The correlation of the maximum intensity of fluorescence with pigment characteristics of leaves of *Betula pendula*

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ABSTRACT

Using fluorimeter Junior PAM (Heinz Walz GmbH, Germany) the fluorescence parameters of leaves of *Betula pendula* are investigated. A linear dependence of the maximum fluorescence (Fm) of leaves from the ratio of total chlorophylls concentration to concentration of carotenoids is obtained. Such dependence is found for samples collected during the period of vegetation and for simultaneous selection of colored leaves.

Keywords: fluorescence, chlorophyll, carotenoids.

INTRODUCTION

Previously we have found that the dependence of the maximum fluorescence (Fm) of plants from the concentration of total chlorophyll (C_{a+b}) depends on the method of sampling [1]. Such methods are two. One is the simultaneous collection of the leaves of plants with different color [2] and the other is in the process of vegetation [3, 4]. The first method can be realized when the colored leaves appear on the trees. The colored leaves of birch growing on the territory of Krasnoyarsk arboretum appear in late August and exist until the second decade of September. If the number of colored leaves are small at the end of summer so in September they predominate in the crown of a birch. As in 2013, simultaneous selection of leaves was carried out on September 13, it can be argued that the effect of the sampling method on the distinction dependencies of Fm on C_{a+b} is obtained only at the time of the domination of yellowing leaves. Thus, it remains unexplored the existence of above differences at a time when colored leaves do not dominate in the crown of the birch.

From late August until middle of September of 2014 three series of measurements of Fm, the concentration of chlorophylls and carotenoids (C_{car}) in colored birch leaves were performed. In addition, similar measurements were made in the leaves collected during the period of vegetation of birch (thirteen series of sampling).

In this paper we are presented and analyzed dependences of F_m on C_{chl} and C_{a+b}/ C_{car}.

2. MATERIAL AND METHODS

The object of the investigation was the leaves of the three trees of birch (*Betula pendula)*. Trees grew on the territory of the arboretum of the Institute of forest SB RAS (Akademgorodok, Krasnoyarsk). During the vegetation samples were collected in May-September of 2014. Three leaves from each tree are taken for analysis. First, we measured the fluorescence of chlorophyll. Then a circular plate with a diameter of 8 mm cut from the middle part of each leaf. All nine plates were mixed together, and pigments were extracted from this sample.

Colored leaves were collected on 28 August, 7 and 13 September. The collected leaves were classified by their color, which was visually determined. The pictures of these leaves are presented in figure 1. 3-5 measurements of intensity of chlorophyll fluorescence were performed on each leaf. Then nine round plates with a diameter of 8 mm were cut from the middle part of each leaf. All nine plates were mixed together, and pigments were extracted from this sample.

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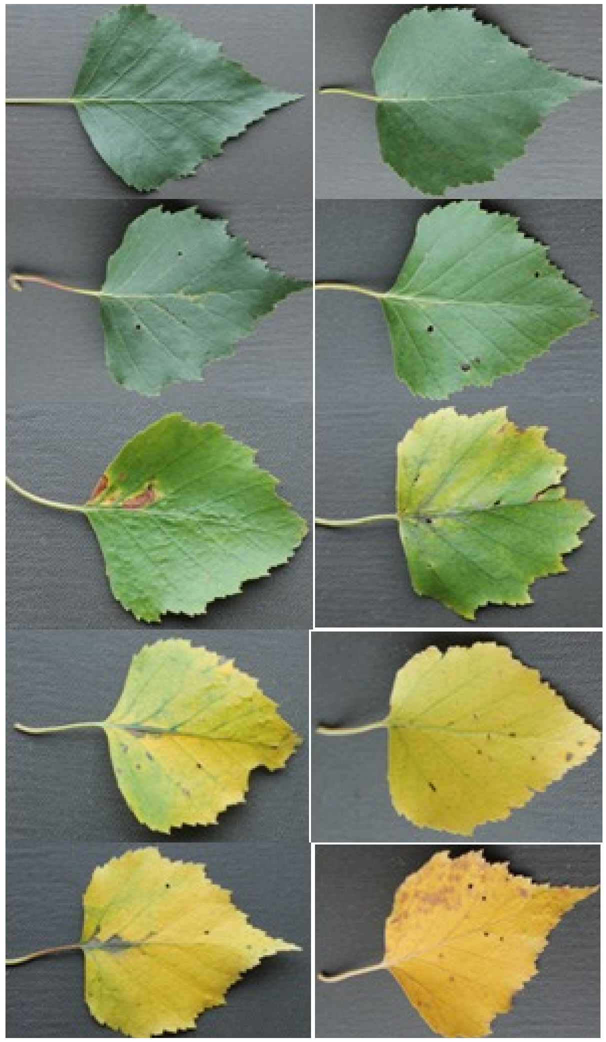

Figure 1. The pictures of colored birch leaves collected on August 28 of 2014

Pigments were extracted with 96% ethanol. Absorption spectra were recorded on a UVICON spectrophotometer 943 (Contron Instruments, Italy). The concentration of chlorophyll was calculated by the formulas [5]:

$$\text{Chl } a \text{ (mg/l)} = 13{,}7\ D_{665} - 5{,}76\ D_{649},$$

$$\text{Chl } b \text{ (mg/l)} = 25{,}8\ D_{649} - 7{,}60\ D_{665},$$

$$\text{Chl } (a + b) \text{ (mg/l)} = 6{,}10\ D_{665} + 20{,}0\ D_{649},$$

where D_{649} and D_{665} - optical density of the extracts of pigments at the wavelength of 649 nm and 665 nm, respectively. Carotenoid content was calculated by the formula [6]:

$$C_{CAR} \text{ (mg/l)} = 4{,}695\ D_{440,5} - 0{,}268\ (\text{Chl(a+b)}),$$

where $D_{440,5}$ - the optical density of the extracts of pigments at a wavelength λ=440,5 nm.

For the measurement of Fm a modulating pulse PAM fluorimeter (Junior PAM, “Heinz Walz GmbH, Germany) is used. Measurements were performed after 15 min of dark adaptation of the leaves.

Processing of experimental data was performed using software complex of Statistica 6.

3. RESULTS AND DISCUSSION

In 2014 a decrease of the light period and periodically lowering the air temperature below 10 ^{O}C are observed since the appearance of colored leaves (end of August) to complete yellowing of the crown of a tree (Fig. 2). These two factors caused a decrease of the intensity of photosynthesis [7]. During this period a decrease of the concentration of chlorophyll, the value of ratio of the total chlorophylls concentration to carotenoids and Fm are observed (Fig. 3-6).

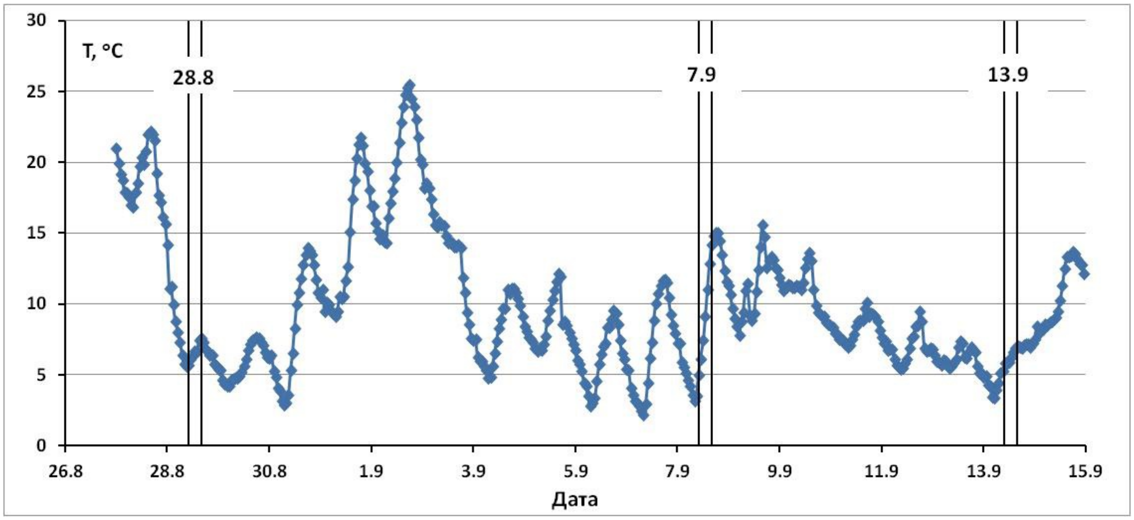

Figure 2. The change of air temperature in Krasnoyarsk from 27 August to 15 September 2014. Narrow the gap between the vertical lines shows the temperature during the selection of colored leaves: August 28, 7 and 13 September

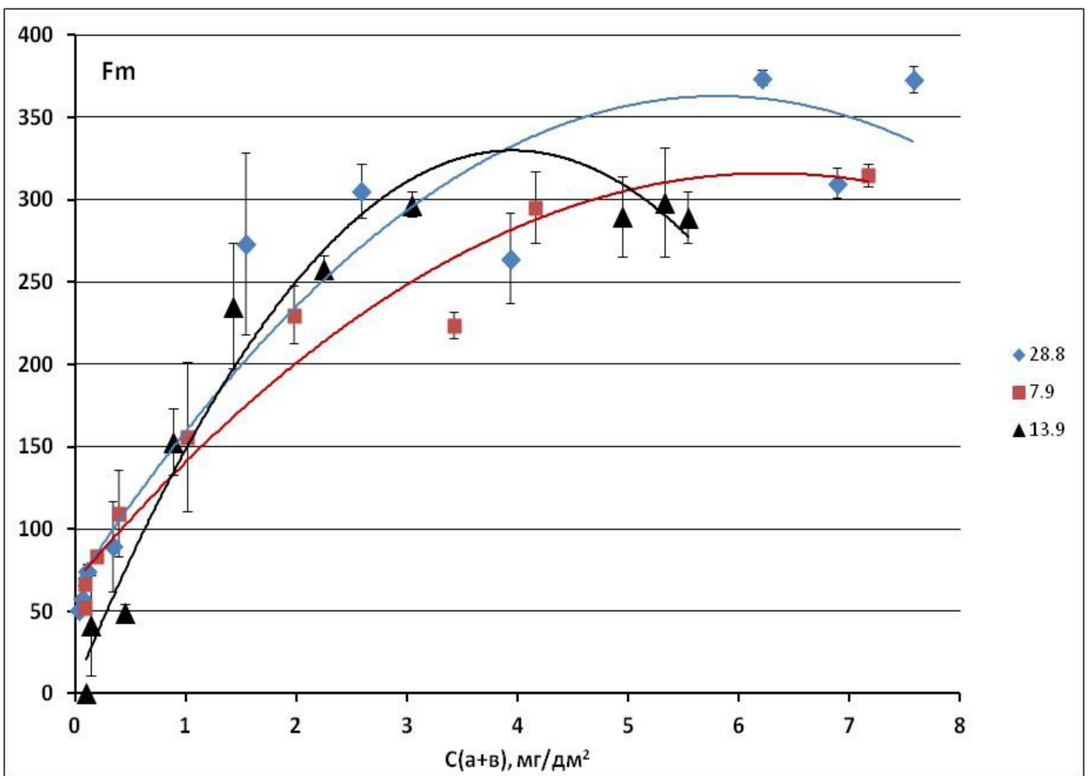

Figure 3. The dependence of Fm from the total chlorophylls concentration for simultaneous sampling on 28 August, 7 and 13 September

The dependence of Fm from the concentration of C_{a+b} in leaves collected on 28 August, 7 and 13 September, well approximated by quadratic functions 1-3, respectively.

$$y = -8{,}815x^2 + 102{,}36x + 65{,}767 \qquad R^2 = 0{,}91 \qquad (1)$$

$$y = -6{,}3x^2 + 79{,}091x + 67{,}824 \qquad R^2 = 0{,}95 \qquad (2)$$

$$y = -20{,}774x^2 + 164{,}35x + 4{,}8329 \qquad R^2 = 0{,}97 \qquad (3)$$

Extremes of equations 1-3 are 5,80; 6,28; 3,92 mg/dm^2. If we consider that the accuracy of determining the concentration of chlorophyll in our experiments does not exceed 8%, then we can talk about the reliable distinction of the extremes of equations 1-3. This means that the dependences of Fm from the concentration of $C_{a+в}$ in leaves collected on 28 August, 7 and 13 September are differed.

It should pay attention to the fact that dependences of F_m from $C_{a+в}/C_{car}$ (Fig. 4) are well approximated by linear equations 4-6.

$$y = 54{,}007x + 59{,}462 \qquad R^2 = 0{,}95 \qquad (4)$$

$$y = 47{,}543x + 65{,}415 \qquad R^2 = 0{,}95 \qquad (5)$$

$$y = 54{,}073x + 42{,}009 \qquad R^2 = 0{,}89 \qquad (6)$$

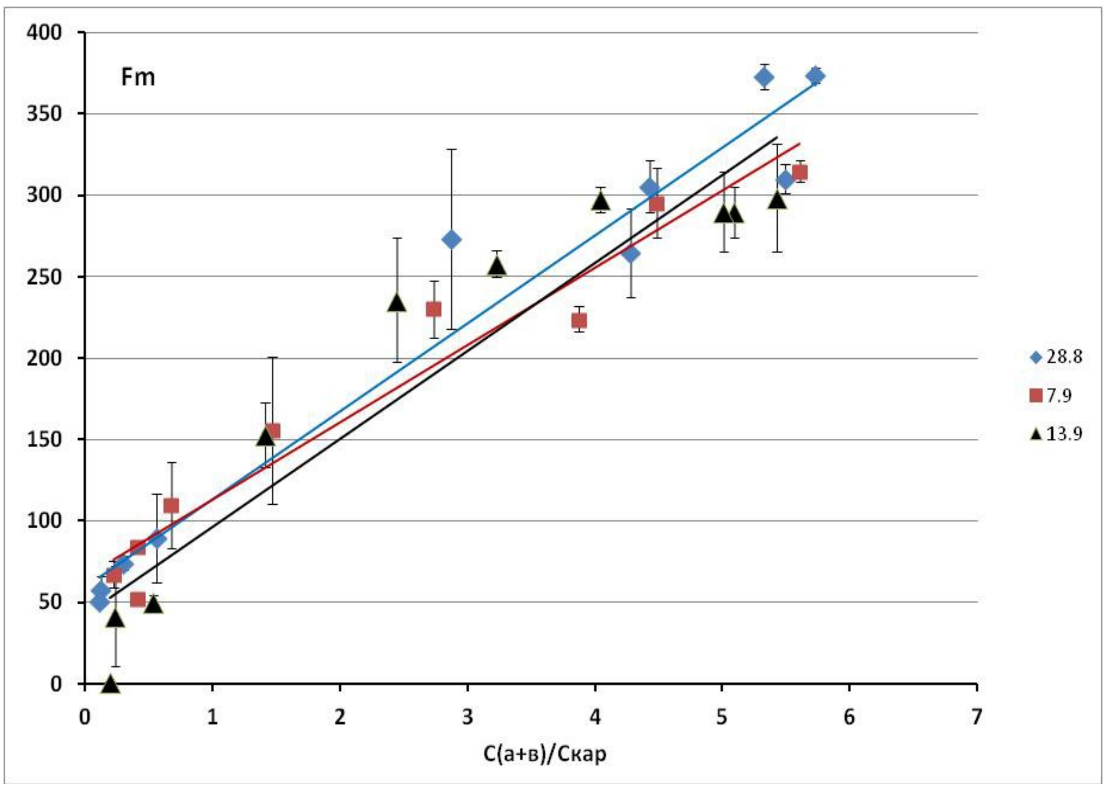

Figure 4. The dependence of Fm from ratio of total chlorophylls concentration to carotenoids for simultaneous sampling on 28 August, 7 and 13 September

The available literature we were unable to find a linear dependence of Fm from $C_{a+в}/C_{car}$ nor for any plant species. To explain the obtained linearity we will try to do as a result of further work.

Dependences of Fm from $C_{a+в}$ and $C_{a+в}/C_{car}$ during vegetation of birch are shown in Figures 5, 6. They are approximated by equations 7 and 8, respectively.

$$y = -4{,}2367x^2 + 64{,}539x + 90{,}137 \qquad R^2 = 0{,}83 \qquad (7)$$

$$y = 45{,}189x + 87{,}252 \qquad R^2 = 0{,}85 \qquad (8)$$

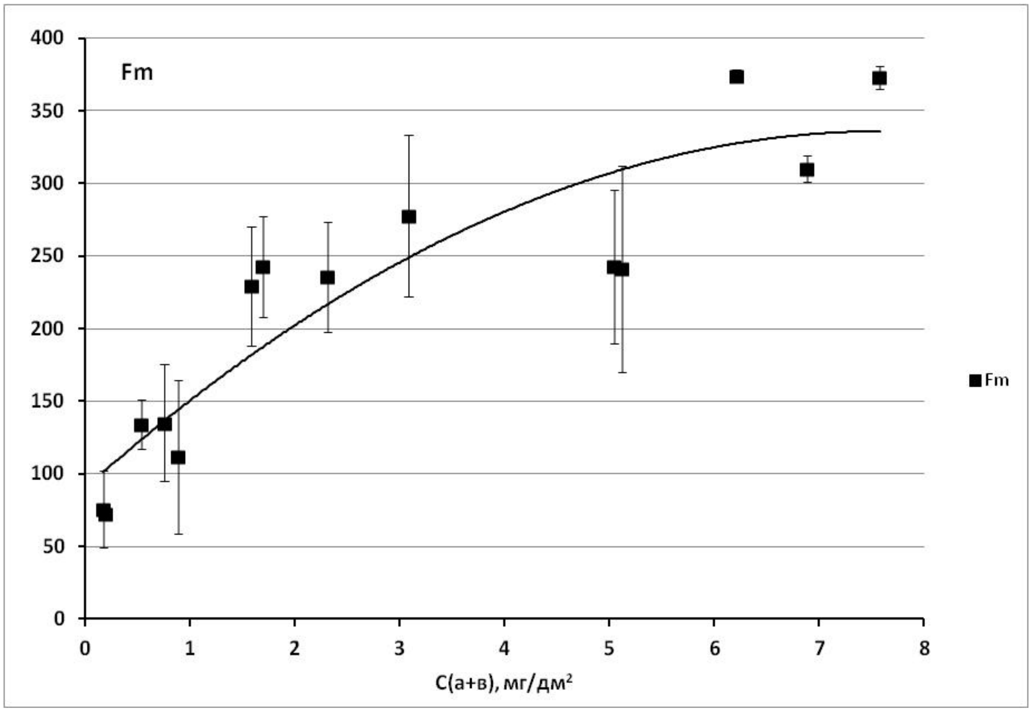

Figure 5. The dependence of Fm from the total chlorophylls concentration during the vegetation

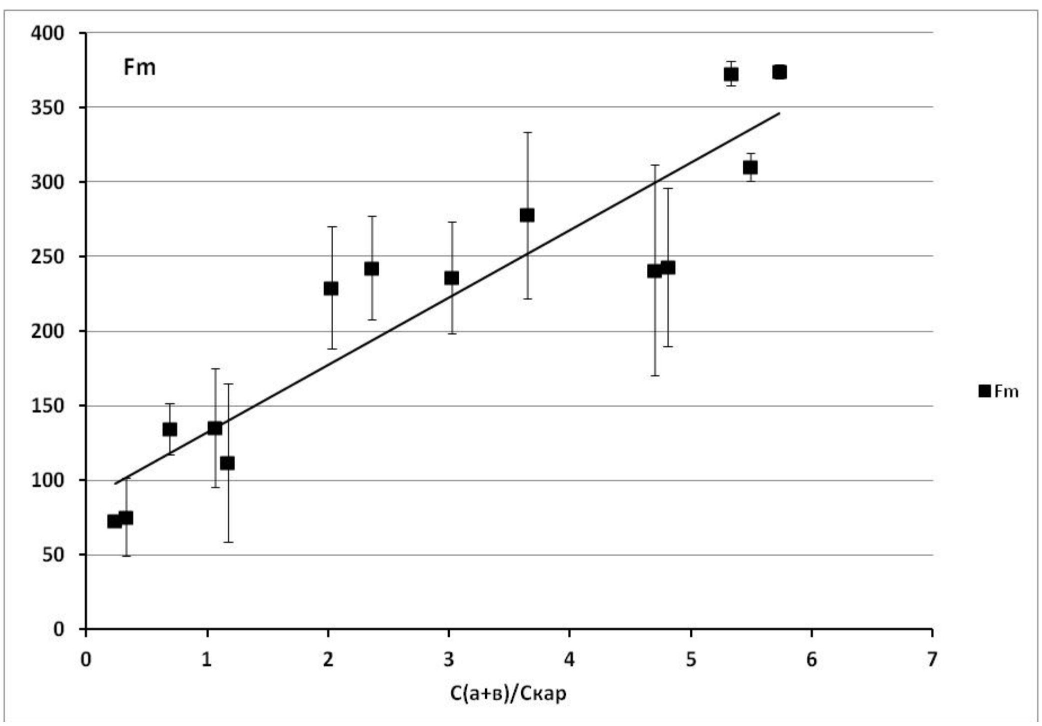

Figure 6. The dependence of Fm from ratio of the total chlorophylls concentration to carotenoids during the vegetation

The extremum of equation 7 is 7.62 mg/dm^2. It is significantly different from the extrema of equations 1-3. This fact on the one side confirms our earlier conclusion [1] that the dependence of Fm from the concentration of $C_{a+в}$ depends on the method of sampling, on the other hand complements it: none of the Fm dependence from the concentration of $C_{a+в}$, obtained in one-stage sampling, does not coincide with the analogous dependence obtained during the vegetation.

The dependence of F_m from $C_{a+в}/C_{car}$ during the vegetation of birch is described by the linear equation 8, in which in contrast to equations 4-6, the lowest coefficient of variable (45,189) and highest constant (87,252).

By using analysis of covariance (STATISTICA 6) was determined the level of significance of regression equations 4-6 and 8. It is equal to 0.8444, indicating no significant difference between the four data sets of dependence of Fm from $C_{a+в}/C_{car}$. Thus, method and time of sampling not affect on the type of dependences of F_m from $C_{a+в}/C_{car}$ unlike the dependence of Fm from $C_{a+в}$.

In [8] the chlorophyll meter SPAD-502 is used, which allows to measure chlorophyll content in relative units (Spad reading) (Chl_{rel}) in the range 0-100. The dependence of Chl / Car from Chl_{rel} ([8], Fig 2b) and the dependence on Fm from Chl_{rel} ([8], Fig 3b) are obtained. Both dependences are described by an exponential function. Consequently, a linear relationship between Chl / Car and Fm exists. This fact is an indirect proof of the existence of a linear dependence of Fm from $C_{a+в}/C_{car}$ for other plants (C. *canephora* Pierre).

4. CONCLUSIONS

1. Three of dependence of Fm from the concentration of $C_{a+в}$ obtained in one-stage sampling statistically significantly different extremes: in each series of observations maximum value of Fm correspond to different concentrations of the total chlorophylls. In addition, these three relationships are different from those that obtained during vegetation of birch leaves.

2. A linear dependence of Fm from $C_{a+в}/C_{car}$ is obtained. The nature of this relationship is not dependent on the method of selection of leaves for analysis.

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