

Fluorescence Methods for Estimation of Post-Fire Response of Pine Needles

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Abstract

This paper discusses the possibility of using fluorescent methods to assess the thermal effects on Scots pine (*Pinus sylvestris* L.) needles' assimilation apparatus. Assimilation apparatus of pine needles was proved to be able to reestablish after exposure to convective, simulating the effect of ground fire heat flow, though the recovery rate depends on the impact force. The analysis of fast and delayed fluorescence characteristics revealed differences in the thermostability of the Scots pine needles evidencing certain modification of physiological processes in plants under the influence of stress factors with a positive acclimation effect. The Scots pine needles grown after ground fire are more resistant to the repeating sublethal temperature, and this effect is maintained during the next growing season.

Key words: fluorescence, forest fires, heat stress, *Pinus sylvestris*, resilience, Siberia.

Introduction

Forest fires are one of the main recurrent factors that have a significant impact on forest ecosystems throughout the world. Being characterized by highly frequent fires, the boreal forest fires have always been a major evolutionary factor. Annually, from 4.5 to 27 thousand forest fires occur in forest and steppe zones of Siberia, most of them being ground ones (Ivanova *et al.* 2014). In the recent decades there has been seen a close correlation between the air temperature dynamics and the total number of forest fires, which is reflected in the damage area increase (Ponomarev *et al.* 2012; Kharuk and Ponomarev 2016). Distribution of forest fires in Siberia is uneven, they happen mainly in the areas occupied by light coniferous taiga, which may be related to the intensity and duration of droughts in the taiga subzones (Valendik *et al.* 2014).

Most studies have shown that the fire influences forest stands not only directly, but also through unmediated habitat alteration. Fires disrupt the natural balance between the

individual components of ecosystems and make considerable changes in the appearance of today's forests, namely they cause changes of tree species, affect the age structure of forest stands, alter and violate the species composition of the grass-shrub layer (Tsvetkov 2013; Girs 1982; Gabysheva 2014). They lead to the disruption of the natural soil structure, changes in power and even morphological characteristics of genetic horizons (Turetsky and Wieder 2001; Tsibart and Grennadyev 2008). After the fire has gone, environmental conditions change rapidly resulting in improved temperature control, accompanied by the permafrost degradation and mineralization of soil organic matter, which entails the increasing amount of available moisture and nutrients, and as a consequence, the progressive radial growth of trees for a few subsequent years (McRae *et. al.* 2006; Yevdokimenko 2008; Tarasov *et. al.* 2008).

Woody plants, in particular pine plantations, die completely only under very heavy fire. Crawling low- or medium intensity ground fires are not always devastating and can have a positive impact on the individual components of forest ecosystems (Verkhovets 2000; Tsvetkov and Buryak 2014). For example, in some countries there is a practice of prescribed burning preceding logging for several years, which may further contribute to the rebirth of young saplings. In addition, a number of authors consider it appropriate to have a burning in the thick forest floor and moss cover to reduce the fire hazard and eliminate harmful insects in the forest floor

Currently, much attention is paid to the study of adaptive responses and mechanisms of plant resistance to various stress factors. The impact of high temperatures on the vegetative organs during forest fires may be referred to as extreme stress factor with a short duration of action. Many authors demonstrate in their works that under the influence of high temperatures plants go through multiple structural and functional changes, among which changes in the photosynthetic apparatus are fairly important (Titov and Talanov 2010; Wang and Hu 2014.).

Numerous studies on the effect of heating on the individual parts of the plant point out its critical influence on a number of physiological and biochemical processes and indicators, in particular the synthesis of stress proteins, the composition of phytohormones and polyamines, the rate of assimilates transport, the expression of several genes (Kulaeva 1997; Xin *et.al.* 1994; Wang *et.al.* 2004).

Thus, Sudachkova and co-authors showed in their work (2016) that following artificial, simulating medium - intensity fire burning in plantations of Scots pine, the trees demonstrate the primary reaction to the pyrogenic stress for a few months after exposure to the fire. The reaction implied a sharp decline in chlorophyll concentration in needles, increased concentration of hydroperoxyl, reduced concentration of low molecular weight

carbohydrates and the weakening activity of most members of the antioxidant system in the layer next to cambium (Girs 1984; Sudachkova *et al.* 2016). At the same time low-intensity ground fires do not cause irreversible consequences in the layer next to cambium among dominating species, while the subordinate part of forest stand is extensively damaged (Sudachkova *et al.* 2015).

Some researchers are devoted to studying the impact of heat stress on the electron transport system, photosystems functioning, photosynthetic activity, gas exchange and chlorophyll fluorescence in plants (Owen and Mark 2003; Wang and Hu 2014). It is known that photosynthesis is one of the most sensitive to heat physiological processes, so even a short-term temperature rise due to ground fire can become a significant stress factor for them (Wataru and Kouki 2014). This damage to photosystems is often the first reaction to the thermal stress. A method for detecting the chlorophyll fluorescence is a convenient and promising way of getting information about the primary reactions of photosynthesis, which allows researchers to quickly estimate the impact of extreme temperatures on the needle mesophyll; it is believed that the state of the photosynthetic apparatus largely corresponds to the general state of the plant.

In addition to substantial cellular protection mechanisms against heat shock, there are some other leading to plants' morphological adaptations. In particular, the thickness of the protective layer of dead bark, which plays a momentous role in the protection of live stem tissues from thermal damage (Valendik *et.al.* 2006). On this basis the most resistant to fire species are larch and pine. Apart from that, the key fire resistance factors of wood species normally include: the species of tree, the tree age, height, diameter, location of the crown.

Nowadays, the main task is not only to define a parameter that can be used to assess the sensitivity of photosynthesis to heat stress, but also to estimate the preservation of acclimation effects of trees after exposure to fire. So the purpose of our study was to determine the possibility of using fluorescent methods to determine the photosynthetic activity and the ability of needles' assimilation apparatus to recover after Scots pines were subjected to heat stress during ground fire.

Materials And Methods

Study Area and Objects

In a fire it is the tree crown which is directly exposed to short-term convection heat. According to G.I. Girs (1982), during the combustion the crown temperature depending on

combustion intensity can range from 33°C (ground fire of low intensity) to 52°C (crawling ground fire of medium intensity).

For experiments on modeling convective flow, simulating the effect of ground fire, we used 15–25 year old saplings of Scots pine, grown outside the city of Krasnoyarsk. Collecting of saplings (branches of the lower part of the crown) was carried out in a single day. The branches in a jar with water were delivered to the laboratory within one hour.

Commonly more attention is paid to studying plants' adaptive responses to heat stress, while the ability of plants to recover under stress conditions has been studied to a lesser extent so far. Comparing the samplings of *Pinus sylvestris* L., growing in the same climatic and soil conditions, but differing in the duration of post-fire period, we could distinguish acclimation effects appearing after the stress.

The studied area was located in a forest-steppe zone of Krasnoyarsk region in the territory of “Pogorelskiy bor” station of Sukachev Forest Institute, Siberian Branch of the Russian Academy of Sciences (56°22'07.48" N 92°57'17.95" E)

The object of research was pine saplings (I age class) from the rich in herbs forest with abundant pleurocarpous moss. Area for sampling selected for the study was the land plot where in 2014 the ground fire was occurred. Control sample for comparison was the plot which was not exposed to fire (Table 1). Ground fire intensity was measured by the average height of scorch (a charred bark surface) on the tree trunks. (Kurbatsky 1962; Tsvetkov 2006).

Tab.1.

From each plot there were chosen 8 model trees growing in the same conditions with the same characteristics of moisture, light, viability, with no visible damage from xylophagous insects or dieback. Saplings' collection from all the studied sites was carried out in a single day (Grigoriev and Andreev 2012). A lopper was used to cut 3 branches from the lower part of the crown of each tree (average height of sapling was 2.5m). The cut saplings in jar with water were delivered to the laboratory within one hour.

Research Methods

Since the determination of the crown temperatures during the fire is methodologically quite difficult, all the experiments were performed on the cut branches. Samples' heating was modeled by convective heat transfer from the flame of a gas burner with constant burning

intensity. The duration of heating was 2, 5 and 10 minutes at temperatures of 45°C, 50°C and 55°C.

To create a sustainable convective flow with set temperature we used the following installation (Fig. 1), where the gas burner gave rise to convective flow. Installation allows one to create a steady convective flow, which simulates the fire impact on a tree branch (Valendik and Kosov 2008).

Fig.1

The sampling (4) was fastened horizontally on the flow regulator (3), the thermocouple (6) was fixed directly under the sapling in the center flow where the temperature corresponded to the set one. The flow temperature was recorded at intervals of one second.

To measure the flow temperature per unit time we used autonomous datalogger EClerk-USB-K with the measuring element of prefabricated chromel-alumel thermocouple (TII.AX (K)-K11).

To evaluate the photosynthetic productivity of forest stands *in vivo* and in laboratory one must have a component that can serve as a characteristic of the photosynthetic activity. When examining the eco-physiological photosynthesis fast fluorescence parameters can act as such indicators (Molchanov 2012). Fast fluorescence parameters were measured before (control measurement) and immediately after the convective heat flow influence by fluorometer Junior-PAM (Walz, Germany) for pulse-amplitude modulated fluorometry. The ratio of F_v/F_m was used as an estimate of the maximum quantum yield of photosystem II photochemistry (Maxwell and Johnson 2000). Fluorescence parameters were calculated using WinControl program. Measurements were carried out in 5 biological and 3 analytical replications for each temperature and heating time.

To assess the needles' stability in terms of post-fire conditions, fast (F_v/F_m) and delayed fluorescence relative parameters were taken into account, as they show the needles' photosynthetic activity. Parameters of the delayed fluorescence were measured by fluorometer "Photon-10", taking into consideration guidelines for the selection and analysis of the Scots pine needles (Grigoriev *et al.* 1996; Grigoriev and Andreev 2012).

To determine the stability of woody plants to high temperatures and their reestablishing in the post-fire period we applied method of artificial stress exposure, heating needles at temperatures higher than their physiological optimum. According to the data obtained in experiments on convective heating of needles, the authors observed reversible inhibition of photosynthesis with the gradual recovery process at temperatures up to 55°C,

which is consistent with G.I. Girs's results (1982). During the experiment, heating of the cut branches with the lower parts immersed in the water containers was performed in climatostat (climate chamber) equipped with an internal fan for uniform heating of samples at sublethal temperatures – 43°C, 45°C and 47°C. These temperatures did not lead to the needles' shrinkage. The duration of heating was 10 minutes, and then fluorescence indicators were immediately measured.

After heating in climatostat and on convective heater, the saplings were left under laboratory conditions at relative humidity of $\approx 60 \pm 5\%$ and air temperature of $\approx 24 \pm 2^\circ\text{C}$. After that, the recovery of their photosynthetic activity was observed during 4 days, thus, it was possible to detect the needles' thermostability, the ability of cells to activate defense mechanisms and to resist heat damage, which determines wood resilience during fire and after it (Lange 1964).

The concentration of photosynthetic pigments was measured in the biennial needles by spectrophotometer SPEKOL1300 Analytik Jenna AG after extraction in 85% acetone (Gavrylenko and Zhigalova 2003). The measurement was conducted at an average sample of each model tree with three promptitude parameters.

Statistical analysis was performed by STATISTICA 9 software. Statistically significant differences were determined by Student's t-test where $p \leq 0.05$.

Results and Discussion

Photosynthesis belongs to thermolabile processes whose suppression occurs at relatively low temperatures (Girs 1984). According to the researches, it has a high capacity for repair the damage. PSII is considered to be the most vulnerable part of the photosynthetic apparatus (Murata *et. al.* 2007). As a consequence, changes in photosynthetic activity during exposure to high temperatures and after it may signal about the functional stability of the assimilation apparatus.

Study of the thermostability of biennial Scots pines' needles showed that temperatures of 45°C and 50°C are not lethal to cells regardless of heating duration. Thus, the effect of 45°C temperature, which exceeds the photosynthetic optimum of photosynthesis, did not have a significant impact on the maximum quantum yield of PSII photochemistry, the values of which remained the same throughout the laboratory exposure evidencing that at low intensity exposure to heat there can be a direct continuation of photosynthetic activity.

Reversible photoinhibition followed by a gradual recovery process was observed at 50°C, the important factor was the duration of heat exposure. So after 5-minute heating at 50°C the level of reparation for the next three days was higher than that after 10-minute warming-up at the same temperature, while short 2-minute heating at 50°C did not lead to any noteworthy suppression of the indicator against the control measurement.

After exposure to sublethal temperature of 55°C for 5 and 10 minutes (Fig. 2) there was a sharp decrease in the fast fluorescence parameter, which was on average 55% and 72% against the control level. During experimental day 3 in laboratory exposure photosynthesis repair was detected; it was almost the initial level but only for the needles after 2-minute heating at temperature 55°C. On the third day after 5-minute heating photosynthesis gained 50% of the initial intensity. The irreversible reduction in photochemical activity of PSII occurred only after 10-minute heating at 55°C, followed by the complete drying of needles a day after heating.

Fig.2

Thus, the observations showed that photosynthesis in pine needles is relatively resistant to the damaging effects of high temperatures. Repair of photosynthetic activity took place after a partial suppression at temperatures of 45°C, 50°C and 55°C. As regards the recovery speed of photosynthesis, the important factor was the duration of damaging heating.

According to the cited studies, it was found that the temperature deviation from the optimum, typical of a species, affects the quality and quantity of pigment fund of the plastid apparatus, the structural organization of chloroplasts and their functional activity (Girs 1984; Sudachkova *et.al.* 2016).

Fig.3

Needles' heating leads to a slight fall in the amount of chlorophyll and carotenoids (Fig. 3), which indicates the absence of degradation. Small changes in the pigments' content in the period after exposure to high temperatures depend on the heat intensity. So after short heating at 45°C and 50°C there is almost no decrease in the content of pigments against the control level. Under the stress influence on the needles at 55°C the amount of chlorophyll a + b and carotenoids decreased by 30% on average from the control level on the day after heating. As a result, our studies showed plasticity of the Scots pine pigment apparatus in response to high temperatures, as well as the possibility of repair.

Therefore, during the experiments simulating the effect of ground fire and conducted to study the impact of convective flow on the Scots pine assimilation apparatus the needles' primary thermal stability was detected. During registration of fast fluorescence parameters and determination of the pigments' quantitative content it was proved that after removing the damaging factor the Scots pine assimilation apparatus can recover, while the rate of recovery depends on the impact force.

The above data are the result of short-term experiments, when the after effects were observed within a few days, so there is a question about the recovery period duration for the normal metabolism in the leaves, the presence of acclimation effects at the organism level after stress and their duration. For that reason we conducted experimental studies on the saplings of *Pinus sylvestris* L., being in the same climatic and soil conditions, but differing in the duration of post-fire period.

According to the classification by Kurbatsky (1962), model trees of the I age class Scots pine from a sample plot 1 (SP) experienced low-intensity ground fire, as the average flame height for the site did not exceed 0.5 m.

During the experiment on the effects of stress temperatures (43°C, 45°C and 47°C) on the branches of Scots pine, there was marked a reduction of the relative parameter of the delayed fluorescence and the maximum quantum yield of PSII photochemistry in response to the action of temperature.

Natural heat resistance of plants depends on several factors, including the leaf age. According to G.I. Girs (1982), evergreens after overwintering lose age differences in heat resistance, so in our work we used the average values for 2 and 3 year old needles, as heating embraced samplings fully, not partially.

Fluorescence indicators of Scots pine needles at room temperature ($\approx 24^\circ\text{C}$) do not show significant differences between the sample plots throughout the whole study period (Fig. 4 A, B, C). However, upon heating photosynthetic activity changes in varying degrees.

Fig. 4 shows in detail the fast and delayed fluorescence parameters of the Scots pine needles growing on the sample plot after ground fire that occurred in May 2014 (SP1). Short 10-minute heating of saplings at 45°C leads to the suppression of photosynthetic activity both among trees having grown in the control sample plot and the trees which were exposed to ground fire a year ago.

For samples taken in June 2015 (Fig. 4A), 10-minute heating at the temperature of 45°C led to a decrease of the delayed fluorescence relative parameter against the control level by 25% for trees from the control plots and by 19% for trees exposed to heat during ground

fire. Ratio Fv/Fm also decreased in comparison with the control level. Even greater decrease was observed in photosynthetic activity for the needles from all plots the next day after heating. This reaction was possible due to the fact that the plants were in a phase of shoot growth, and the flow of plastic substances (metabolites) was directed to the support of growth. It may be due to the cells containing a smaller amount of the thermal protector. Recovery of photosynthetic activity almost to the control level occurred on 2-3 days; with higher values of the delayed fluorescence and Fv/Fm of needles exposed to stressful temperatures in the fire in 2014 (the differences were statistically significant for $p \leq 0.05$).

Figure 4B shows the simulated effects of high temperature (45°C) on chlorophyll fluorescence of pine needles that was investigated in July 2015. The parameter of delayed fluorescence at room temperature had the lowest values compared to the previous and subsequent period of study. It is known that photosynthesis is the thermolabile process, and falloff in intensity occurs at relatively low temperatures which are not fatal to the cell (Konovalov and Semenov 1990). Thus, high daily average and daytime temperatures in July could have affected the decrease in photosynthetic activity as a whole. High daily average air temperature had no effect on the maximum quantum efficiency (Fv/Fm), which was consistently high (about 0.7-0.8), attesting to the high efficiency of the primary PSII photochemistry.

Ten minute heating at the temperature of 45°C did not lead to a significant decrease of delayed fluorescence against the control level. Such thermal stability may result from early cell defense reactions in order to prevent damages due to high daytime temperatures. Trees in the summer vegetation phase are adapted to high air temperatures. Neither Fv/Fm decreased by about 12 % in relation to the initial levels for the needles of pine trees from all plots. Fig. 3B shows that 3 days after exposure to the damaging factor there was an increase in photosynthetic activity under the laboratory exposure against the control level. In contrast, the lower values were characteristic of the trees in the control plots, which were not exposed to fire. It may be concluded that ground fire in 2014 boosted the tree's ability of repair after the action of damaging temperatures had finished.

Data obtained for saplings selected in September are shown in Fig. 4B. During 10-minute heating of saplings at the temperature of 45°C there was equal suppression of needles' photosynthesis for the control area and for the area subjected to fire. Under the laboratory exposure ratio Fv/Fm and the relative parameter of delayed fluorescence for needles from SP2 control are reestablished at the original level during the first experimental day. Repair of the photosynthetic activity of the needles from SP1 also happened on the first experimental day,

at the same time on second and third days there was recorded an increase in fluorescence level by 40% on average in comparison to the control level, which is reflected in the increased relative parameter of delayed fluorescence. Such differences between the sample plots in response to short-term exposure to temperature above physiological optimum for photosynthesis may stem from the fire impact on SP1 forest stand in the past. So this could have conditioned the heat resistance of the needles, which is important for maintaining the functional activity after the stress and during repeated exposure to temperatures.

The value of the maximum quantum yield of (Fv/Fm) photochemistry in the needles, which were selected in July and September, shows no significant differences between SP1 exposed to ground fire in 2014 and the control plot. Specifically, after repeated exposure to stress, the ratio Fv/Fm remained the same for both tree plots. That is why the current study does not demonstrate the possible application of this parameter for estimating the duration of persistent metabolic changes in the trees.

Fig. 4

Tab.2.

The data of comparative analysis of the chlorophyll *a+b* content (see Table 2) for the forest stand which experienced ground fire in 2014 (SP1) testify to the decrease in the number of pigments in July and September. According to G.I. Girs (1982), the following year after the fire, the chlorophyll content in needles at the beginning of the growing season (June) should exceed the control level, which is consistent with our data (2.26 mg/g and 2.06 mg/g of dry weight for SP1 and SP2 control respectively). There were almost no vivid changes in the content of carotenoids in general after the low intensity fire damage. Differences in the quantitative content of the pigments are not statistically valid. Hence, we can conclude that the pigment complex is resistant to short-term heating at high temperatures during the fire of low intensity, which ensures the preservation of functional activity after the stress.

Conclusion

The experimental studies revealed some differences in the dynamics of the needles' heat resistance during immediate exposure to damaging high temperatures and in the post-fire period. While registering the fluorescence parameters, it was found that certain physiological

processes in plants can be modified by stressful events. These changes may have a positive effect in case of repeated stress factors.

Chlorophyll fluorescence may act as an indicator of structural and functional properties of photosynthetic membranes in the plant leaves. In particular, the relative parameter of delayed fluorescence may express the duration of conservation the metabolic changes of photosynthetic apparatus for several years after a fire, whereas the ratio of F_v/F_m reflecting the PSII photochemical efficiency can be applied for registration of the immediate response of the needles' assimilation apparatus to heat stress.

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Tab. 1. Characteristics of sample plots (SP)

№ SP	Forest stand	Average indicators				Fire period	Growth class / estimate productivity/ fullness
		Diameter [cm]	Height [m]	Height up to the crown [m]	Strength, type of fire/ height of scorch [cm]		
SP1	10 Pines	11.7±1.1	7.8±0.4	2.9±0.2	weak ground/ h=23.0±9.0	May 2014	I /Ia/0.6
SP2 control	10 Pines	12.0±1.6	7.9±0.6	3.0±0.2	-	-	

Tab. 2. Chlorophyll a + b and carotenoids content, mg/g of dry weight

	Chlorophyll a + b content, mg/g of dry weight		Carotenoids content, mg/g of dry weight	
	SP1	SP2 control	SP1	SP2 control
June	2.26±0.12	2.06±0.40	0.29±0.02	0.28±0.03
July	2.07±0.15	2.24±0.11	0.24±0.01	0.25±0.004
September	2.11±0.30	2.19±0.30	0.26±0.01	0.25±0.03

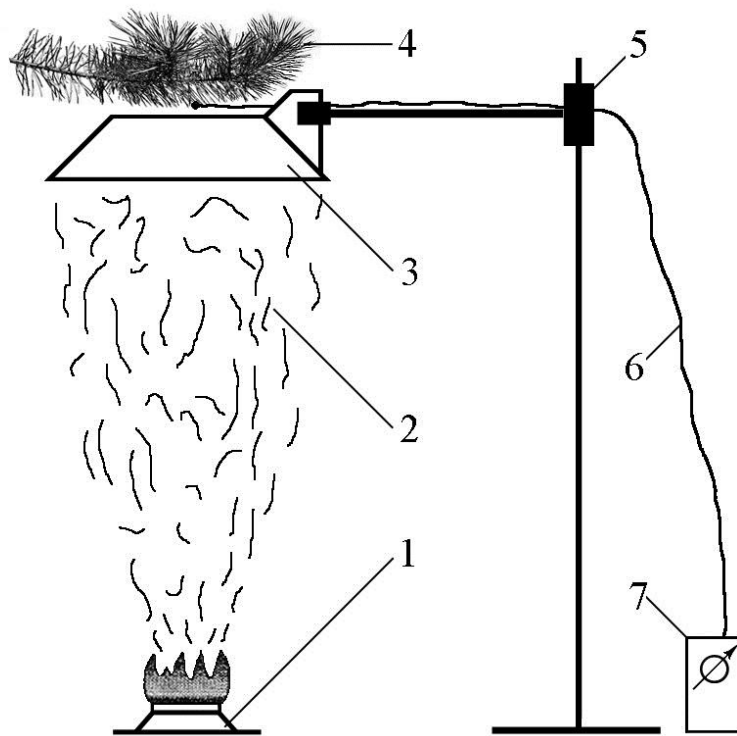
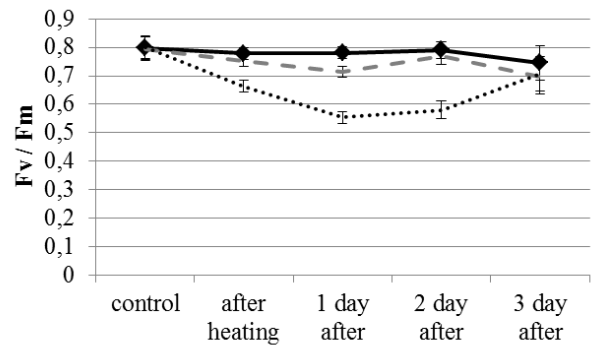
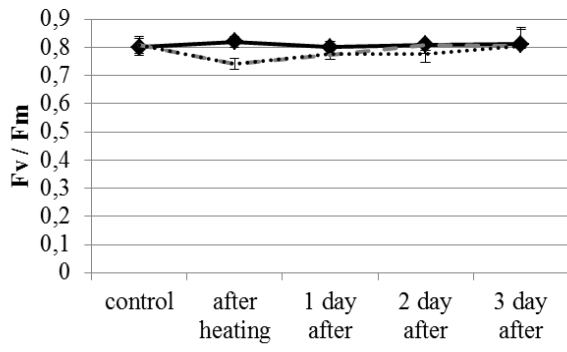
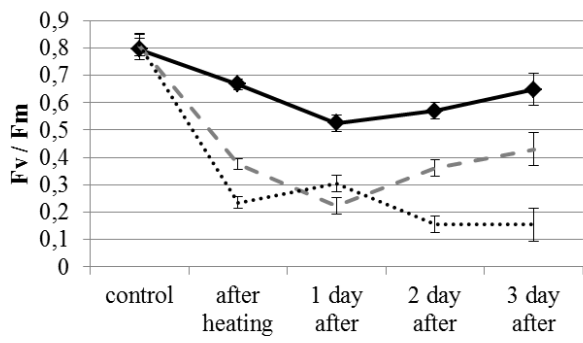


Fig. 1. Convection Heater Installation for Samples:
1 – gas burner, 2 – convective flow, 3 – flow regulator,
4 – sample, 5 – stand with holder, 6 – thermocouple,
7 – datalogger



—◆— 2 min / 45 °C — — 5 min / 45 °C 10 min / 45 °C

—◆— 2 min / 50 °C — — 5 min / 50 °C 10 min / 50 °C



—◆— 2 min / 55°C — — 5 min / 55 °C 10 min / 55 °C

Fig. 2. Changes in fast fluorescence after needles' exposure to convective flow

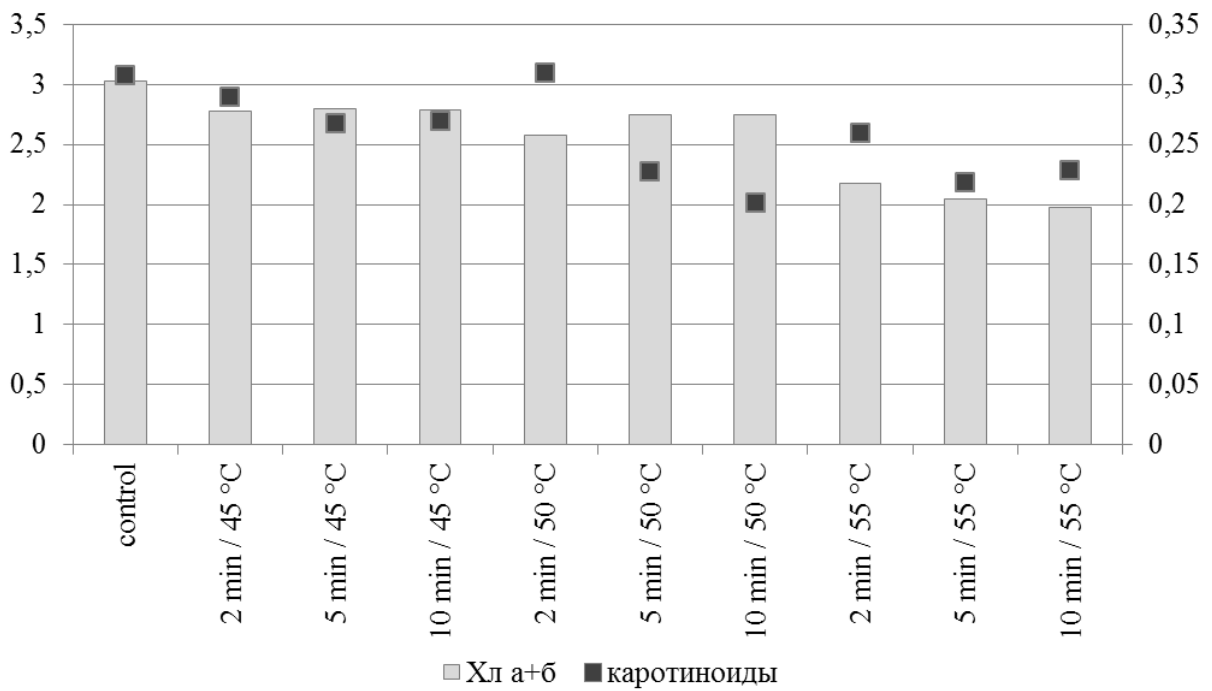
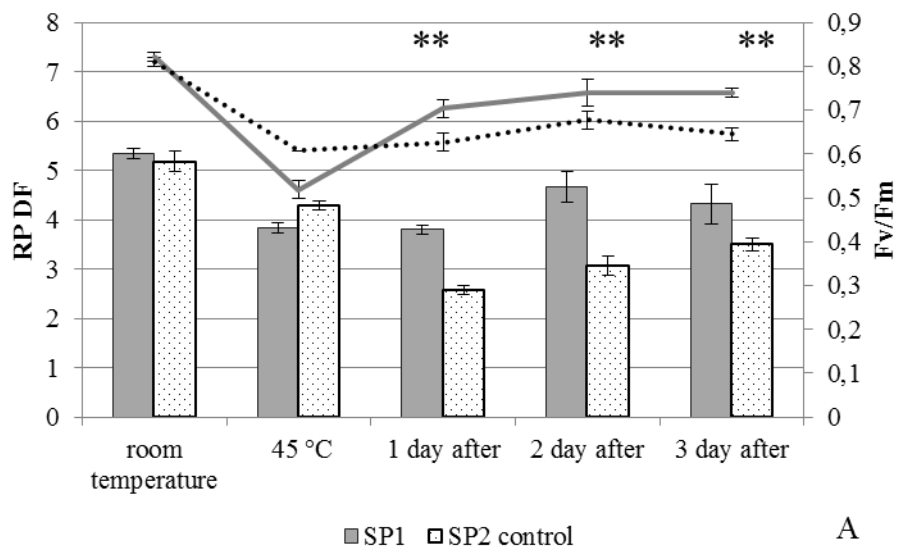
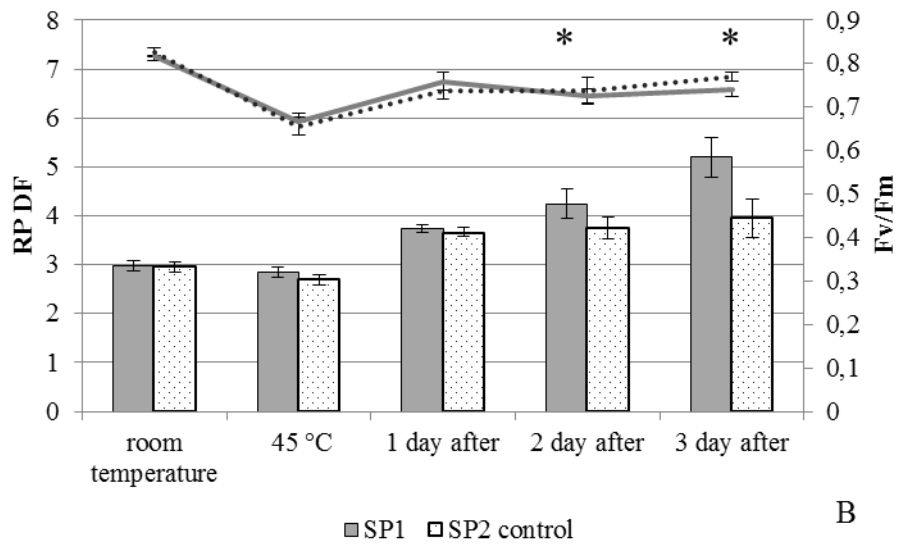


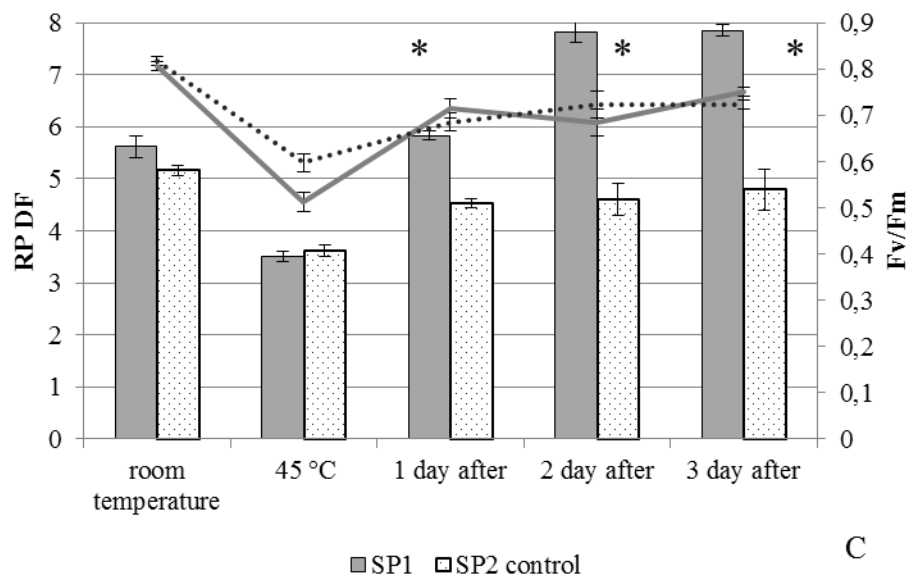
Fig. 3. Changes in the chlorophyll a + b and carotenoids content a day after heating, mg/g of dry weight (control level – the original content of pigments prior to the exposure to high temperatures).



A



B



C

* Statistically significant differences ($P < 0.05$) for (RP DF)

** Statistically significant differences ($P < 0.05$) for (Fv/Fm) and RP DF

Fig. 4. The average of relative delayed fluorescence (RP DF) and PSII quantum yield (Fv/Fm) for the Scots pine needles being exposed to the temperature of 45°C in laboratory conditions, study periods: A – June; B – July; C – September.