Tree bud parameters in a false spring

Bud biomass was higher in black pine and sorbus than in apricot and chokecherry trees on 7 April 2017 ($F_{5,12}$=11.36, P=0.0003) (Figure 5). However, the difference among species disappeared on 14 April ($F_{5,12}$=4.53; P=0.0149). On 21 April, bud biomass was also greater in sorbus trees than in other trees except for that in black pine ($F_{5,12}$=24.15; P<0.0001). Bud biomass was also greater in chokecherry trees than in apricot trees on 21 April. On 28 April, bud biomass was still greater in sorbus trees than in other trees except for black pine ($F_{5,12}$=24.84; P<0.0001). Bud biomass was also greater in chokecherry trees than in apricot trees.

Soluble sugar content in buds was greater in peach trees than in other trees on 7 April ($F_{5,12}$=38.00; P<0.0001). Bud soluble sugar content was also greater in peach trees than in other trees on 14 April ($F_{5,12}$=85.57; P<0.0001) (Figure 6A). Soluble sugar content was also greater in apricot buds than in the rest of other tree species on 14 April.

Starch content in buds did not show significant difference among tree species on 7 April ($F_{5,12}$=1.40; P=0.2907) (Figure 6B). Bud starch content was higher in peach and willow trees than in other species on 14 April ($F_{5,12}$=94.35; P<0.0001). Thereafter, starch content was highest in chokecherry trees among all tree species on 21 April ($F_{5,12}$=19.50; P<0.0001).

As a result of summing soluble sugar and starch contents, bud NSC content was higher in peach trees than that in black pine, sorbus, and willow trees on 7 April ($F_{5,12}$=1.40; P=0.2907) (Figure 6C). On 14 April, bud NSC content was highest in peach trees among all species and that in willow trees was higher than the rest of tree species ($F_{5,12}$=113.88; P<0.0001). Chokecherry trees had the highest bud NSC content among all species on 21 April ($F_{5,12}$=22.87; P<0.0001).

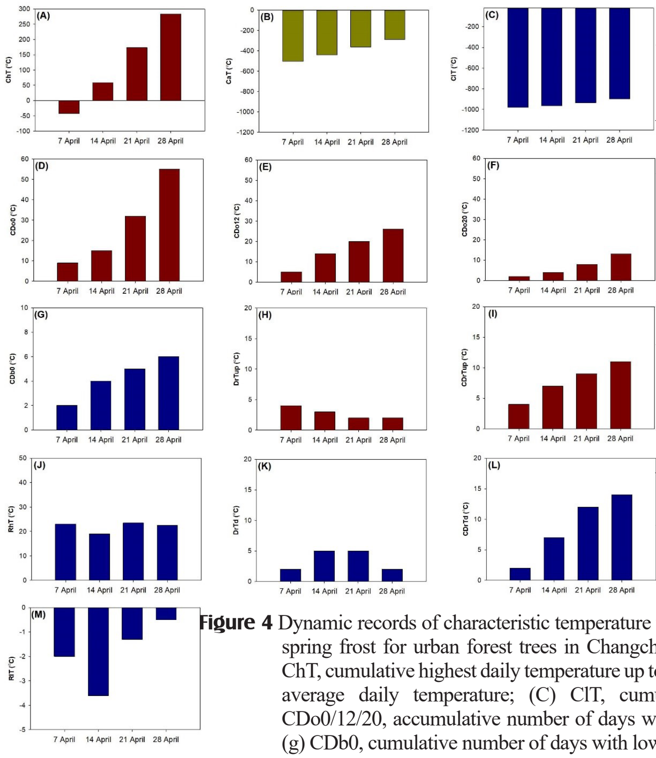

Figure 4 Dynamic records of characteristic temperature that may contribute to the impact from a late-spring frost for urban forest trees in Changchun, Northeast China. Abbreviations are: (A) ChT, cumulative highest daily temperature up to the investigating day; (B) CaT, accumulative average daily temperature; (C) ClT, cumulative lowest daily temperature; (D,E,F) CDo0/12/20, accumulative number of days with lowest daily temperature over 0/12/20°C; (g) CDb0, cumulative number of days with lowest daily temperature below 0°C; (H) DrTup, days of recent rising-up temperature prior to the investigating date; (I) CDrTup, cumulative days of recent rising-up temperature prior to the investigating date; (J) RhT, recent highest temperature prior to the investigating date; (K) DrTd, days of recent declining-down temperature prior to the investigating date; (L) CDrTd, cumulative days of recent declining-down temperature prior to the investigating date; (M) Rlt, recent lowest temperature prior to the investigating date.

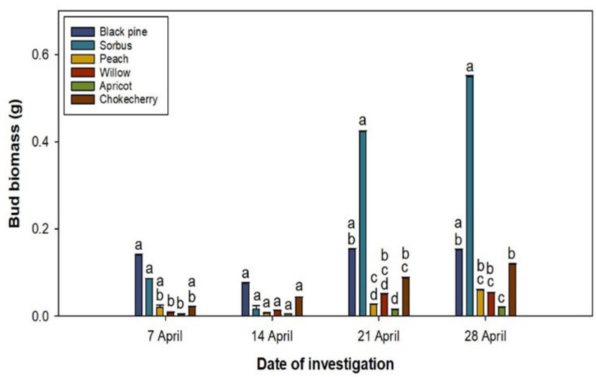

Figure 5 Repeated measures on bud biomass in black pine (*Pinus tabuliformis* var. *mukdensis*), sorbus (*Sorbus pohuashanensis* [Hance] Hedl.), peach (*Prunus persica* L. var. *persica* f. *rubro-plena* Schneid.), willow (*Salix babylonica* L.), apricot (*Prunus sibirica* L.), and chokecherry (*Padus virginiana* 'Canada Red') trees on 7th, 14th, 21st, and 28th April of 2017 at Changchun city, Northeast China. Different letters labeled on error bars (standard deviation) in one investigating date indicate significant difference at 0.0125 level (adjusted from 0.05 by the Bonferroni method due to four times of repeats) according to Tukey's honest significant difference test using log-transformed data.

RC was highest in black pine buds among all tree species on 7 April ($F_{5,12}$=27.08; P<0.0001) (Figure 7). On the same day, bud RC was also higher in sorbus and chokecherry trees than in peach, willow, and apricot trees. On 14 April, bud RC was higher in black pine trees than in other tree species except for that in sorbus ($F_{5,12}$=20.67; P<0.0001).

Bud biomass had a negative relationship with solu-ble sugar and NSC contents (Table 2). In contrast, any pairs of soluble sugars, starch, and NSC con-tents had a positive relationship with each other. RC had a negative relationship with starch and NSC contents (Table 2).

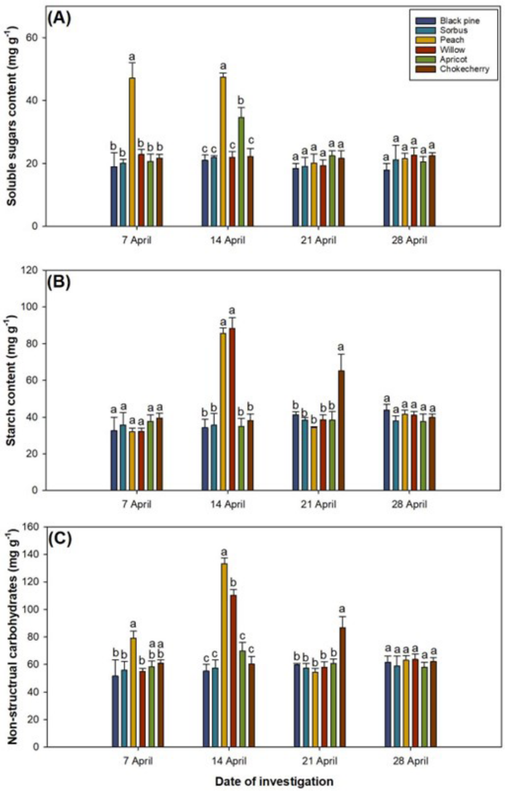

Figure 6 Repeated measures on contents of soluble sugars (A), starch (B), and non-structural carbohydrates (C) in buds of black pine (*Pinus tabuliformis* var. *mukdensis*), sorbus (*Sorbus pohuashanensis* [Hance] Hedl.), peach (*Prunus persica* L. var. *persica* f. *rubro-plena* Schneid.), willow (*Salix babylonica* L.), apricot (*Prunus sibirica* L.), and chokecherry (*Padus virginiana* 'Canada Red') trees on 7th, 14th, 21st, and 28th April of 2017 at Changchun city, Northeast China. Different letters labeled on error bars (standard deviation) in one investigating date indicate significant difference at 0.0125 level (adjusted from 0.05 by the Bonferroni method due to four times of repeats) according to Tukey's honest significant difference test.

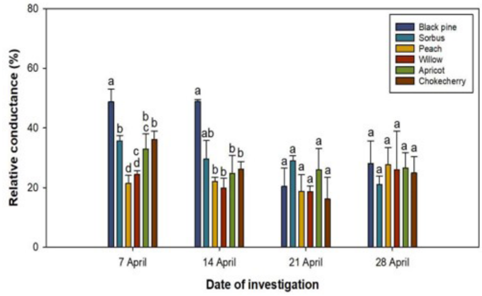

Figure 7 Repeated measures on relative conductance in buds of black pine (*Pinus tabuliformis* var. *mukdensis*), sorbus (*Sorbus pohuashanensis* [Hance] Hedl.), peach (*Prunus persica* L. var. *persica* f. *rubro-plena* Schneid.), willow (*Salix babylonica* L.), apricot (*Prunus sibirica* L.), and chokecherry (*Padus virginiana* 'Canada Red') trees on 7th, 14th, 21st, and 28th April of 2017 at Changchun city, Northeast China. Different letters labeled on error bars (standard deviation) in one investigating date indicate significant difference at 0.0125 level (adjusted from 0.05 by the Bonferroni method due to four times of repeats) according to Tukey's honest significant difference test.

Table 2 Pearson correlations between any pair among variables of biomass, soluble sugar content, starch content, non-structural carbohydrates (NSC) content, and relative conductance (RC) in buds of six species of landscape trees in Changchun city, Northeast China, across four investigating days in April of 2017 (*n*=72).

	Cf	Sugar	Starch	NSC	RC
Biomass	*R* [1]	**-0.40521** [2]	-0.17298	**-0.30776**	0.10655
	P [3]	**0.0004**	0.1462	**0.0085**	0.373
Sugar	*R*	1	**0.28772**	**0.64892**	-0.19693
	P		**0.0143**	**<.0001**	0.0973
Starch	*R*		1	**0.91539**	**-0.31439**
	P			**<.0001**	**0.0072**
NSC	*R*			1	**-0.33254**
	P				**0.0043**
RC	*R*				1
	P				

Note: [1]R, correlation coefficient; [2]Values in bold font indicate significant results; [3]P, probability of correlation. Cf: coefficient

Principal component analysis on spring temperature and bud parameters

In black pine buds, the first PC accounts for 75.95% of total variation and the second 18.37%.

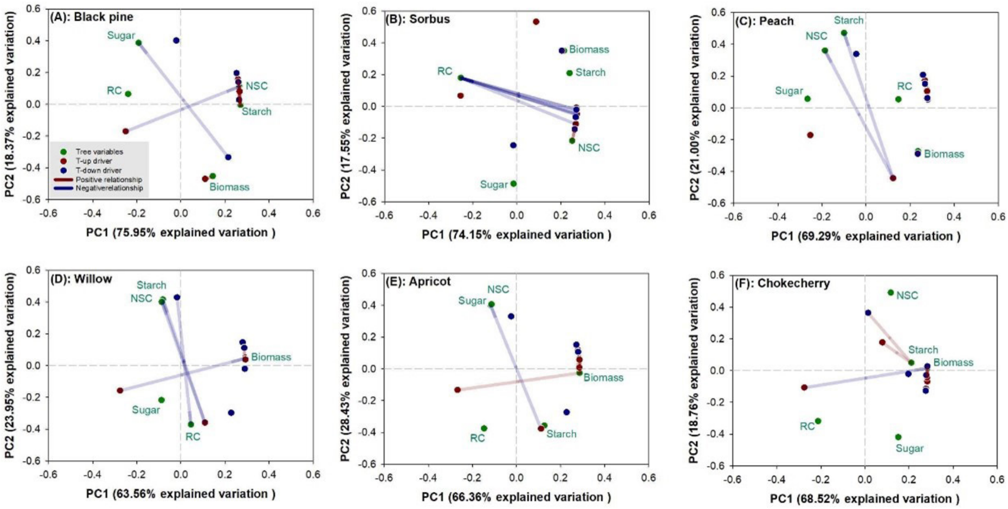

Figure 8 Interaction of eigenvalues of the first two principal components (PCs) about variables of biomass, soluble sugar content (sugar), starch content (starch), non-structural carbohydrates (NSC) content, and relative conductance (RC) in buds of black pine (*Pinus tabuliformis* var. *mukdensis*) (A), sorbus (*Sorbus pohuashanensis* [Hance] Hedl.) (B), peach (*Prunus persica* L. var. *persica* f. *rubro-plena* Schneid.) (C), willow (*Salix babylonica* L.) (D), apricot (*Prunus sibirica* L.) (E), and chokecherry (*Padus virginiana* 'Canada Red') (F) trees subjected to determinant drivers of temperature rising (Temperature up driver; dots in red) or declining (Temperature down driver; dots in blue). Bands of polygons indicate significant relationship between tree variables and temperature drivers with transparent red and blue colors as positive and negative relationships, respectively.

The first PC diverged bud parameters into contrasting groups of soluble sugar and RC as one group versus starch and NSC as the other (Figure 8A). The second PC diverged bud biomass from soluble sugar content. Both starch and NSC contents had a positive relationship with most cumulative degree of temperature parameters except for the relationship between bud starch content and CDb0 (Table S1).In sorbus buds, the first PC accounts for 74.15% of total variation and the second 17.55%. The first PC diverged bud RC from biomass and starch and NSC contents (Figure 8B). The second PC diverged soluble sugar content from biomass. Bud RC had a negative relationship with cumulative degree of temperature variables below 20°C (Table S2). RC also had a negative relationship with CDrTup. In contrast, bud NSC content had a positive relation-ship with CDb0 and CDrTup and another positive relationship was found between bud biomass and RlT.

In peach buds, the first PC accounts for 69.29% of total variation and the second 21.00%. The first PC diverged bud RC from soluble sugar content (Figure 8C). The second PC diverged bud biomass from NSC and starch contents. Both bud starch and NSC contents had a negative relationship with RhT, but bud biomass had a positive relationship with RlT (Table S3).

In willow buds, the first PC accounts for 63.56% of total variation and the second 23.95%. The first PC diverged bud biomass and soluble sugar con-tent (Figure 8D). The second PC diverged starch and NSC contents from RC in buds. Bud biomass had a positive relationship with ChT, CaT, and CDrTd, but a negative relationship with DrTup (Table S4). Both starch and NSC contents had neg-ative relationships with RhT. In addition, the rela-tionship between RC and DrTd was also negative.

In apricot buds, the first PC accounts for 66.37% of total variation and the second 28.43%. The first PC diverged bud biomass and other parameters (Figure 8E). The second PC diverged starch content and RC as one group from the other one with solu-ble sugar and NSC contents in buds. Bud biomass had a positive relationship with ChT, CaT, CDo0, CDo20, and CDrTd (Table S5). Bud starch content also had a positive relationship with RhT. In con-trast, bud starch content had a positive relationship with RhT.

In chokecherry buds, the first PC accounts for 68.52% of total variation and the second 18.76%. The first PC diverged bud biomass and RC (Figure 8F). The second PC diverged soluble sugar content from NSC contents in buds. Bud biomass had a positive relationship with ChT, CaT, CDo0, CDb0, CDrTup, and CDrTd (Table S6). In contrast, bud biomass had a negative relationship with DrTup. Bud starch content had a positive relationship with RhT and DrTd.

Discussion

In accordance with our first hypothesis, we found that the temperature fluctuation in the spring of 2017 showed typical characters of a false spring. This was partly characterized by the extraordinary increase of highest temperature on 93–95 days of year (2–4 April) in comparison with the historical records of the recently past decade. Similar methodology was used to successfully characterize the late spring frost for European beech in 2011 (Principe et al. 2017).

Although spring temperature kept increasing since March, days of continuously increasing temperature declined before each of the investigating days in April. This suggests that days with advanced increase of temperature decreased with time with a contrasting relationship with rising temperature. Therefore, the extraordinary temperature increase can be taken as an abnormal advance of warm spell that dehardened new shoots (Rodrigo 2000, Park 2016, Chmielewski et al. 2018, Vitasse et al. 2018). We also found an advanced increase of daily lowest temperature in a broad time range of 91–96 days of year (31 March to 5 April). Our results concur with previous findings that daily lowest temperature increased more than the highest temperature in an advanced warm spell (Augspurger 2013).

Daily lowest temperature was also found to be extremely higher than historical records in the 2007 false spring event in eastern USA (Gu et al. 2008). The sudden decline of daily lowest temperature to -3.6 °C (103 days of year and 12 April) was the sign of the late spring frost. The sudden decline of lowest temperature increased days of declining temperature in mid-April. According to the long-term observation, the frost conditions were equated with a lowest temperature of -1.7°C or lower (Augspurger 2013). However, the post-warming lowest temperature was also found on 100 days of year (9 April) and nearly days in early April had the historical records of lowest temperature that touched this value. Here, we reserve to give more absolute conclusions about the frost occurrence depending on daily lowest temperature. At least, we can characterize the late spring frost event in our case, during the time from 95 days of year (5 April), that occurred to a possible freeze on 103 days of year (12 April) or later.

In temperate forests, a false spring was usually characterized by temperature in March and early April and sometimes extended to early May. Most of current records agree to the temperature characters found in our study. A set of 31-year long satellite monitoring data on temperate regions of USA revealed that the first 92 days (up to 1 April) of the year was defined as the range of spring frost days (SFD) as the total number of spring frost days (Kim et al. 2014). Records of spring temperature from 1979 to 2010 in USA further indicated a standard deviation of ±18 days for SFD from mid-March to mid-April. Accordingly, a number of 31 out of 124 years from 1889 to 1992 also indicated a late spring frost occurrence in late March and early April at Trelease Woods (40°9' N, 88°10' W), Urbana, Illinois, USA in April (Augspurger 2013). A real-time monitoring on 24-ha forest in this area found out a specific late spring frost event happened on 97 days (6 April) of the year 2007 (Augspurger 2009). The 2007 false spring also generated severe effect of freeze event on forest trees in Missouri Ozark Site (38°45’ N, 92°12’ W), Ozark, Missouri (Wood & Gu 2020) and Walker Branch Watershed (35°58’ N, 84°17’ W), Oak Ridge, Tennessee (Meyers 2020). Frost happened on trees in Missouri Ozark Site and Walker Branch Watershed 98 (7 April) and 99 (8 April) days of the year 2007 (Gu et al. 2008). The occurrence of false spring in these pieces of evidence from USA generally overlapped the period of our study in spite of different latitudinal locations. In Asia, a severe spring frost ever occurred in Morioka (39°46’ N, 141°08’ E), Japan in the morning of 111 days (21 April) of the year 2011 (Awaya et al. 2009). All typical forests in Northeast USA (40°–47° N, 66°–81° W) suffered frost from 129 to 131 days of the year (9–11 May) 2010 (Hufkens et al. 2012). It was also reported that a false spring happened in Schachtenau (48°57’ N, 13°25’ E), Bayerischer wald, Germany, where 35 deciduous trees suffered frost damage on 123 (3 May) and 124 (4 May) of the year 2011 (Menzel et al. 2015). We did not record daily temperature in May; hence we have no idea whether typical frost may have happened after mid-April. Future work is suggested to extend the investigating period up to May.

As we surmised none of the tree species in this study showed apparent symptoms of frost injury in April although a false spring had been characterized by daily temperature. The use of internal carbohydrate metabolites was diverged in tree buds for biomass production and frost loss as depletion of soluble sugars and starch, respectively. The two divergences of carbohydrate metabolism had no relations with each other because biomass was not related to RC as well. However, biomass was found to have a negative relationship with soluble sugar content in buds, which concurs with previous findings and both suggest a tradeoff between soluble sugar consumption and new assimilation for biomass consumption (D'Andrea et al. 2019). Starch is a necessary substrate for inspiration that is used by trees in spring (Wei & Guo 2017). The negative relationship between starch and NSC contents in buds and RC suggests the intensive consumption of carbohydrate to counter frost (Ögren et al. 1997). As RC represents the extent of freeze injury (L’Hirondelle et al. 2006), starch depletion in buds can also be taken as a physiological consumption in response to late spring frost.

On 7 April, buds were subjected to a warm spell; thereafter on 14 April did buds come across the sudden decline of temperature that generated a frost damage. RC in black pine buds was higher than that in all broadleaf species on both days of 7 and 14 April, which, however, was not result from a relationship with any temperature characters. These findings suggest that high RC in black pine buds resulted from the nature of fast dehardening that promoted electric leakage release that was irrelevant from any perception of temperature. In addition, Black pine buds also did not show any apparent symptoms caused by frost injury at the same time. This further confirms that Black pine has resistance to the late spring frost. As days of spring temperature accumulated, NSC content increased with a synchronization of starch production and accumulation as a substance to fuel countering dehardening (Pagter & Arora 2013). However, increasing temperature further promoted the consumption of carbohydrate; subsequently cumulative days of declining temperature maintained starch accumulation and reinforced NSC reserve. The sudden decline of lowest temperature benefited a preparation of soluble sugar accumulation that can be used from starch hydrolyzation when experiencing spring frost (Charrier et al. 2018). According to these results, we cannot accept our second hypothesis.

Peach tree buds accumulated greater soluble sugar content than black pine and other broadleaf species in days of 7 and 14 April, when RC in peach tree buds was lowest among buds in all species. Neither soluble sugar nor RC in peach tree buds had any relationships with spring temperature. Hence, high sugar content in peach buds resulted from an insignificant requirement for being used to produce biomass. This can be further confirmed regarding the nature of a late phenology with soluble sugar accumulation during the time of a low demand for the break of dormancy. Late phenology of peach trees can also be characterized through the dynamic change of bud biomass, which did not show any increasing trend until 21 April. It was the increase of recent values in lowest temperature that drove the increase of bud biomass in peach trees, which concurs to findings by Scheifinger et al. (2003). Therein, it was found that the increase of lowest temperature had a greater contribution to the form of spring frost than highest temperature. Starch content in peach tree buds showed an only response to the highest temperature in close days. Low values of highest temperature close to freezing days resulted in the accumulation of starch in buds of peach trees. Dual abundances in both soluble sugar and starch together led to NSC the highest in peach trees among all species.

As another *Prunus* spp., apricot also showed high content of soluble sugars after the freeze event occurrence. Apricot bud biomass increased in the middle of April which was followed by a later phenology than black pine and other broadleaf species. During this process bud biomass of apricot trees synchronized with the dynamic of change in cumulative average to high temperature. Other studies also found that trees from Rosaceae family had a late phenology indicated by the development of budburst from late spring frost (Chmielewski et al. 2018, Vitasse et al. 2018). It was the low level of recent highest temperature in freezing days that caused the sudden increase of soluble sugar content in apricot buds on 14 April. Soluble sugars are the direct substrates that involved in the primary metabolism for assimilating demand (Ögren et al. 1997, D'Andrea et al. 2019). High temperature would simulate the use of soluble sugar for assimilation. The persistence of low temperature would mediate sugar depletion. However, the low level of recent highest temperature also limited starch accumulation in apricot buds. Overall, apricot bud developed with days of cumulative degree of temperature, but the highest temperature would impair soluble sugar reserve although no sign of bud development was apparent.

Bud biomass in willow trees also increased with the number of cumulative days of average or highest daily temperature. However, willow tree phenology was not affected by number of cumulative high-temperature days. As the number of recent temperature-increasing days was reduced with time, willow bud biomass increased by contrast. This went in parallel with cumulative days of recent temperature decline and two types of temperature changing dynamics together revealed that willow tree phenology was easily to be driven by high temperature in spring. The phenology of willow trees can be sensitive to warming temperature in early spring, but high temperature in mid-to-late spring would stimulate seed release from catkin while bud biomass was reduced accordingly (Hubalek 2016, Perry et al. 2020). On 14 April, the sudden increase of starch content in willow buds was caused by the low level of recent highest temperature during freeze. This was the reason of high level of NSC content in willow buds. In contrast, the number of days with a continuously temperature decline decreased RC in willow buds, revealing the damage of temperature decline on willow buds.

RC in sorbus buds was lower than that in black pine buds on 7 April. Sorbus bud RC was also higher than that in peach and willow trees at the same date. However, sorbus was the only species whose bud RC had no difference from that in black pine on 14 April, when the difference of bud RC disappeared between sorbus and other species. One may surmise that the freeze caused damage on sorbus buds and increased bud RC. Instead, it was the continuously accumulation of days with temperature below 12°C that reduced RC in sorbus buds. Our results reveal that sorbus buds were not responsive to spring frost because the leakage from buds naturally declined with the solar term. Thomas & Sporns (2009) also found that sorbus is the least sensitive to spring frost among deciduous species.

On 21 April, starch content in chokecherry buds suddenly increased to be the highest among all tree species. In the same day, chokecherry showed a sudden increase of catkin growth which was the result of recent high temperature and concurs with results in another study on birch trees (Jochner et al. 2013). Bud starch content in chokecherry trees also increased in synchronization with recent days of declining temperature, which increased to the maxima on 21 April. Regarding another synchronization of bud biomass with recent days of increasing temperature, chokecherry would benefit more from the time exposed to low temperature than high temperature. Since mid-April, the average daily temperature had increased to a level of about 15°C and continuously growth of high temperature would stimulate the consumption of starch in buds. Not like temperature fluctuation in early spring, the decline of late April temperature meant the mitigation from high temperature rather than decline to below freezing point. Overall, we can accept the third hypothesis.

Conclusions

In this study, a false spring in a city of Northeast China was characterized as an extraordinary warm spell of daily highest temperature and a sudden decline of lowest temperature at times of early- and mid-April, respectively. Compared to black pine, most of broadleaf species showed higher resistances in buds to late spring frost and only apricot accumulated less biomass in April. Peach reserved greater carbohydrate content in buds than black pine at the time of post-chill, while apricot and willow had greater soluble sugars and starch contents in buds, respectively. Overall, no apparent symptom of frost injury was found. Advanced warm spell brought more pronounced impact on bud burst and development through impairing carbohydrate metabolism than the sudden decline of minimum temperature. All physiological responses, however, vanished at late April. Willow was the only species that showed the tendency to suffer frost damage on buds by the amount of days with continuously decreasing temperature. The maximum daily temperature at the early time of year should be concerned in predicting extraordinary warming event in spring that may cause more severe late frost event for ornamental trees.

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