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Quantitative Characteristics of the Phases of Winter Dormancy of Conifer Species at a Site in Central Siberia.

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ABSTRACT

Traditionally the registration of seasonal changes in plant growth and development has been carried out phenologically, i.e. qualitatively using visual inspection. However since the process of plant acclimatisation to winter dormancy involves reversible biochemical and physiological changes at the level of cells, quantitative methods can be applied to determine the duration and the depth of winter dormancy in perennial plants. We used a method based on detecting thermally induced changes in the zero-level fluorescence (TICZF) on needles from four Siberian coniferous trees. Needles of *Picea obovata* and *Abies sibirica* recover from the state of winter dormancy much faster than those of *Pinus sibirica* and *Pinus sylvestris*. The photosynthetic apparatus in the needles of *A. sibirica* may be damaged during the spring period, characterized by unstable weather, when after several days of warm weather the plants prematurely recover from winter dormancy. We conclude that under conditions of climate warming tree species like *A. sibirica* may suffer from severe diebacks due to desiccation caused by premature break of winter dormancy.

Keywords: *chlorophyll fluorescence, photosynthetic pigments, abscisic acid, Picea, Abies, Pinus.*

1. Introduction

It has been recognized for a long time that winter dormancy phenomena in trees are very complex (Perry 1971). Their regulation involves a large number of phytohormones, enzymes and metabolites. Temperature is not the only factor that causes initiation and cessation of dormancy, other factors are photoperiod, nutrition, water, an array of chemicals, and shock treatments. Defining when a tree is in a dormant state is not easy (Perry 1971). This problem has become especially important for studying the effect of climate change on tree physiology. The question of the effect of a warming climate on trees has been raised a long time ago (e.g. Cannell and Smith, 1986, Hänninen 1995; Heide 2003), and the influence of global warming on trees at mid- to high latitudes is becoming more clear now (Wilmking et al. 2004; Bonan 2008; Ohse et al. 2012): for example the growing season of trees has increased by ~11 days since the 1960s (Menzel and Fabian 1999). Rapid climate changes can greatly influence tree metabolism and development, and climate-related traits of tree species, such as timing of growth and reproduction should be studied in that regard (Alberto et al., 2013)

Some studies suggest that trees in boreal regions may suffer from severe frost damage as a result of premature recovery from dormancy during mild spells in winter and early spring due to climate warming (Cannell and Smith 1986; Hänninen 1990, 1991; Kellomäki et al. 1995; Saxe et al. 2001). It is generally acknowledged that more studies are needed for addressing questions of how plants respond to warmer winter environments caused by climatic change (e.g. Hänninen, 2006; Harrington et al.

2010), since temperature in boreal and temperate regions is expected to rise (IPCC, 2013).

At the present time under conditions of changing climate there is a high likelihood of perturbations in the acclimatization mechanisms of plants, in particular in the seasonal photoperiodic reaction in autumn and in the subsequent transition into the state of winter dormancy. The increase of autumn and winter temperatures may, via the influence of various phytohormones, negatively affect the ability of trees to enter the deep state of dormancy. The trees that have not fully switched to the state of winter dormancy may resume photosynthetic activity, respiration and transpiration during outbreaks of unusually warm winter weather. This process may lead to desiccation and death of trees during the winter season (Grigoriev and Pakharkova, 2001).

In Central Siberia the forest forming conifers are Siberian fir (*Abies sibirica* Ledeb.), Siberian spruce (*Picea obovata* Ledeb.), Scots pine (*Pinus sylvestris* L.), and Siberian pine (*Pinus sibirica* Du Tour). Traditionally the registration of seasonal changes in plant growth has been carried out phenologically, i.e. qualitatively using visual inspection. For example the time of opening of buds and the flushing of leaves or needles was observed and was empirically linked with the accumulated sum of positive temperatures (Shultz, 1981).

Qualitative studies carried out in the National Reserve "Stolby" near Krasnoyarsk in spring during the years 1952-2008 revealed time shifts of seasonal changes in plant growth in the last two decades by an average of 2-15 days for various

tree species (Ovchinnikova et al. 2010). However no quantitative assessment of various phenophases of Siberian conifers has been carried out yet, including the hard-to-detect periods of autumn photoperiodic reaction and the subsequent transition into the deep state of winter dormancy.

It is well-established that during the transition from the phase of active vegetation into the phase of winter dormancy the cells of photosynthesizing parenchyma undergo a number of changes. These changes may include changes in the following: the amount and the proportions of various pigments, an increase in the fraction of unsaturated fatty acids in membranes, an increase in the concentration of mono- and oligosaccharides, the synthesis of cryoprotective proteins, an increase in the viscosity of cytoplasm and changes in the structure of the photosynthetic apparatus (Alberdi and Corcuera 1991; Öguist and Huner 2003; Maslova et al. 2009; Petrov et al. 2011). Changes in the assembly of the photosynthetic apparatus are mirrored in changes of fluorescent signals emitted at different temperatures (thermally induced fluorescence changes, see Material and Methods).

This paper has the goal of quantifying the state of winter dormancy for Siberian conifers by the thermally induced change of the chlorophyll fluorescence signal in order to assess whether under conditions of climate warming trees are more likely to prematurely break of winter dormancy and to be accordingly damaged.

2. Study Area and Experimental Methods

2.1 Study area

The National Reserve "Stolby" located right outside of the Krasnoyarsk city to the south-west was selected as the study area (55°57'N, 92°46'E). Annual dynamics of temperature for this site (Fig. 1) are characterized by a pronounced long period of negative temperatures (ca. six months) when the active functioning of woody plants is not possible.

The "Stolby" reserve is located in the temperate climatic belt and is subjected to the influence of North Atlantic winter cyclones. The multi-year data from the meteostation "Stolby" (536 meters a.s.l.) give the following average values: the mean annual temperature at the reserve is -1.2 °C. The coldest month is January, with a mean temperature of -17.6 °C and the warmest month is July, with a mean temperature of +16.2 °C. During the existence of the reserve the maximum registered temperature was +31.6 °C and the minimum temperature was -44.9 °C. On average during 147 days in a year the temperature does not drop below 0 °C. The first day when temperature drops below zero is between August 28 and October 9 (on average September 17) and the last day is between May 19 and June 12 (on average May 29). The duration of the vegetation period, when during the daytime the temperature is equal to or greater than 5 °C is 138 days. The annual amount of precipitation is 686 mm, with 31.3% during winter, 27% during summer and 22.7% during spring and 19% during autumn. A permanent snow cover exists on average 124 days per year, its

average depth is 94 cm. The maximum depth of snow cover is observed at the end of March.

2.2 Sample collection

Two-year old healthy looking needles of gymnosperms were collected during two periods: 1) September 2010 through April 2011 and 2) September 2011 through April 2012. Samples were collected from the middle part of the crown at a height of ~3.5-4 meters. For our study we employed 5 trees. The same 5 trees were used for the whole study period. The samples were collected from the same trees at the different sampling campaigns. Four sub-samples were taken from four perpendicular directions around the tree and were mixed subsequently to make one sample per tree. After taking needles from each tree we mixed needles from all five trees to make a combined sample. Three samples for measurements were taken from the combined sample (that represented all 5 trees).

The reason for taking two-year old needles rather than one-year old was that the photosynthetic apparatus of one-year old needles is not mature, i.e. it is not fully developed. Therefore fluorescence measurements on two-year needles, which are characterized by fully developed photosynthetic apparatus, are more representative.

2.3Determination of the thermally induced fluorescence changes

2.3.1 Theoretical background

The state of the photosynthetic apparatus (PSA) may be utilized as an effective indicator characterizing the physiological condition of plants as a whole. Use of the fluorescence temperature curve (Ilík et al. 1997), i.e. changes in the zero-level

fluorescence as a function of temperature, in order to evaluate the dormancy state is based on the difference in these curves in summer and winter time (Gaevsky et al. 1991). The condition of PSA may also allow to monitor various hidden changes taking place during the transition of trees in and out of winter dormancy (Gaevskiy et al. 1991). In order to study the transition of plants into the state of dormancy and the exit out of this state we applied the method based on recorded profiles of thermally induced changes in the zero-level fluorescence (TICZF).

The theoretical foundation of the method lies in the change of the aggregation of the components of the photosynthetic apparatus when temperature decreases to subzero values, which is manifested in the qualitative change of the TICZF curves. During the period of active metabolism there are two peaks observed on the graph of zero-level fluorescence versus temperature: low temperature, related to the activity of the chlorophyll-protein complex of photosystem-2 and high-temperature, related to "flaring" of the chlorophyll-protein complex of photosystem-1, as its reaction centers are deactivated (Fig. 2a). At the transition into the state of winter dormancy a qualitative change of the shape of the curve is observed: there is no longer a low-temperature maximum (Fig. 2b), which leads to the reduction of the ratio of low- and high-temperature maxima of fluorescence (i.e. coefficient R_2, see below). According to the modern view all the processes of transformation of light energy, including fluorescence, are related to the membranes of thylakoids of chloroplasts. They are composed of liquid crystals with "icebergs" of protein complexes. The speed of

capture of quanta by the reaction centers of PS-1 essentially does not depend on the condition of the reaction center (if extreme conditions are not considered). Two primary acceptors of PS-1 transfer the electron to the secondary acceptors so quickly that under ordinary conditions it is not possible to accumulate the primary receptors in reduced form. At room temperature PS-1 does not produce fluorescence, and PS-2 and the light-accumulating complex are the main fluorescence units.

It has been shown that the ability of plants to resist the influence of high temperatures is determined by the stability of their photosynthetic apparatus. The damaging influence of high temperatures is manifested in the damage of the structure and functions of the chlorophyll-protein complexes and in the change of the effectiveness of the transfer of the excitation energy between complexes (Gaevskiy et al., 1991). One of the express methods of the estimation of these phenomena is based on determining the thermally induced changes in the zero-level fluorescence (TICZF). The zero level of fluorescence is measured when acceptors of PS-2 are in the oxidized state (i.e. when they are “open”). The general view of the TICZF curve is shown in Figure 2a. Let us consider the main processes that are responsible for generating this type of a curve.

The fluorescence intensity at 40-55 °C (low temperature peak) is determined by the action of heating on the structure and functioning of PS-2 components. The functional connection between the antenna complex of chlorophyll-protein complexes and its energy trap, i.e. an open reaction center, is disturbed during heating. At this

time damage of the reaction center of PS-2 is possible. In the phase of the falling of the signal caused by heating, there is a functional and also possibly structural separation of the light-accumulating chlorophyll-protein complex of PS-2. At this time there is also a reduced efficiency of energy transfer from chlorophyll-b to chlorophyll-a. In order to estimate this phenomen the relative magnitude of the low-temperature peak can be used:

$$R_1 = (F_{LT} - F_{25})/ F_{LT}, \quad (1)$$

where F_{LT} is the fluorescence intensity at the low temperature peak and F_{25} is the fluorescence intensity at 25°C.

There is no fundamental difference for the fluorescent pigment system as to how it "loses" the energy trap due to the thermal breakdown of communication between the reaction centers of the chlorophyll-protein complex and the antenna or due to photochemical reduction of the primary electron acceptor. Therefore the relative magnitude of the low-temperature maximum can be considered as an indicator of the efficiency of energy capture excitation in the reaction centers of PS-2.

The cause of high temperature peak can be the "warm-up" of the fluorescence of the more thermally stable chlorophyll-protein complex of PS-1. Some contribution to the emergence of high-temperature peak can be given by the chlorophyll-protein complex of PS-2 if it is localized in thylakoid membranes and if there is deficit of the chlorophyll-a protein complex (Gaevskiy et al., 1989). The decrease of the quantum efficiency of PS-2 photochemistry above 30°C is due to several effects: a decrease of

the rate of charge separation, an increase of P^+I^- recombination rate constant and a decrease of the stabilization of charges (Briantais et al., 1996).

The ratio of low-temperature and high-temperature peaks (R_2) is determined according to the formula:

$$R_2 = F_{LT} / F_{HT}, \quad (2)$$

where F_{HT} is the fluorescence intensity at high-temperature peak. The R_2 parameter can be used as an indicator of the structural organization of chloroplast membranes. In addition to the parameters mentioned above the coordinates of the low and high temperature peaks on the TICZF graph give an indication of the thermal stability of the photosynthetic apparatus to the damaging effects of high temperatures. The ability to withstand temperatures below zero is a very interesting and rather little-studied property of chloroplasts in the "evergreen" tissues of trees and shrubs.

The transition of chloroplasts into a cryoresistant state (the dormancy state) is characterized by a decline of photosynthetic activity, changes in the chemical composition and the structural organization of chloroplast membranes. The characteristics of this transition vary substantially in different species, which makes it difficult to choose a reliable criterion for assessing cryoresistance of chloroplasts and its seasonal dynamics.

During the period of dormancy in plants a qualitative change in the shape of curves of thermally induced changes in the zero level of fluorescence (Fig. 2b) is observed. This probably points to the different nature of thermally induced

transitions in winter time and in summer time. The "winter" type of the TICZF curve is likely determined not by the quantitative changes of the forms of chlorophyll and of chlorophyll-protein complexes, but rather by special physical and chemical properties of the thylakoid membranes and protein complexes themselves. This assumption is also consistent with a significant reduction in the relative yield of fluorescence in winter samples (Gaevskiy et al. 1991). The kinetic characteristics, the amplitude of the rise of the high-temperature fluorescence and the complete irreversibility of changes driven by the heat changes point to a reason for the increase in fluorescence intensity.

In our earlier studies we have observed typical changes in the thermally-induced response of chlorophyll fluorescence during the periods of transition of *Pinus sylvestris* and *Picea obovata* needles into the state of winter dormancy and the transition out of it (Pakharkova et al. 2009, Pakharkova et al. 2012). It should also be noted that "winter" type of thermograms in the studied chlorophyll-containing tissues is apparently the most universal of the currently known criteria of the cryoresistance state of chloroplasts (Gaevskiy et al., 1991).

The calculated ratio of the low- and high-temperature peaks (R_2) may be used as an indicator of the degree of the depth of dormancy. For the period of winter dormancy R_2 is around 0.08, increasing to around 1.7 during the transition of plants to active metabolism (Gaevskiy et al. 1987).

2.3.2 Measurements

TICZF measurements were carried out in the laboratory within 1 hour after collecting the samples. Transporting of the shoots to the laboratory took place at negative (outdoor) temperatures. For assessing the depth of winter dormancy, needles were artificially driven out of the dormancy in winter period shoots by placing the shoots in containers with water. The shoots were kept this way for 7 days in the laboratory with a 12-hour photoperiod and a temperature of +24 °C. This temperature was maintained both during the light and the dark periods.

TICZF data were obtained using the "Photon-11" instrument (an updated version of the instrument employed in the study by Grigoriev and Pakharkova 2001). A heating rate setting of 8K per minute was used. The ratio of the intensity of the zero-level fluorescence at 50 °C and at 70 °C (coefficient R_2) was used as an indicator of the depth of the dormancy state. The height of the peak is measured relative to the lowest value of the baseline, which reflects the F_0 value at room temperature. Values of the coefficient R_2 below unity indicate that the woody plant is in the state of winter dormancy, whereas values above unity indicate that the woody plant is actively vegetating (Gaevskiy et al. 1987; Grigoriev and Pakharkova 2001). In order to characterize the properties of the photosynthetic apparatus in more detail, the dynamics of the content of photosynthetic pigments have been measured in the winter period. The amounts of chlorophyll a and chlorophyll b, as well as the total carotenoid content were determined using a spectrophotometer (SPEKOL 1300 Analytik Jena AG). The measurements were performed in acetone extracts and calculated on a dry weight basis (Lichtenthaler 1987).

2.4. Determination of the abscisic acid content of needles

As an independent indicator of the dormancy state we measured the content of abscisic acid (ABA) in needles. ABA plays the most important role in regulating the state of dormancy, since it is an inhibitor of bud growth. Growth inhibition induced by ABA is accompanied by suppression of anabolic processes and by acceleration of aging of tissues. In the phase of physiological dormancy the accumulation of ABA in conifers is a necessary factor for successfully surviving low winter temperatures (Feurtado et al. 2004; Duan et al. 2007).

The samples for ABA measurements were taken in the pre-winter period from the needle samples collected for TICZF measurements as described above. The needle samples for ABA measurements were frozen with liquid nitrogen and grounded. The content of ABA was determined using the UHPLC/mass spectrometry system Agilent 1200 (Agilent Technologies®) according to Cutler and Bonetta (2008). The data were converted to correspond to dry weight of the needles.

2.5 Statistical treatment of data

The statistical treatment of the data was carried out using the Microsoft Excel software. We used the following statistical approaches: ANOVA (two-factor with replication), T-test (two-sample assuming equal variances) and correlation.

3. Results and Discussion

The results for R_2 measurements are shown in Figure 3. When comparing the depth of winter dormancy in the investigated species by analyzing the seasonal variation curves of coefficient R_2, some regularities can be observed. All species showed a similar behavior of a gradual decrease in R_2 during autumn, a minimum until late winter and a rapid recovery in early spring. Among the studied conifer species, the earliest timing of transition into the state of dormancy ($R_2 < 1$), the greatest depth of dormancy state and the latest exit out of the dormancy state ($R_2 > 1$) were found for Scots pine. Moreover, the curve for the coefficient R_2 for Scots pine is most flattened, i.e. the depth of the dormancy state for Scots pine is the least dependent on the current temperature of the environment.

Siberian fir, on the contrary, is characterized by the shortest period of winter dormancy and the smallest dormancy depth. The depth of the dormancy state for Siberian fir depends to a large extent on the current temperature of the environment. Intermediate values are found for Siberian spruce and Siberian pine. For example, in 2010-11, the correlation coefficient between the R_2 values and air temperature was 0.70 for *Pinus sylvestris*, 0.73 for *Pinus sibirica*, 0.74 for *Picea obovata* and 0.78 for *Abies sibirica*.

Autumn of 2011 was characterized by an earlier than usual decrease of temperature. Winter of 2012 was characterized by a rather smooth temperature curve, without abrupt temperature fluctuations, which were observed in the year 2010-11.

The curves for the coefficient R_2 also have a smoother appearance with all of the studied species having been in the state of dormancy from the end of December until the end of March (Fig. 3).

In order to obtain more information on the depth of winter dormancy, we were artificially driving the shoots of the studied species out of the state of dormancy under laboratory conditions (at a temperature of +24 °C and12-hour photoperiod during the phase of forced dormancy (second half of February), using 2011 as an example). At the time of collecting samples the air temperature in the study area was about -10 °C. However, it is known that the temperature of the needles may not be exactly the same as the temperature of the ambient air. For example at sub-zero air temperatures on a sunny day the temperature of the needles and bark of the south side of the shoots can reach +5 °C or more at noon for some woody plants (Schaedle and Foot 1971).

The data presented in Figure 3 indicate that for the shoots of *Abies sibirica* the vegetation mode has already started by the middle of February. In the laboratory, in the same way as in nature, the greatest depth of dormancy is displayed by *Pinus sylvestris*, whose needles begin to vegetate on the fourth day only (Fig. 4). Intermediate values were observed for *Pinus sibirica* and *Picea obovata*, which exit out of the dormancy state after two days.

In order to characterize the photosynthetic apparatus in more detail and to assess its ability to recover photosynthetic activity we performed measurements of the dynamics of the content of photosynthetic pigments in needles (chlorophyll-a and

chlorophyll-b, total carotenoid content). Generally there is a decrease in chlorophyll content during the winter period (Fig. 5A) and an increase in the content of carotenoids (Fig. 5B). The decrease in chlorophyll content during winter was more pronounced in *A. sibirica* and *P. sylvestris* than in *P. sibirica* and *P. obovata*; recovery of chlorophyll content after winter was highest in *P. sylvestris.*

It is well-established that the photosynthetic apparatus of evergreen conifers is characterized by a complex system of protective mechanisms, which help avoiding photoinhibition in the conditions of below-zero temperatures. Carotenoids serve as an important component of this complex system. They stabilize membranes of chloroplasts and proteins of the antenna complexes. They also absorb and dissipate the energy of light that is not utilized under these circumstances (Maslova et al. 2009; Öguist and Huner 2003). The maximum carotenoid content in the needles is observed during the winter period, however during the spring period their content is quite high as well. The differences between the species of the groups of dark-needle and light-needle conifers can be followed by the differences in the content of carotenoid in needles. For example, in the needles of *Picea obovata* the content of carotenoids in December is higher by a factor of 1.25 compared to October. At the same time this factor is 1.29 for *Abies sibirica,* 1.43 for *Pinus sylvestris* and 1.57 for *Pinus sibirica*.

Carotenoid content in all species is negatively correlated with R_2 (Table 1). Chlorophyll content, by contrast, has a high positive correlation with this indicator (except *Pinus sylvestris*, which is characterized by winter "yellowing" due to

significant degradation of chlorophyll). This indicates that carotenoids may be functionally related to the depth of winter dormancy.

Processes leading to physiological dormancy as described above are regulated by abscisic acid (ABA). In the pre-winter period, the largest amount of abscisic acid was present in the needles of Scots pine and the minimum content of ABA was observed in Siberian pine and in Siberian fir (Fig. 6). Since lower levels of abscisic acid were measured for fir in the pre-winter period, this may have led this species to enter the state of forced rest earlier. This, in turn, leads to early recovery of the photosynthetic activity and physiological desiccation of fir needles during early spring outbreaks of unusually warm weather.

4. Conclusions.

We conclude that all of the tree species studied are characterized by different depths of dormancy. Dark-needle species, especially fir are characterized by a smaller depth of dormancy and a shorter duration of dormancy. Perhaps in the conditions of climatic warming, the *Abies sibirica* trees emerge from the forced dormancy even during the brief winter thaws, which previously have not been typical for the studied area. Water losses that arise due to the resumption of photosynthetic activity and gas exchange can not be compensated in the winter conditions due to frozen soils, which leads to desiccation and death of the needles.

In our view, one possible reason contributing to the dying of fir forests, which is noted since the last century in many parts of Europe and Russia, can be the disturbance of the normal process of going through the winter dormancy phase due to changing climatic conditions.

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TABLES

Table 1: Correlation coefficient of pigments and R_2

Species	Chlorophyll	Carotenoids
Pinus sylvestris	0,405	-0,830
Pinus sibirica	0,999	-0,981
Picea obovata	0,988	-0,774
Abies sibirica	0,999	-0,844

FIGURE CAPTIONS

Figure 1.Seasonal profile of temperature for the time period from 1927 until 2012 based on the data collected at the meteostation “Stolby”.

Figure 2. Thermally induced changes of the zero-level of fluorescence in chlorophyll-containing tissues in the phase of active vegetation (a) and in the state of winter dormancy (b).

Figure 3. Dynamics of the coefficient R_2 from TICZF curves in needles from 4 Siberian coniferous trees under natural conditions. Temperatures on the days when needle samples were collected are shown for reference (data from meteostation “Stolby”). Error bars show the standard deviation (n = 3). Significant differences ($P<0.01$) are found for all species.

Figure 4. Dynamics of the coefficient R_2 from TICZF curves when needles were artificially driven out of the dormancy state in the laboratory in February 2011. Error bars show the standard deviation (n = 3). Significant differences ($P<0.05$) are found for all species.

Figure5.Content of pigments in needles (a – sum of chlorophyll-a and chlorophyll-b, b – carotenoids; dry weight basis) during the winter half. Error bars show the standard deviation (n = 3). Significant differences ($P<0.05$) are found for all species.

Figure 6. Content of abscisic acid (ABA) in needles (dry weight) during the pre-winter period. Error bars show the standard deviation (n = 3). Significant differences ($P<0.01$) are found for all species.

Figure 1.

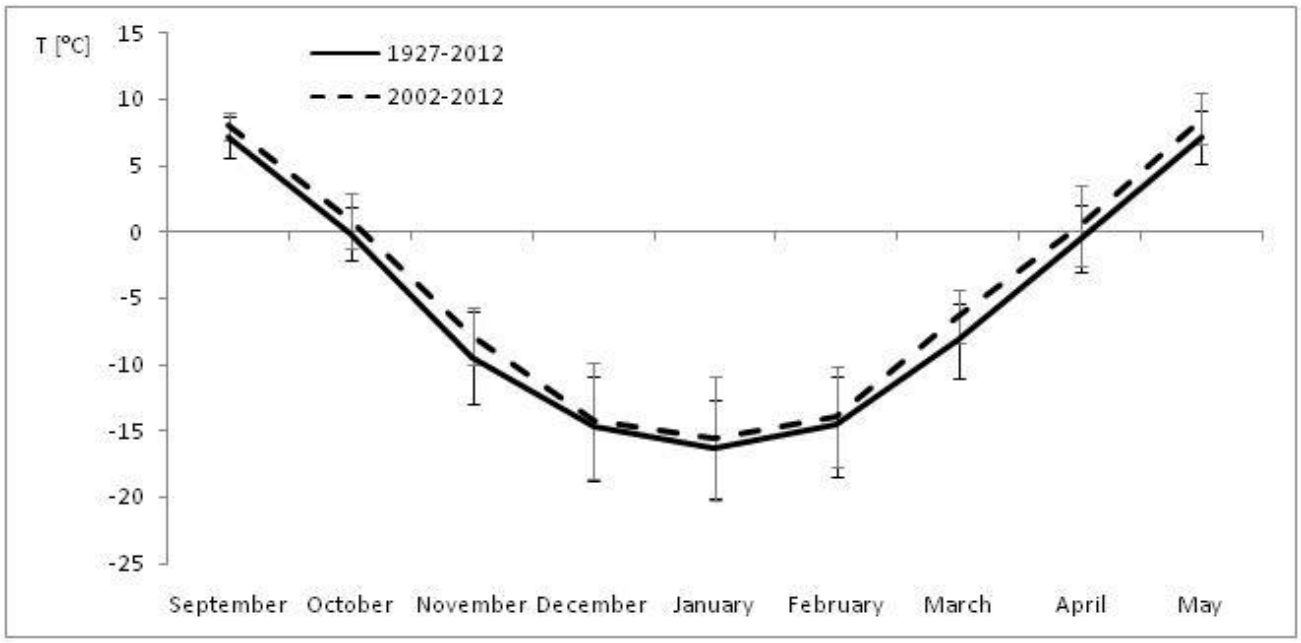


Figure 2.

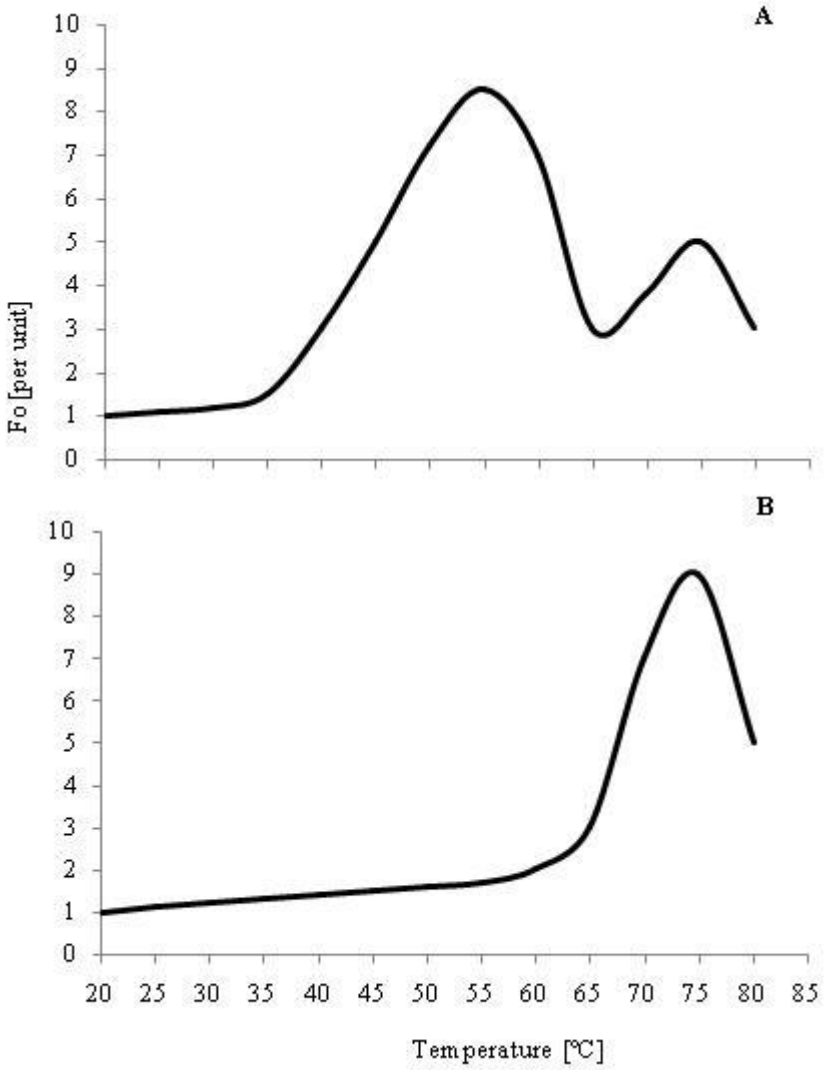

A
B
Fo [per unit]
0
1
2
3
4
5
6
7
8
9
10
20
25
30
35
40
45
50
55
60
65
70
75
80
85
Temperature [°C]

Figure 3.

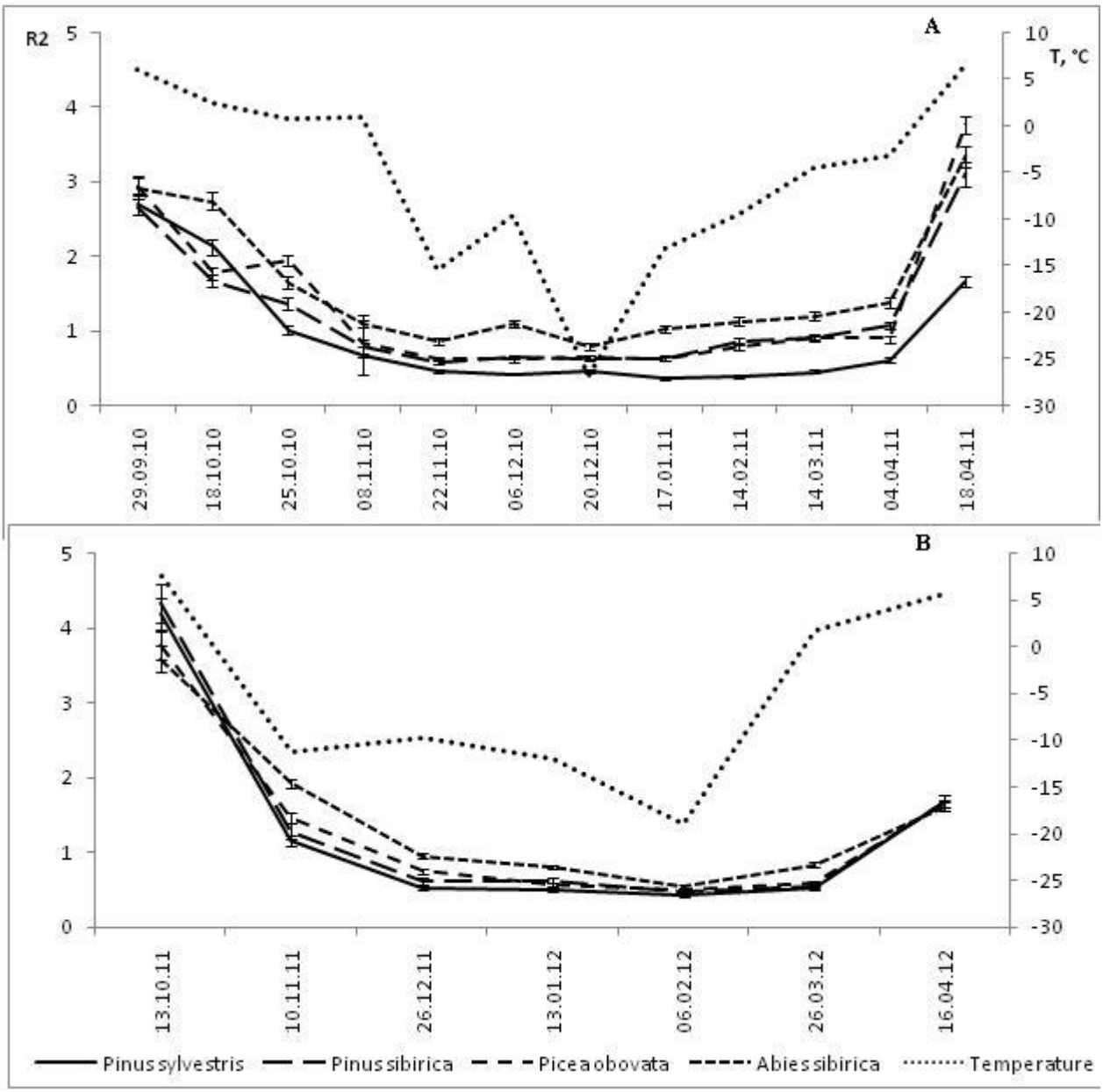

A
R2
T, °C
29.09.10
18.10.10
25.10.10
08.11.10
22.11.10
06.12.10
20.12.10
17.01.11
14.02.11
14.03.11
04.04.11
18.04.11
B
13.10.11
10.11.11
26.12.11
13.01.12
06.02.12
26.03.12
16.04.12
Pinus sylvestris
Pinus sibirica
Picea obovata
Abies sibirica
Temperature

Figure 4.

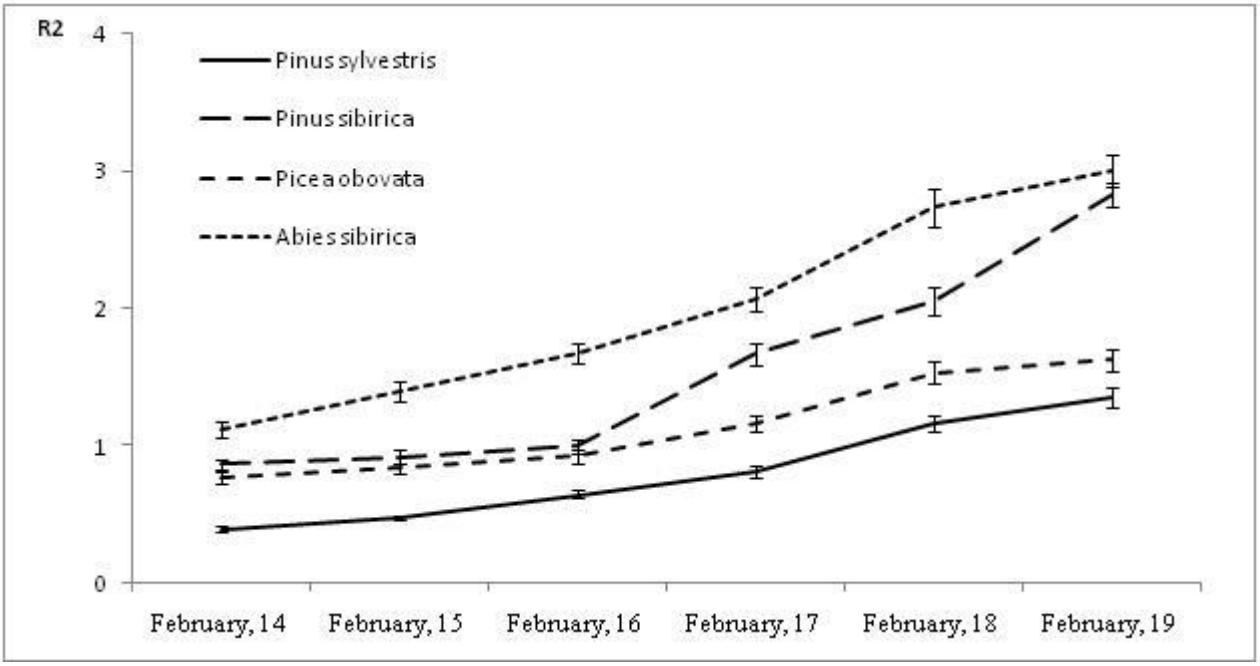

R2
4
3
2
1
0
Pinus sylvestris
Pinus sibirica
Picea obovata
Abies sibirica
February, 14
February, 15
February, 16
February, 17
February, 18
February, 19

Figure 5.

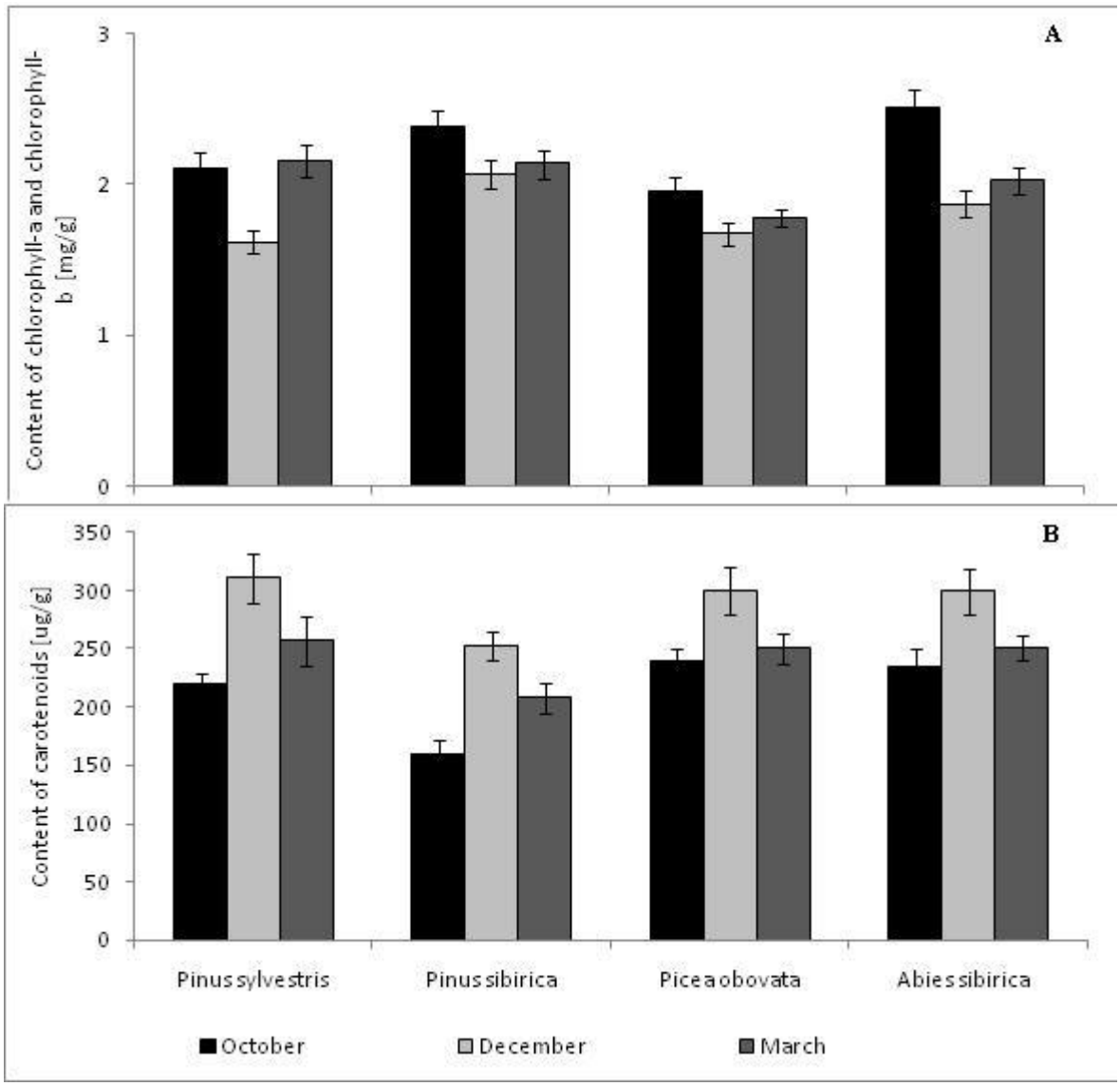
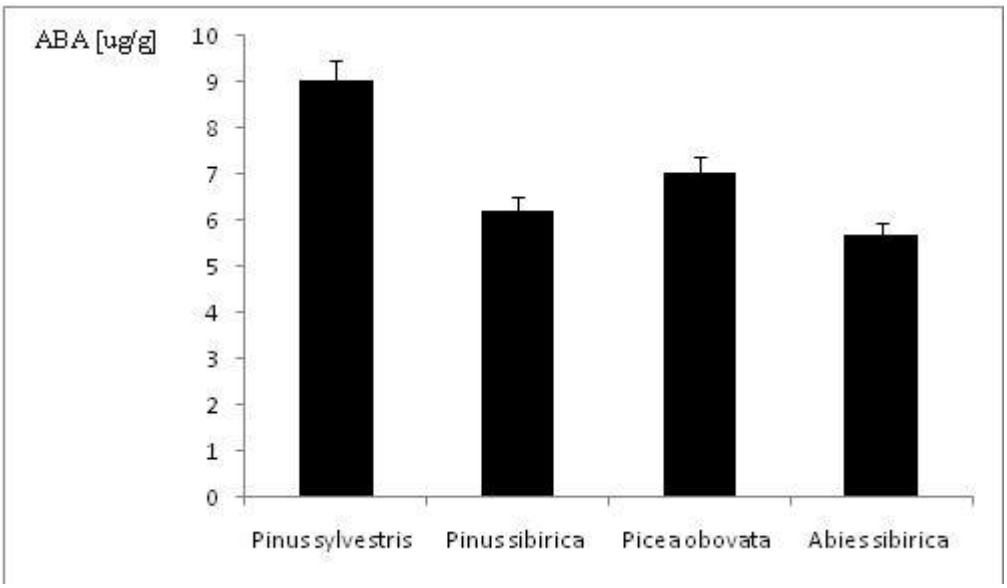

A
Content of chlorophyll-a and chlorophyll-b [mg/g]
3
2
1
0
B
Content of carotenoids [ug/g]
350
300
250
200
150
100
50
0
Pinus sylvestris
Pinus sibirica
Picea obovata
Abies sibirica
October
December
March

Figure 6.


ABA [ug/g]
10
9
8
7
6
5
4
3
2
1
0
Pinus sylvestris
Pinus sibirica
Picea obovata
Abies sibirica