Fluorescent monitoring of small-leaved tree photosynthesis under anthropogenic impact

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The investigation results on the concentration dependence of birch leaf chlorophyll fluorescence are presented. It is established that variation of the F_{682}/F_{734} parameter during the vegetation process is described by two parabolas. One parabola refers to the period of the chlorophyll concentration increase up to its maximum and the second one – to the leaf aging. Minimum of the first parabola is observed during the birch flowering while of the second one – before coloring leaves. Red and far-red fluorescence parameters of birch leaf chlorophyll have been determined, which are more informative for early diagnostics of their state under different environmental conditions.

As is known, due to high absorbability of plants they are capable of cleaning partially atmosphere and soil of pollutants. Reaction of plants to pollution can serve an indicator of environmental state. Therefore, express methods controlling the plant physiological state in different environmental conditions are of interest today.

Adverse anthropogenic impacts on viability of trees, causing irreversible processes in them and leading to their mortality, first manifest themselves in leaf withering. The technique for early diagnostics of pollution impact on the plant photosynthesis is now in the initial stage of development. Different fluorescent methods are applied most often. One of them consists in the following: the intensity is measured in the fluorescence maxima, i.e., in the ranges 682–686 (F_{682}) and 730–742 nm (F_{734}),[1–3] and then the ratio F_{682}/F_{734} is calculated. This parameter indicates the degree of environmental impact on the photosynthetic apparatus of plants.[4,5] The range of F_{682}/F_{734} variation during vegetation of broad-leaved trees was considered in Ref. 6. There exists one more parameter calculated through maximal and stationary fluorescence levels. It is also characteristic of environmental impacts on photosynthetic processes.[7]

The purpose of our work was to study the regularities of variation of the above-mentioned parameters of the birch leaf red and far-red fluorescence in response to different anthropogenic factors.

Leaves of warty birch (*Betula pendula Roth.*) gathered in May–October in Krasnoyarsk Akademgorodok (55°59′ N and 92°46′ E) were the objects of the study. During the vegetation period, adult leaves were taken three at a time from six trees at 12 a.m. of the local time.

Intensity of the leaf chlorophyll fluorescence was measured with the fluorometer.[8] A spectral band 380–540 nm was isolated by a SZS-22 filter. The intensity of exciting light was 180 W/m^2, which is close to daily average illuminance in summer. Before measurements, the cut leaves were kept during 40–60 min in the dark in Petri dishes with a damp filter paper. Fluorescence was recorded at a room temperature.

Maximal F_m and a stationary F_s values of chlorophyll fluorescence were determined from curves of the chlorophyll fluorescence slow induction. Using these values, the R_f index was calculated, which characterizes the leaf potential photosynthetic activity[4]

$$R_f = (F_m - F_s)/F_s.$$

The influence of heavy metals (concentration of 10^{-3} M) and diuron (10^{-5} M) was studied using cut birch branches, the wood parts of which were placed into bottles with corresponding solutions. To prepare solutions, the following salts were used: $NiCl_2$; $CoCl_2 \cdot 6H_2O$; $MgCl_2 \cdot 6H_2O$; $FeSO_4 \cdot 7H_2O$; $CdCl_2$, and diuron (Sigma). The branches were kept for 4 days in natural light conditions.

The chlorophyll concentration dependence of the ratio F_{682}/F_{734} for birch leaf during vegetation are shown in Fig. 1.

Variation of F_{682}/F_{734} during vegetation is described by two parabolas. The first (*a*) characterizes the period when the chlorophyll concentration rises up to its maximum:

$$y = 0.025x^2 - 0.232x + 0.616, \quad R^2 = 0.95$$

while the second (*b*) is for the leaf aging period:

$$y = 0.045x^2 - 0.486x + 1.457, \quad R^2 = 0.99.$$

Minimum of the first parabola is observed in the birch flowering period and of the second one – before the leaf coloring. The maximum of F_{682}/F_{734} has been registered before the leaf fall. The ratio F_{682}/F_{734} for birch during vegetation varies between

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0.1 and 0.9, while for poplar – between 0.1 and 0.3 (Ref. 9). The difference between the turndowns can well be related to either a higher content and greater size of chloroplasts in cells of broad-leaved trees in comparison with small-leaved ones or specific features of the studied higher plants.[2]

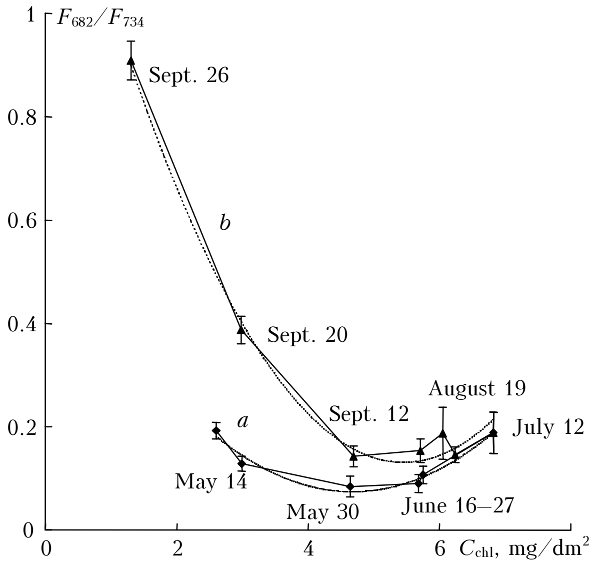

Fig. 1. The ratio F_{682}/F_{734} as a function of chlorophyll concentration in the vegetation period (solid line) and its quadratic approximation (dotted line). Numbers near curves correspond to dates of sampling.

Since the red fluorescence of birch leaf chlorophyll is more intensive than the far-red one under the chosen conditions, the parameter F_{734}/F_{682} was used later on. Its variations under influence of anthropogenic factors on leaves are presented in Table 1. Note, that the contribution of the concentration dependence of F_{734}/F_{682} shown in Fig. 1, is taken into account in Table 1.

Table 1. F_{734}/F_{682} variations for birch leaves

Date	Sound leaves	Leaves affected by pathogenic organisms	Leaves from anthropogenically polluted area*
06.24.05	10.4±0.32	9.26±0.35	8.33±0.27
07.04.05	9.13±0.32	8.27±0.32	7.42±0.43
07.15.05	9.31±0.32	8.47±0.40	8.21±0.24

* $API_5 > 14$.

Further studies have shown that the parameter F_{734}/F_{682} not always indicates the influence of anthropogenic factors on birch leaves. Table 2 does not point to certain variations of the parameter for birch branches grown in solutions of heavy metal salts.

Distinctions are significant only for diuron, which is an inhibitor of electron transport between photosystems. Most herbicides affect in the same way.

Table 2. Variations of fluorescence parameters and leaf area when growing birch branches in solutions of heavy metal salts

Acting agent	F_{734}/F_{682}	$R_{f\,682}$	$R_{f\,734}$	S, % of control
Nickel	9.17±0.46	0	0	49.2
Magnesium	9.98±0.67	3.45±0.19	2.48±0.32	100.0
Cobalt	8.35±0.34	1.47±0.31	1.54±0.20	50.9
Iron	8.15±0.50	2.44±0.44	1.73±0.26	77.4
Cadmium	9.20±0.45	0.86±0.27	0.71±0.35	34.5
Diuron	7.68±0.41	0.10±0.03	0.09±0.02	73.2
Control	9.01±0.49	2.80±0.38	2.32±0.22	100.0

Data from Table 2 were obtained for leaves being at the stage of leaf surface formation. Except magnesium, all the test solutions inhibited growth processes that is seen from leaf area S after 4-day growing in the presence of salts of heavy metals. The indices $R_{f\,682}$ and $R_{f\,734}$ vary in accordance with growth changes. The correlation factors are 0.75 and 0.7, respectively.

The heavy metals and diuron affected biosynthesis of chlorophylls a and b in juvenile leaves in the following way. Pigmentation increased by 44% in cadmium solution, decreased by nearly 15% in presence of diuron, while in other cases the concentration decrease did not exceed 10%. Therewith, leaves in nickel solution became black and withered; in cobalt solution they became black near ribs and in cadmium solution withered.

Then the effect of the test substances on adult leaves was studied. Variations of F_{734}/F_{682} were virtually the same as for juvenile leaves (Table 3). No morphological changes in birch leaves appeared even after 4 days of observations. The chlorophyll concentration was close to the control. The heavy metals influenced the potential photosynthetic activity, but differently than in the case of juvenile leaves. For instance, the magnesium inhibited the activity instead of stimulating it while cadmium had no action at all.

Table 3. Variations of fluorescence parameters for adult leaves, when 4-day growing birch branches in solutions containing salts of heavy metals

Acting agent	F_{734}/F_{682}	$R_{f\,682}$	$R_{f\,734}$
Nickel	9.70±0.29	3.40±0.35	1.28±0.21
Magnesium	9.17±0.47	2.44±0.19	1.18±0.32
Cobalt	8.85±0.29	2.48±0.11	1.53±0.13
Iron	8.75±0.33	1.63±0.44	0.95±0.35
Cadmium	9.82±0.43	3.26±0.13	2.12±0.11
Diuron	7.88±0.10	0.06±0.03	0.00±0.00
Control	9.58±0.52	3.32±0.48	1.72±0.22

Morphological changes in adult birch leaves clearly appeared on the 12th day of the experiment. Table 4 presents variations of chlorophyll concentration in the leaves and fluorescence parameters. As is evident, chlorophyll concentration decreased under action of heavy metals.

It is characteristic, that the R_f value, registered on the 4th day (see Table 3), indicates a deviation from the standard for these salts. Thus, the potential photosynthetic activity of leaves is the parameter, which predicts variations of chlorophyll concentration before their appearance (the 4th and 12th day, respectively). The R_f parameter itself was equal to zero on the 12th day for all solutions except for iron; however, the potential photosynthetic activity in the last case was lower than in the control.

Table 4. Variations of chlorophyll concentration and fluorescence parameters for adult birch leaves, when 12-day growing birch branches in solutions containing salts of heavy metals

Acting agent	Chl (a+b), mg/dm^2	F_{734}/F_{682}	$R_{f\,682}$	$R_{f\,734}$
Nickel	6.08±0.42	5.51±0.13	0	0
Magnesium	6.56±0.38	8.46±0.17	0	0
Cobalt	3.87±0.27	6.99±0.09	0	0
Iron	2.35±0.18	7.14±0.14	2.49±1.10	1.94±0.59
Cadmium	1.98±0.15	2.78±0.13	0	0
Diuron	7.10±0.49	8.96±0.10	0	0
Control	7.49±0.52	8.16±0.28	3.20±0.84	2.03±0.32

The F_{734}/F_{682} parameter for adult leaves decreased on the 12th day of observations in solutions with nickel, cobalt, cadmium, and iron ions, while remained equal to the control for magnesium and diuron.

The data obtained evidence the fact that the F_{734}/F_{682} parameter for birch leaf varies in response to attack by pathogenic organisms, treatment of juvenile leaves by herbicides, and industrial atmospheric pollution. However, it does not change up to 4 days when growing trees in presence of salts of heavy metals. These substances cause morphological changes in juvenile leaves on the 4th day and in adult leaves on the 12th day. Early precursors of the changes are the indices reflecting the potential photosynthetic activity at λ = 682 and 734 nm.

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