

RESEARCH PAPERS

Seasonal Changes in Chlorophyll and Carotenoid Content in Needles of Scots Pines (*Pinus sylvestris* L.) Exposed to the Thermal Field of a Gas Flare

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Received June 25, 2020; revised July 2, 2020; accepted July 2, 2020

Abstract—Seasonal changes in chlorophyll (Chl) and carotenoid (Car) content were analyzed in needle samples from young Scots pines (*Pinus sylvestris* L.) growing in the thermal field zone at various distances from the petroleum gas flare. Experiments were performed in Khanty Mansi Autonomous Area (Yugra) of Russia throughout the autumn–winter–spring period. Two hypotheses were subject to verification: (1) the thermal field of petroleum gas combustion flame imitates the influence of climate warming on plants, thus affecting the pigment complex of the photosynthetic apparatus (PSA) in pine needles; (2) transformations of PSA pigment complex in pine needles throughout the autumn–winter–spring period are sensitive to a long-term rise in ambient temperature by 1–2°C. In the winter period, the seasonal dynamics of certain PSA parameters comprised maxima and minima that are supposedly due to the regulation of Chl and Car content upon changes in air temperature. In trees growing under divergent thermal conditions, seasonal changes of these parameters were not synchronous. Analysis of seasonal changes revealed that winter-related transformations of the PSA structural–functional condition in needles are subject to consistent variations at different distances from the gas flare. The PSA activity of needles throughout the studied period was higher near the flame and decreased with the distance from the gas flare. When Chl content in needles decreased in autumn–winter, the smallest reduction was observed in the location near the gas flare (site I) where temperature was 1–2°C higher than the background level. The intermediate decrease in pigment content was noted at a moderate distance from the flame (site III), while the most pronounced decrease occurred at the largest distance from the gas flare (site VII). At the same time, the Chl *a/b* content ratio in needles was consistently lower for trees located at site I than at site VII. Hence, the amount of light-harvesting complexes in chloroplasts from trees grown at site I was higher than the amount of photosystems in the same chloroplasts. The Chl (*a + b*)/Car ratio in needles of trees grown near the gas flare (site I) was higher than in locations III and VII. This ratio displayed two significant peaks in the winter dynamics, which was supposedly due to the sufficient preservation of green pigments. Divergent seasonal changes in Chl and Car content and their dissimilar correlations with air temperature indicate that the pools of these pigments are controlled by different mechanisms. The actual Chl content, determined by the balance of pigment degradation and synthesis, should depend on the environmentally modified Chl synthesis and on the protective function of Car. Photosynthesizing cells produce additional amounts of carotenoids during autumn–winter, thus preventing the photodegradation of Chl in the period from autumn to late spring.

Keywords: *Pinus sylvestris*, chlorophyll, carotenoids, effect of temperature, effect of climatic changes, seasonal changes of pigment apparatus in needles

DOI: 10.1134/S1021443721020187

INTRODUCTION

The structural–functional organization of the pigment apparatus in leaves is subject to seasonal dynamics that reflects the adaptation of the photosynthetic apparatus (PSA) to variable environmental condi-

tions. The mechanisms of PSA seasonal transformations and their relation to primary reactions of photosynthetic energy conversion are insufficiently understood. The study of these processes in evergreen coniferous plants inhabiting climatic zones with cold winters revealed several regular features. Two main stages of the annual cycle include the decline in chlorophyll *a* and *b* (Chl *a* and Chl *b*) content in the autumn–winter period and the restoration of these

Abbreviations: Car—carotenoids; Chl *a*—chlorophyll *a*; Chl *b*—chlorophyll *b*; Chl (*a + b*)—total content of chlorophyll *a* and *b*; LHC—light-harvesting complex; PSI—photosystem I; PSII—photosystem II; PSA—photosynthetic apparatus.

pigment pools upon the resumption of active vegetative growth in spring and summer [1–4]. In the Middle Urals, the highest Chl content in 2-year-old needles of young pine trees (*Pinus sylvestris* L.) is attained in summer. The content of this pigment decreases in September–February, with the decline extending to March–April, and recovers in May [1]. The PSA of conifers grown at northern latitudes is characterized by the predominance of Chl bound to light-harvesting complexes (LHC) and by a comparatively low content of Chl associated with photosystems [4, 5]. This dominance, related to high light-absorbing capacity of needles, compensates for the low level of green pigments accumulated under these conditions.

The content of carotenoids (Car) in needles of some spruce, fir, and pine species increases in winter and decreases in spring [2, 4–8]. In the majority of coniferous species, the ratio of total Chl to Car content decreases in winter, supposedly, due to the protective function of carotenoids. These pigments protect the assimilatory apparatus from photodynamic destruction at low temperatures by virtue of quenching singlet oxygen, by capturing and dissipating the excitation energy of chlorophyll molecules, and by stabilizing the physical state of membranes and antenna complex proteins [9, 10]. The role of carotenoids apparently increases when physiological and biochemical processes in needles are retarded at low temperatures. In coniferous plant species, the photoprotection during winter–spring period is mainly based on zeaxanthin-dependent thermal dissipation of excitation energy in the reaction centers of photosystem II (PSII) [2, 11].

Qualitative and quantitative changes of chloroplast pigments in conifer needles during transition to winter dormancy are accompanied by structural rearrangements in the cells of photosynthetic tissues. The granal stacks disintegrate gradually, the number of thylakoids in chloroplasts decreases, the vacuoles become smaller and undergo fragmentation, the plastids are relocated within the cell, and the transformation of membrane structures takes place [12–14]. In the needles of Siberian spruce (*Picea obovata* Ledeb.) and blue spruce (*Picea pungens* Engelm.), starch grains disappear from mesophyll cells, and chloroplasts change their shape and location [15]. Winter conditions lead to the inhibition of PSII photochemical activity, suppression of noncyclic electron transport in chloroplasts [16, 17], partial destruction of chlorophyll–protein LHC [18], and changes in the lipid composition of chloroplast membranes [19, 20]. In addition to these events, the needles of Scots pine showed successive changes in temperature-dependent fluorescence yield of Chl *a* under fully opened PSII reaction centers [1, 21] as well as changes in intensity and induction kinetics of the millisecond component of Chl *a* delayed fluorescence arising in PSII [22]. In the Middle Urals, the decreased photochemical activity of PSA extends over the entire winter period, and the lowest activity was observed in February.

The temperature regime is one of the main environmental factors affecting the pigment apparatus [23–25], the carbon sequestration rate, and the productivity of forest ecosystems in the boreal zone. Because of the global climate changes, ambient temperature in some regions rose by 1–2°C over several decades (<https://unstats.un.org/sdgs/report/2019/The-Sustainable-Development-Goals-Report-2019.pdf>). The average annual air temperature in the Russian Federation increased at a rate of 0.47°C per 10 years in the period from 1976 to 2018 [26]. Exploring the impact of climatic changes on intact natural objects encounters significant technical and methodological problems. A promising approach to studying the influence of climate warming on plants is to analyze the condition of trees growing in the minimally disturbed natural environment within the thermal field of a long-operating petroleum gas flare [27]. Observations in areas of the thermal influence of a gas flare provide information on the complex adaptive responses occurring in PSA of plant leaves upon warming of the environment.

While elaborating the design of our research, we put forward two working hypotheses. (1) The thermal field of petroleum gas flare imitates the effect of climate warming on plants, thus affecting the PSA pigment complex in needles of Scots pine (one of the main forest-forming species in the northern taiga subzone of the taiga zone). (2) Transformation of PSA pigment complex in pine needles during the autumn–winter–spring period is sensitive to a long-term increase in ambient temperature by 1–2°C. To test hypothesis 1, we attempted to reveal differential seasonal dynamics of PSA pigment complex in needles of Scots pine trees growing under divergent thermal regimes (at different distances from the gas flare). At the largest examined distance from the flame, the environmental temperature was identical to that of the undisturbed environment. To verify hypothesis 2 using seasonal dynamics patterns, we searched for consistent differences in winter-related transformations of the structural–functional condition of PSA in pines grown at different distances from the gas flare. In accordance with the above hypotheses, this work aimed at comparative analysis of seasonal changes in the content of chlorophylls and carotenoids in the needles of Scots pine trees growing at various distances from the petroleum gas flare in the zone of its thermal field during the autumn–winter–spring period.

MATERIALS AND METHODS

The study was performed on the area of Pokachi district forestry of the Megion forestry of Khanty-Mansi Autonomous Area (Yugra). The climate of Khanty-Mansi region is continental. The average annual air temperature in the area of our studies was 3.6°C over the past 10 years. The coldest month is January (average temperature of –22°C) and the warmest month is July (average temperature of 16.9°C). During

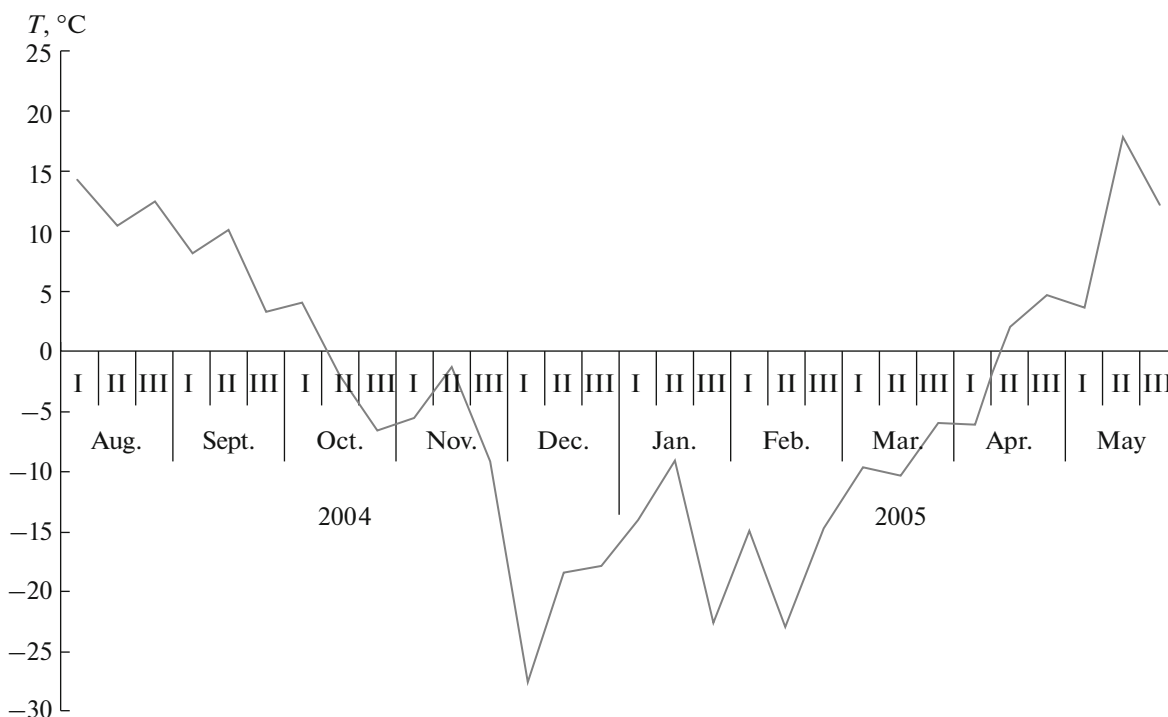


Fig. 1. 10-day average air temperatures during the research period. Data are based on the archives of Khanty-Mansiysk weather station (Weather services: weather archive, <http://pogoda-service.ru>).

winter period, significant changes were observed in average daily temperatures, as calculated by averaging data points over 10-day intervals (Fig. 1). The frost-free period lasts for 98 days, and the growing season continues for 131 days. The average annual precipitation is 492 mm, with the highest level in July–September (239 mm). The area remains snow-covered for 201 days, and the largest height of snow cover (77–85 cm) is observed in the fourth week of March.

A sample plot was set up according to standard techniques in locations of young Scots pine (*Pinus sylvestris* L.) stands growing near the gas flame of the fourth booster pumping station of the Pokachi oil field. The naturally regenerated tree stand grew in the place of the formerly cut mature pine forest. The experimental plot was divided into seven subplots (sites I–VII). All subplots were 10 m wide and 60 m long and were oriented perpendicular to the radius from the gas flare. The inner borders of sites I, III, and VII were located at distances of 70, 90, and 130 m from the gas flare, respectively. The aggregation of trees under study was described as pine–lichen forest, single-storey pure stand (10 Pine) aging 12–15 years; it belonged to the quality classes III–IV; the stand density was 2900–6400 trees per 1 ha; average tree diameters ranged from 2.5 to 5.6 cm and the average heights were 1.3–2.3 m in various subplots [27].

Ambient temperature at site VII corresponded to the natural thermal background. Owing to the thermal field of the gas flare, air temperatures at sites I and VII

differed by approximately 1.5°C at a height of 1.3 m from the ground; the difference in soil temperatures between these locations was as high as 6°C [28]. These values are typical for the general configuration of the thermal field. Because of the mosaic microrelief and heterogeneity of vegetation on each site, the temperatures varied in different locations and underwent changes throughout the day–night cycle and over the growing period. Since the increasing trend in temperature with approaching the gas flare is reliable, we studied the effect of the thermal field by establishing subplots located at different distances from the gas flare.

We examined the pigment apparatus of needles that were formed in the previous growth year. Samples of needles were taken in the period from August 15, 2004, to May 30, 2005, at a height of 1.5 m once or twice a month from the lateral branches in the middle part of the crown. Branches facing the gas flare were selected on five representative trees (trees of medium diameter and height) grown at sites I, III, and VII. The samples were stored for 24–26 h in a thermostat where temperature was kept at the level of air temperature at sampling time. Because of the large volumes of sampled materials, the needles remaining on branches inevitably changed their condition after defoliation, but this influence was minimized since needles were evenly sampled from different parts of the branches. The averaged sample method was used for analyses. Each measurement was carried out in three replicates. Pigments were extracted with 80% acetone from the homogenate of needles at 4–6°C. The pigment con-

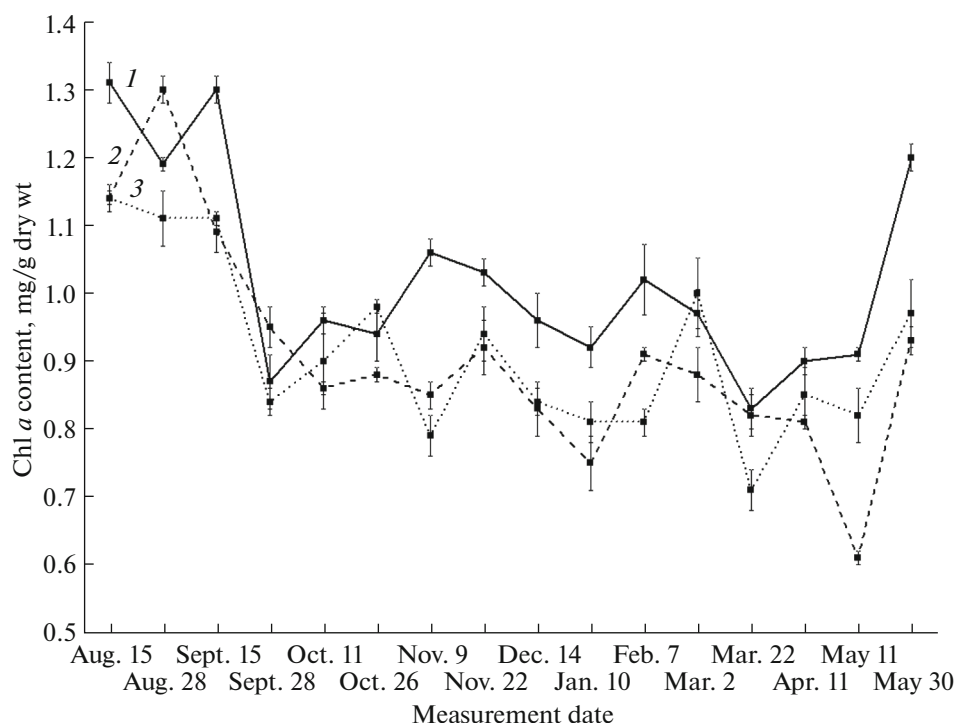


Fig. 2. Seasonal dynamics of Chl *a* content in *Pinus sylvestris* L. needles on research sites I (1), III (2), and VII (3).

content was determined by measuring the absorbance of filtered extracts with an SF-46 spectrophotometer (LOMO, Russia) at wavelengths of 470, 646.8, and 663.2 nm [29]. The pigment content was expressed per unit weight of completely dried needles. Proportions of LHC, PSI, and PSII content in chloroplasts was assessed by using the Chl *a/b* ratio. The pigment apparatus was also characterized by the ratio of total chlorophyll to carotenoid content (Chl (*a + b*)/Car).

The results were statistically treated using Statistica 8.0 (StatSoft Inc., 2007) and Excel (Microsoft, 2007) standard software packages. Correlation and regression analyses were applied. The graphs for the dynamics of measured and calculated parameters show mean values and their standard errors. Correlations between seasonal dynamics of the pigment parameters and changes in air temperature were revealed using 10-day average temperatures. When the sampling was performed at the beginning of the 10-day interval, we used temperature data for the previous 10-day period.

RESULTS

Seasonal changes of Chl *a* content in needles (Fig. 2) comprise three main stages. The first stage (from mid-August to mid-September) is marked by the highest Chl *a* content. The second stage starts at the end of September as a sharp decrease in Chl *a* content and continues until the first 10 days of May. By the end of the second stage, the Chl *a* content reduced to the

lowest relatively stable level; the Chl *a* content in pine needles in this period was identical at sites III and VII, while it was significantly higher at site I. The Chl *a* content at site I exhibited two peaks (in November and February) that remained below the summer levels. The third stage begins at the end of May; the Chl *a* content in needles increases during this stage to the values of August at site I and tended to increase to summer values at sites III and VII. The seasonal patterns of Chl *a* in trees from different sites were discernibly similar despite different absolute values. This similarity was substantiated by high pairwise correlations for trees from different sites ($r = 0.74\text{--}0.81$, $P < 0.05$). The seasonal changes in Chl *a* content at all locations correlated with the dynamics of air temperature ($r = 0.58\text{--}0.67$, $P < 0.05$).

Seasonal changes in Chl *b* content in needles (Fig. 3) were largely similar to the dynamics of Chl *a* in terms of timing of various stages. Throughout the observation period, the Chl *b* content was relatively high at site I, low at site VII, and displayed intermediate values at site III (in the latter case, the values sometimes approached those at site VII). Unlike Chl *a* pattern, the Chl *b* content in location I was subject to larger recovery at the second stage. The Chl *b* content during winter peaks reached the records typical of August. The seasonal changes in Chl *b* content at site III occupied an intermediate position between those at sites I and VII. This observation is consistent with the results of correlation analysis that revealed no significant correlation between Chl *b* dynamics at sites I and III, in

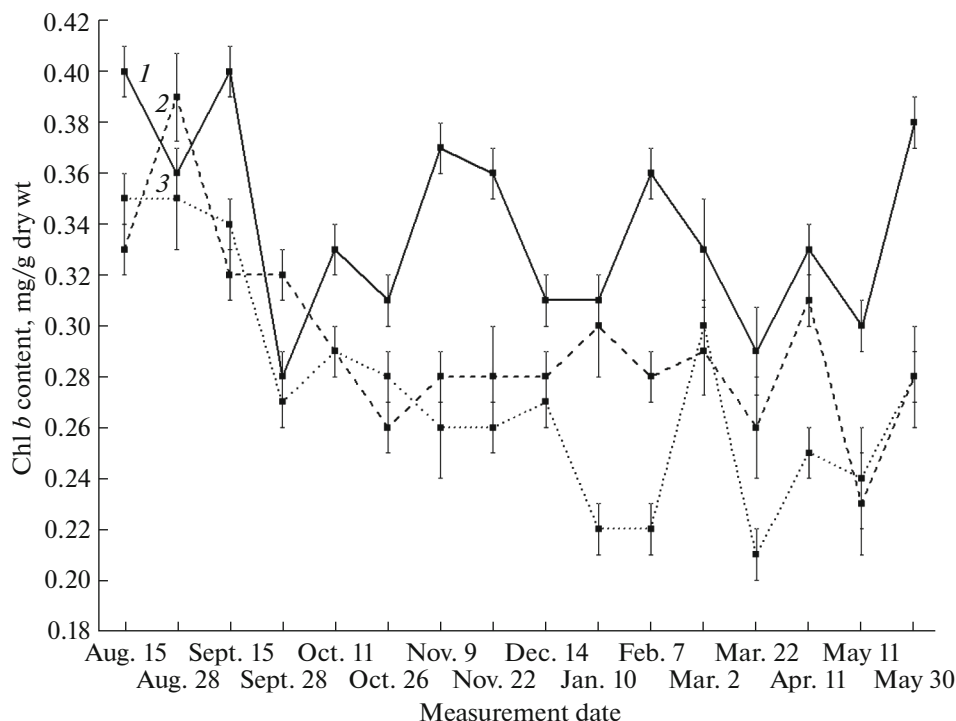


Fig. 3. Seasonal dynamics of Chl *b* in *Pinus sylvestris* L. needles on research sites I (1), III (2), and VII (3).

contrast to appreciable correlations between sites I and VII ($r = 0.59$, $P < 0.05$), as well as between sites III and VII ($r = 0.67$, $P < 0.05$). It should be noted that the coefficients of correlation between Chl *b* content in needles and air temperature were nearly equal for sites I and III ($r = 0.5$, $r = 0.45$, $P < 0.05$), whereas the correlation coefficient was slightly higher at site VII ($r = 0.69$, $P < 0.05$).

We analyzed seasonal changes in Chl *a/b* ratio (Fig. 4) with emphasis on three stages of PSA transformation in needles and found deviating patterns of this ratio in different locations. The Chl *a/b* ratios changed most smoothly (without large variations) at site I. The first stage (from August to mid-September) in this location was characterized by stable Chl *a/b* ratio with its subsequent decrease (until mid-October). Thereafter, the second stage began at which the Chl *a/b* ratio in needles was persistently low. During the third stage in May, the Chl *a/b* ratio recovered to summer values. The Chl *a/b* ratios at site VII were less stable throughout the observation period, although a weak positive linear trend was noted. For example, in the period from December 14 to January 10, the Chl *a/b* ratio in needles increased sharply (by 20% with respect to the value recorded in December). Variations of Chl *a/b* ratio at site III were larger, and the linear trend of its values was negative. During winter, the Chl *a/b* values at site III usually occupied an intermediate position between the respective values at sites I and VII. During the third stage in May, the Chl *a/b* ratio recovered to the level typical of August. It should be noted that the

lack of substantial similarity between seasonal dynamics of Chl *a/b* ratio in pine needles from different sites is supported by the absence of reliable pairwise correlations between them. Sufficiently high correlation between Chl *a/b* ratio and air temperature ($r = 0.65$, $P < 0.05$) was observed for site I located at the closest distance to the flare, but the correlation between these parameters was substantially weaker for sites III and VII ($r = 0.43$ and 0.3 , respectively, $P < 0.05$).

The Car content in needles increased in September (Fig. 5). Later, the Car content stabilized at sites I and III until the beginning of February and remained at a constant level until mid-May. Thereafter, Car content decreased at the end of May to the level observed in August.

The Car content in needles from site VII varied throughout the winter and decreased in May like in samples from other sites. Throughout the examined period, the Car content in needles from site I located at the closest distance to the gas flare was generally higher than in the most distant location VII. At the same time, the seasonal dynamics of Car content at site VII correlated with that at sites I and III ($r = 0.71$ and 0.64 , respectively; $P < 0.05$); however, no pairwise correlation between sites I and III was observed. It should be noted that the Car content in pine needles did not correlate with air temperature at any location.

In the seasonal dynamics of Chl (*a + b*)/Car ratio (Fig. 6), three stages were evident, and they were similar to Chl *a* and Chl *b* patterns (decrease—relative stabiliza-

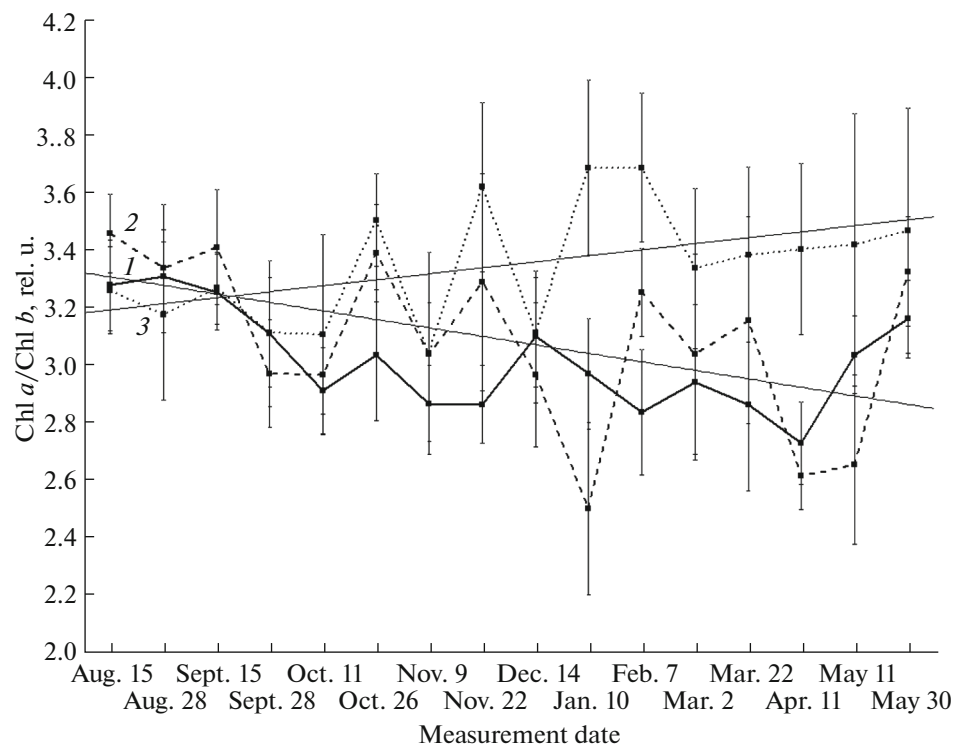


Fig. 4. Seasonal dynamics of Chl *a/b* content ratio in *Pinus sylvestris* L. needles on research sites I (1) , III (2) , and VII (3).

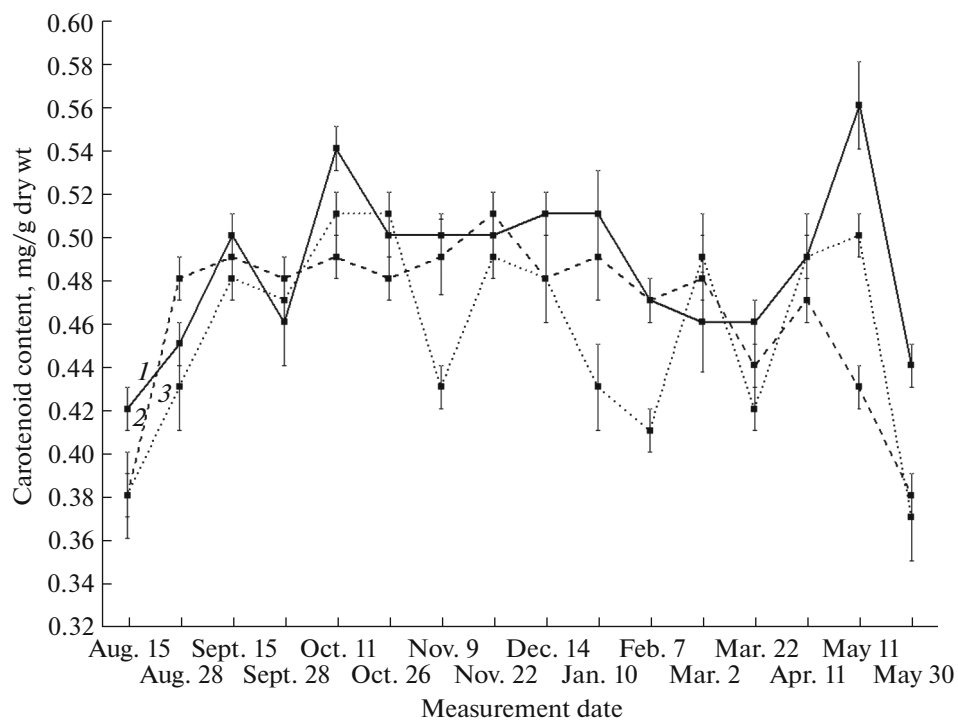


Fig. 5. Seasonal dynamics of carotenoid content in *Pinus sylvestris* L. needles on research sites I (1) , III (2) , and VII (3).

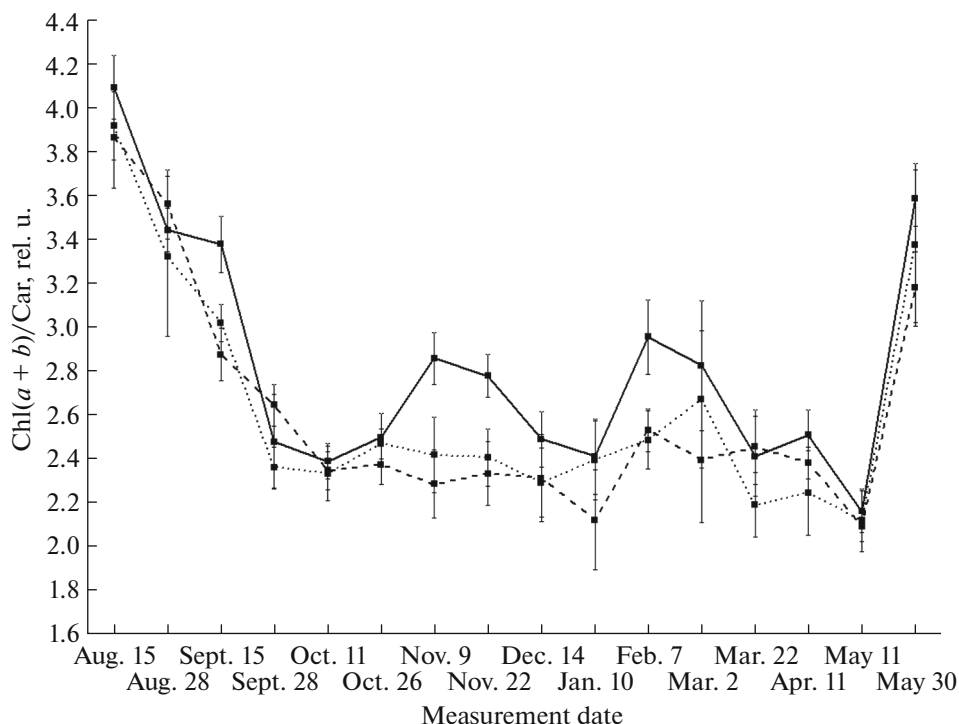


Fig. 6. Seasonal dynamics of Chl ($a + b$)/Car ratio in *Pinus sylvestris* L. needles on research sites I (1) , III (2) , and VII (3).

tion—increase). At the same time, the Chl ($a + b$)/Car ratio decreased significantly at all examined sites during August–September, after which it remained at a relatively stable low level until the first 10 days of May. By the end of May, the Chl ($a + b$)/Car ratio tended to recover to the level typical of August. It should be noted that significant increases in Chl ($a + b$)/Car ratio were observed in November and February at the closest distance to the gas flare (site I), and these increases were due to the accumulation of Chl b on the background of invariable carotenoid pool. The Chl ($a + b$)/Car ratios for sites III and VII varied insignificantly during winter. For all observation sites, the lowest Chl ($a + b$)/Car ratios were achieved at the beginning of May. High values of linear pairwise correlations for the seasonal dynamics of Chl ($a + b$)/Car ratios at all three sites—I, III, and VII—are particularly worth noting ($r = 0.91–0.96$, $P < 0.05$) as well as a significant correlation between Chl ($a + b$)/Car ratio and air temperature ($r = 0.6, 0.69$, and 0.66 respectively; $P < 0.05$).

DISCUSSION

Analysis of seasonal changes in Chl a and Chl b content in pine needles showed that these changes were generally similar in all observation sites (the highest Chl content was attained in the last month of summer and its lowest level was observed in winter) in consistency with the published data [1]. The Chl a and Chl b content recovered partly at the end of May, and this recovery was most pronounced at site I located near the gas

flare. The highest Chl content in pine needles, coincident with the active state of PSA, occurred at the end of the growing season and beginning of the next year's growth season. The Chl content in needles decreased consistently in autumn, which was due to lowered temperatures, transition of trees to winter dormancy, and inhibition of biochemical processes. The release of winter dormancy began in spring after the local minimum in Chl content was attained at the end of March.

The current knowledge on sequential seasonal changes in the pigment complex of needles, on structural rearrangements of PSA at the cell and the chloroplast levels, and modulation of Chl a fluorescence parameters in the autumn–winter period indicates the likely occurrence in conifer needles of active low-temperature adaptive events, including pigment biosynthesis. Winter temperatures in the investigated locations were not constant (deviations from average values by $10–15^{\circ}\text{C}$ and even larger were noted in some months), and such temperature changes might affect the physiological condition of PSA. This influence is indicated by correlations between seasonal dynamics for the majority of PSA parameters in needles and ambient temperature. Further evidence came from the existence of transient rises and drops of these parameters, even in the middle of winter. The content of green pigments in pine needles near the gas flare (site I) was, on average, comparatively high during the entire studied period and decreased with the distance from the flare. This feature is the main evidence in favor of our

first hypothesis. Moreover, a statistically significant partial recovery of Chl *a*, *b* pools in needles was observed in winter at site I. This proves an elevated PSA activity in needles at site I compared to other locations and, accordingly, a significant influence of the gas flare on the condition of the pigment complex. The highest Chl *b* content in needles at location I, its lowest level at location VII, and intermediate values at location III in winter suggest that (1) the effect of the flame extends to a distance of at least 100 m and (2) the flame influence at site III is weaker than at site I. The observed transient rises and drops in the seasonal dynamics of Chl *a*, *b* content in pine needles at site I and the less pronounced reliable changes of these parameters at sites III and VII provide evidence for the ongoing seasonal changes in the pigment complex throughout the winter.

At pine stand locations remote from the gas flare, the average Chl *a/b* ratios in needles were higher than at site I positioned close to flame. The seasonal dynamics of Chl *a/b* ratio revealed the winter-associated decrease in relative amount of LHC in chloroplasts compared to the amount of PSI and PSII. This phenomenon was most pronounced at the remote (background) site and was less conspicuous near the gas flare. The consistently low Chl *a/b* ratio in needles at site I in winter was related to the partial restoration of Chl *b* pool, which accounts for the elevated amount of LHC. While the background depression of pigment apparatus in winter was observed in pine needles from all locations, the largest degree of pigment degradation was found at site VII, i.e., outside the zone of thermal field influence of the gas flare; the least degradation was noted at site I, and the degradation of moderate severity was noticed at site III. In all examined sites, the proportion of LHC and photosystems was mainly regulated via changes in the pool of Chl *b*. The differences in paired correlations of Chl seasonal dynamics between individual sites provide further evidence that winter-related adaptive events in PSA of needles are not coincident for trees located at various distances from the petroleum gas flare.

The winter-related increase in Car content in pine needles is consistent with the results of other studies on pigment apparatus in evergreen plants [30] and is ascribed to the carotenoid-dependent protection of chlorophyll molecules from photodegradation during this period. The role of carotenoids is particularly important at low temperatures when the rates of physiological and biochemical processes in the cells of needles are retarded. It should be noted that the correlation analysis of seasonal dynamics of Chl and Car content in needles did not reveal significant relationships in all examined locations. In addition, the Car content, unlike that of Chl *a* and Chl *b*, showed no correlation with ambient temperature. These facts indicate that the extents of Chl and Car pools are regulated in comparatively independent ways.

A relatively stable elevated level of Car in needles in the period from September to April was only observed at the sites located close to the gas flare, while individual rises and falls in Car seasonal dynamics were observed at site VII. These fluctuations of Car content seem to arise because the regulation of the Car pool proceeds differently in the absence (site VII) and the presence (sites I and III) of the thermal field of the gas flare.

The decrease in Chl (*a + b*)/Car ratio in needles during the autumn–winter period was also observed by other researchers [11]. According to the published data, this parameter starts decreasing in October upon lowering the average daily temperature below 5°C. General similarity in seasonal dynamics of this parameter for different observation sites, as confirmed by the high degree of their mutual correlations, indicates the importance of Chl (*a + b*)/Car ratio for adaptation mechanisms associated with the transition of chloroplast pigment complex to winter dormancy. During this transition, the Chl (*a + b*)/Car ratio of 2.4–2.8 in needles apparently preserves the primary events of light energy transformation in PSA to a sufficient level. The occurrence of local maxima in Chl (*a + b*)/Car ratio in November and February in needles sampled at site I confirms the elevated PSA activity near the gas flare in winter. These local maxima arise as a result of the increase in Chl *a* and Chl *b* content (under a relatively constant Car content), which indicates that the balance of synthesis and decomposition of chlorophylls is shifted toward synthesis in these months.

Analysis of seasonal changes in the content of individual pigments and their ratios indicates that the adaptive reactions of PSA in pine needles proceed continuously throughout the autumn–winter period and depend on the distance from the flare; this proves the validity of our second hypothesis. In addition, the results uncover basic trends in the transformation of chloroplast pigments in pine needles during the autumn–winter–spring period and provide new viewpoints on the processes and mechanisms underlying these trends. Upon the seasonal drop in ambient temperature, physiological and biochemical processes are decelerated but not arrested, which is characteristic of PSA condition in winter. At the same time, the photooxidation of chlorophylls reduces Chl content to a certain level that is determined by the carotenoid protective role and biosynthesis of individual pigments. The amounts of chlorophylls integrated in LHC and chloroplast photosystems are kept close to the average proportion under various environmental conditions, including low temperatures, because this proportion is controlled by the systems that monitor the content of green pigments. The amount of carotenoids in LHC and photosystems increases in winter, which ensures the level of photosynthetic pigments sufficient for the minimally active condition of PSA in needles. Regulation of Car content occurs, to a large degree, independently of the control of chlorophyll content. Nevertheless, the Chl (*a + b*)/Car ratio is kept balanced at

a certain range. The impact of gas flare, associated primarily with the increased air temperature, elevates the activity of chloroplast pigments since it improves the adaptive potential of PSA by regulating the pools of individual pigments in needles.

Analysis of the results led us to the following conclusions. (1) Seasonal changes of pigment composition in chloroplasts of Scots pine needles throughout the late summer–autumn–winter–spring period are associated with lowering of ambient temperature below the physiological optimum of active growth; seasonal pigment changes represent a series of adaptive processes in PSA of needles in response to changes in environmental conditions. Multiple observations during the examined period provided new information about seasonal changes in the pigment apparatus. Transient maxima and minima in the seasonal dynamics of pigment composition were found to occur during winter; they are presumably associated with the regulation of Chl and Car content upon changes in ambient temperature.

(2) The thermal field of gas flare affects the adaptation processes that determine the seasonal dynamics of pigment composition in the needles of Scots pine trees at different distances from the flame. Its action modulates the seasonal dynamics of all studied PSA parameters in pine needles. The PSA activity of needles during the examined period was higher near the flare and decreased with the distance from the flame. In the subplot located near the gas flare (site I) where the temperature in the autumn–winter period was 1–2°C higher than in the natural environment (site VII), the seasonal decrease in Chl content was less obvious than at the farthest distance from the flare. At the intermediate distance from the flame (site III), these effects were less pronounced than at site I. The Chl *a/b* ratio at site I was consistently lower than at site VII, which indicates an increased proportion in the amount of LHC to photosystems in chloroplasts. The Chl (*a + b*)/Car ratio at site I was higher than at sites III and VII and featured two reliable peaks, which was apparently caused by the increased retention of green pigments due to the protection of chlorophylls from photodegradation near the gas flare. Our results confirmed the validity of both working hypotheses.

(3) On the basis of differential seasonal changes in the content of Chl and Car in needles, the established correlations of the seasonal dynamics of Chl *a* and Chl *b* with air temperature regardless of the distance to the flame, and the absence of such correlation for carotenoids, we suppose that the seasonal dynamics of Chl and Car are regulated by different mechanisms. The Chl content determined by the balance of Chl decomposition and synthesis would depend on environmental factors, including temperature, as well as on the protective functions of carotenoids, the rate of Chl synthesis, and the PSA activity in general. The above balance controls the proportions of chlorophylls

associated with photosystems and LHC in chloroplasts. In trees exposed to an undisturbed environment, these proportions undergo seasonal variations around the average level. In the autumn–winter period, carotenoids are additionally accumulated in photosynthesizing cells, which reinforces the protection of chlorophylls from photodegradation. The regulation of Car content apparently follows the pattern of Chl (*a + b*)/Car ratio that drops to its minimum and remains low in the period from autumn to late spring.

ACKNOWLEDGMENTS

We are grateful to leading engineer G.N. Novoselova (Ural State Forestry Engineering University) for invaluable assistance in conducting laboratory experiments.

FUNDING

This work was supported by the Comprehensive Program of the Ural Branch of the Russian Academy of Sciences for 2018–2020 (project no. 18-4-4-10) and by a state assignment to the Institute Botanic Garden of the Ural Branch of the Russian Academy of Sciences.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of humans or animals. This article does not contain any studies involving animals performed by any of the authors.

REFERENCES

1. Krivosheeva, A.A., Shavnin, S.A., Kalinin, V.A., and Venediktov, P.S., Influence of industrial pollution on seasonal changes in chlorophyll content in the needles of Scots pine, *Fiziol. Rast.*, 1991, vol. 38, p. 163.
2. Ensminger, I., Sveshnikov, D., Campbell, D.A., Funk, C., Jansson, S., Lloyd, J., Shibistova, O., and Öquist, G., Intermittent low temperatures constrain spring recovery of photosynthesis in boreal Scots pine forests, *Global Change Biol.*, 2004, vol. 10, p. 1. <https://doi.org/10.1111/j.1365-2486.2004.00781.x>
3. Yatsko, Y.N., Dymova, O.V., and Golovko, T.K., Pigment complex of winter and evergreen plants in the middle taiga subzone of the European Northeast, *Bot. Zh.*, 2009, vol. 94, p. 1812.
4. Tuzhilkina, V.V., Photosynthetic pigments of Siberian spruce needles in the middle-taiga forests of the European northeast of Russia, *Sib. Lesn. Zh.*, 2017, no. 1, p. 65. <https://doi.org/10.15372/SJFS20170107>
5. Yatsko, Y.N., Dymova, O.V., and Golovko, T.K., Violaxanthin cycle pigment de-epoxidation and thermal dissipation of light energy in three boreal species of evergreen conifer plants, *Russ. J. Plant Physiol.*, 2011, vol. 58, p. 169. <https://doi.org/10.1134/S1021443711010249>
6. Ottander, C., Campbell, D., and Öquist, G., Seasonal changes in photosystem II organisation and pigment

- composition in *Pinus sylvestris*, *Planta*, 1995, vol. 197, p. 176.
<https://doi.org/10.1007/BF00239954>
7. Wang, K.-Y., Kellomäki, S., and Zha, T., Modifications in photosynthetic pigments and chlorophyll fluorescence in 20-year-old pine trees after a four-year exposure to carbon dioxide and temperature elevation, *Photosynthetica*, 2003, vol. 41, p. 167.
<https://doi.org/10.1023/B:PHOT.0000011948.00870.db>
 8. Tuzhilkina, V.V., Pine needle pigment complex in the forests of the European Northeast, *Lesovedenie*, 2012, no. 4, p. 16.
 9. Kalituho, L., Rech, J., and Jahns, P., The roles of specific xanthophylls in light utilization, *Planta*, 2007, vol. 225, p. 423.
<https://doi.org/10.1007/s00425-006-0356-3>
 10. Öquist, G. and Huner, N.P.A., Photosynthesis of overwintering evergreen plants, *Annu. Rev. Plant Biol.*, 2003, vol. 54, p. 329.
<https://doi.org/10.1146/annurev.arplant.54.072402.115741>
 11. Sofronova, V.E., Dymova, O.V., Golovko, T.K., Chepalov, V.A., and Petrov, K.A., Adaptive changes in pigment complex of *Pinus sylvestris* needles upon cold acclimation, *Russ. J. Plant Physiol.*, 2016, vol. 63, p. 433.
<https://doi.org/10.1134/S1021443716040142>
 12. Khodasevich, E.V., Arnautova, A.I., and Myshkovets, S.N., Ultrastructural organization of chloroplasts related with the reversible degradation of the pigment pool in conifers, *Fiziol. Rast.*, 1978, vol. 25, p. 810.
 13. Martin, B. and Öquist, G., Seasonal and experimentally induced changes in the ultrastructure of chloroplasts of *Pinus sylvestris*, *Physiol. Plant.*, 1979, vol. 46, p. 42.
<https://doi.org/10.1111/j.1399-3054.1979.tb03183.x>
 14. Maslova, T.G., Mamushkina, N.S., Zubkova, E.K., Bubolo, L.S., and Tyutereva, E.V., Structural and functional characteristics of the photosynthetic apparatus of the leaf of winter-green plants during the year-round vegetation, *Bot. Zh.*, 2015, vol. 100, p. 1142.
<https://doi.org/10.1134/S0006813615110022>
 15. Ovsyannikov, A.Yu. and Semkina, L.A., Seasonal changes in the activity of photosystem II and localization of chloroplasts in the plant cells of the needles of the genus *Picea* (Pinacea), *Bot. Zh.*, 2014, vol. 99, p. 971.
 16. Öquist, G. and Martin, B., Inhibition of photosynthetic electron transport and formation of inactive chlorophyll in winter stressed *Pinus sylvestris*, *Physiol. Plant.*, 1980, vol. 48, p. 33.
<https://doi.org/10.1111/j.1399-3054.1980.tb03215.x>
 17. Öquist, G. and Ogren, E., Effects of winter stress on photosynthetic electron transport and energy distribution between the two photosystems of pine as assayed by chlorophyll fluorescence kinetics, *Photosynth. Res.*, 1985, vol. 7, p. 19.
<https://doi.org/10.1007/BF00032919>
 18. Öquist, G., Martensson, O., Martin, B., and Malmberg, G., Seasonal effects on chlorophyll-protein complexes isolated from *Pinus sylvestris*, *Physiol. Plant.*, 1978, vol. 44, p. 187.
<https://doi.org/10.1111/j.1399-3054.1978.tb08616.x>
 19. Novitskaya, Yu.E., Seasonal and age-related dynamics of the main lipid fractions in Scots pine needles, in *Fiziologo-biokhicheskie issledovaniya sosny obyknovnoi na Severe* (Physiological and Biochemical Studies of Scots Pine in the North), Petrozavodsk, 1987, p. 39.
 20. Öquist, G., Seasonally induced changes in acid lipids and fatty acids of chloroplast thylakoids of *Pinus sylvestris*, *Plant Physiol.*, 1982, vol. 69, p. 869.
<https://doi.org/10.1104/pp.69.4.869>
 21. Gaevskii, N.A., Sorokina, G.A., Gol'd, V.M., and Mirol'yubskaya, I.V., Seasonal changes in the photosynthetic apparatus of woody and shrub plants, *Fiziol. Rast.*, 1991, vol. 38, p. 685.
 22. Shavnin, S.A. and Fomin, A.S., Seasonal changes in chlorophyll fluorescence of Scots pine needles, *Fiziol. Rast.*, 1993, vol. 40, p. 209.
 23. Leverenz, J.W., The effects of illumination sequence, CO₂ concentration, temperature and acclimation on the convexity of the photosynthetic light response curve, *Physiol. Plant*, 1988, vol. 74, p. 332.
<https://doi.org/10.1111/j.1399-3054.1988.tb00639.x>
 24. Huner, N.P.A., Elfman, B., Krol, M., and McIntosh, A., Growth and development at cold-hardening temperatures. Chloroplast ultrastructure, pigment content, and composition, *Can. J. Bot.*, 1984, vol. 62, p. 53.
<https://doi.org/10.1139/b84-009>
 25. Strand, M. and Öquist, G., Inhibition of photosynthesis by freezing temperatures and high light levels in cold-acclimated seedlings of Scots pine (*Pinus sylvestris* L.). Effects on the light-limited and light-saturated rates of CO₂ assimilation, *Physiol. Plant*, 1985, vol. 64, p. 425.
<https://doi.org/10.1111/j.1399-3054.1985.tb08517.x>
 26. Bardin, M.Yu., Ran'kova, E.Ya., Platova, T.V., Samokhina, O.F., Egorov, V.I., Nikolaeva, A.M., Gromov, S.A., Alekseev, G.V., Radionov, V.F., Aleksandrov, E.I., Dement'eva, T.V., Korshunova, N.N., Lавrov, A.S., Sterin, A.M., Khokhlova, A.V., et al., Report on the specific climate in the Russian Federation in 2018. http://www.meteorf.ru/upload/pdf_download/o-klimat-rf-2018.pdf.
 27. Shavnin, S.A., Yusupov, I.A., Montile, A.A., Golikov, D.Yu., and Montile, A.I., Effect of increased ambient temperature on the growth rate of young pine forests in the impact zone of a petroleum gas flare, *Russ. J. Ecol.*, 2009, vol. 40, p. 1.
<https://doi.org/10.1134/S1067413609010019>
 28. Shavnin, S.A., Yusupov, I.A., Artem'eva, E.P., and Golikov, D.Yu., The influence of temperature increase of the environment on the formation of terrestrial vegetation near a gas torch, *Izv. Vyssh. Uchebn. Zaved., Lesn. Zh.*, 2006, no. 1, p. 21.
 29. Lichtenthaler, H.K., Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, in *Methods in Enzymology*, Colowick, S.P. and Kaplan, N.O., Ed., San Diego: Academic, 1987, vol. 148, p. 350.
[https://doi.org/10.1016/0076-6879\(87\)48036-1](https://doi.org/10.1016/0076-6879(87)48036-1)
 30. Sudachkova, N.E., Milyutina, I.L., and Romanova, L.I., Adaptive responses of Scots pine to the impact of adverse abiotic factors on the rhizosphere, *Russ. J. Ecol.*, 2009, vol. 40, p. 387.
<https://doi.org/10.1134/S1067413609060022>

Translated by A. Bulychev