INVESTIGATION OF THE PLANT FLUORESCENCE INDUCED BY THE BIHARMONIC OF A Nd:YAG LASER

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Experimental investigations of the fluorescence of tree crowns in the red spectral range induced by the laser radiation with a wavelength of 0.53 μm *have been carried out in summer-fall. Dependence of the quantum yield of chlorophyll a fluorescence on plant species has been established and investigated. Seasonal variations of the fluorescence intensity have the tendency to complete decay for the deciduous trees and to insignificant variations for the coniferous trees. Application of the fluorescence method to the remote specific categorization of the plant tissue and determination of the chlorophyll content is shown to be promising.*

The methods of laser sensing capable of remote contactless measurements are being increasingly used in various fields of science. Specifically, definite potentialities for determination of physiological state of plants under natural rather than laboratory conditions have laser radar systems[1] (lidars). Among the physical methods, attractive from the viewpoint of monitoring of the physiological functions of plants and using lidars, the methods of fluorescence analysis of plant tissue[2] upon exposure to a sounding laser beam should be mentioned. Thus, the chlorophyll fluorescence is widely used for the investigation of the state of photosynthesis of plants. For the classical model it is commonly assumed that green plants under normal conditions fluoresce as a result of energy deactivation by excited chlorophyll *a* molecules with the emission maximum at 685 nm.

We investigated the fluorescence intensity of some plants near 685 nm under natural conditions induced by the biharmonic of a laser on Nd:YAG crystal with a wavelength of 532 nm. The peculiarity of these investigations is the use of the green radiation, which is less intensively absorbed by cell membranes and is more informative for the determination of chlorophyll content,[4] as sounding one. Application of pulsed (10–15 ns duration) sounding radiation with the intensity 1–3 kW/cm^2 provides a means for an analysis of the most intensive fast fluorescence of nanosecond duration.[2]

DESCRIPTION OF MEASUREMENTS

Experimental investigations were carried out with a lidar having two receiving telescopes, one of which was adjusted to the detection of fluorescence signals near 685 nm. Block diagram of the lidar is shown in Fig. 1.

The laser *1* generates light pulses at a wavelength of 1.064 μm. Then laser radiation is converted into the biharmonic and directed to the investigated object *7*.

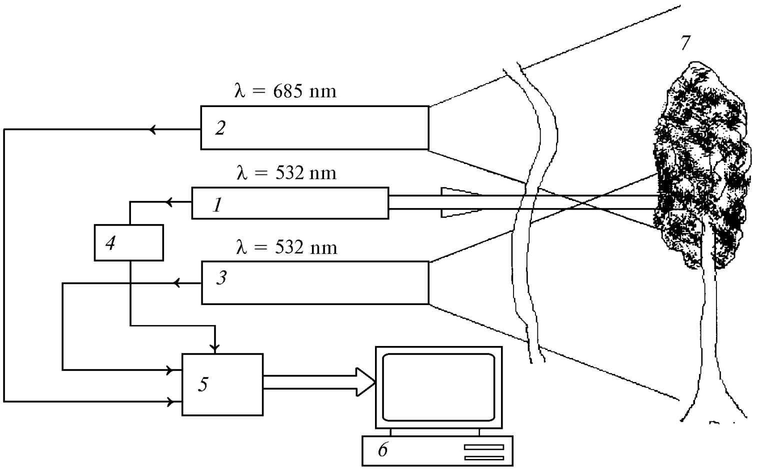

FIG. 1. Block diagram of lidar measurements of the plant fluorescence under natural conditions.

In the process of interaction with leaves, a light beam is partially scattered without changing of the wavelength and a part of the incident light energy is absorbed and converted into the Stokes component of fluorescence radiation with maximum at a wavelength of 0.685 μm. A part of inverted and scattered radiation is recorded by the receiving optical systems *2* and *3*, respectively, where the spectral selection of the received radiation is made with bandpass optical filters.

In connection with the fact that the levels of radiation received at different wavelengths are essentially different, neutral light filters are inserted into the receiving optical channel at $\lambda = 0.53$ μm to adjust the signal amplitudes at the exit. Then the signals are fed into the input of the 8-bit analog-to-digital converter *5* with a sampling frequency of 20 MHz, which is triggered by an output signal from an optical sensor built into the laser. Because the duration of the received signal is approximately 30\~ns, the spectrum of a reflected pulse occupies the frequency band of the order of 30 MHz.

Feasibility of application of the ADC with a sampling frequency of 20 MHz is provided by the controllable delay *4*, which is used to shift the time of ADC triggering from the pulse transmission moment. Thus, the time of data recording is adjusted to maxima of lidar return signals. From the exit of the ADC, signal digital codes enter the IBM PC–AT/286 *6*, in which they are registered, preliminary statistically processed, and recorded on a disk. Lidar specifications are given below.

Laser pulse energy, mJ	10–20
Duration of the radiation pulse, ns	10–15
Laser beam divergence, mrad	6
Diameter of the receiving telescope, cm	15
Field-of-view angle, mrad	9
Half-bandwidth of the filter centered at 685 nm, nm	50
Number of bits of the ADC	8
Sampling frequency, MHz	20

Registered in the receiving channel, lidar signals are described by the known laser sensing equation[5] which for the fluorescence channel can be written in the form

$$F(685) = \\ = F_0(532)\,\frac{532}{685}\,\frac{1 - A(532)}{4\pi}\,\frac{S}{L^2}\,\varphi\,T(532)\,T(685)\,, \qquad (1)$$

where F_0 is the peak power of a laser pulse, A is the albedo, S is the aperture of the receiving objective, L is the distance, T is the transparency of the atmospheric layer between the lidar and investigated plant at the given wavelength, and φ is the quantum yield of fluorescence. Here, it is assumed that the brightness body of fluorescence has the omnidirectional spherical form; moreover, the laser beam is completely covered by the plant canopy not only across the beam section, but also along the pulse length, at a distance of the half pulse length of the sounding laser pulse.

Radiation detected by the lidar on the sounding wavelength is given by the formula

$$F(532) = F_0(532)\,(S/L^2)\,T^2(532)\,d(532)\,, \qquad (2)$$

where d is the reflection coefficient (brightness coefficient[6]).

The reflection coefficient d and the albedo A are related as parameters that characterize the scattering in the same directions (in this case, backscattering) and the scattering in all directions. In general, this interrelation may be rather complicated (for example, cloud cover and water surfaces). However, for some models of the surface the relation between A and d takes specific form. In particular, the plant canopy for wide optical beams is satisfactory described by the Lambertian model.[7–9] Considering this approximation, Eq. (2) assumes the form

$$F(532) = F_0(532)\,(S/L^2)\,T^2(532)\,\frac{A(532)}{2\,\pi}\,. \qquad (3)$$

Now we examine the ratio of powers of laser signals given by Eqs. (1) and (3)

$$\frac{F(685)}{F(532)} = \frac{1}{2}\,\frac{1 - A(532)}{A(532)}\,\varphi\,\frac{532}{685}$$

or

$$f = \frac{F(685)}{F(532)} = 0.39\,\frac{1 - A}{A}\,\frac{T(685)}{T(532)}\,\varphi\,. \qquad (4)$$

Analyzing the last equation, we note that the fluorescence power f is directly proportional to the quantum yield of fluorescence. Usually, the albedo of wood plant near 532 nm lies within[7–10] 0.1–0.2 resulting in the range of variations of $(1 - A)/A$ from 4 to 9. Values of the transparency at wavelengths of 685 and 532 nm for short (up to several hundreds of meters) ranges are close to each other; therefore, $T(685)/T(532) \sim 1$. Considering the above assumptions, the parameter f in the first approximation characterizes the quantum yield of fluorescence induced by the laser at $\lambda = 532$ nm multiplied by the coefficient lying within the limits 1.5–3.5. For specific values of A, the coefficients f and φ take exact fixed values.

Considering that we record the fluorescence signal with the bandpass optical filter, it is well to bear in mind that the obtained data refer to the band 50 nm wide centered at a wavelength of 685 nm rather than to the narrow spectral interval. If we address to the typical fluorescence spectra of the plant tissue,[1] we may state that such a filter band comprises about 30–60% (depending on a specific plant category) of fluorescent energy of chlorophyll *a*. In our experiments, we measured the ratio f that characterizes the power of fluorescence near 685 nm according to Eq. (4).

Among most significant factors influencing the accuracy of measurements of the plant fluorescence characteristics, we can classify the shot and background noise, error of ADC digitizing, and systematic error due to uncertain knowledge of the parameters of optical attenuators in the channel at 532 nm.

The total error of individual measurement of the parameter f can be written in the form

$$\delta F = \sqrt{\delta D^2 + \delta K^2 + \delta P^2} ,$$

where δD is the error caused by combined effect of shot and background noise, δK is the error of ADC digitizing, and δP is the error in determining the optical filter characteristics. In this case, we should consider that δD and δK are random and δP is systematic in character. The measurements were carried out in the dark when the background noise level was very small and could be neglected. Objects of sounding were at distances of about 50–100 m; therefore, the fluorescence signals had significant amplitudes and the relative level of shot noise was no more than 3–5%. Error of digitizing is conventionally determined by the discrete levels of one or two least-significant bits and for the 8-bit ADC does not exceed 1%. Optical characteristics of the light filters were determined on the basis of comparative measurements, which were compared with the rated data.

An analysis showed that the transmission coefficients of the light filters may be determined with the error no more than 4%. Considering that the random errors in determining the average values of F obey the Student law and that during the experiment we performed no less than 300 measurements for each type of trees, we obtain that random error for the measurement cycle did not exceed 2%. Thus, the total error of determining the average value of $F(532)$ did not exceed 6%. It did not exceed 2% for $F(685)$ and 8% for the average value of the ratio f.

When interpreting the measurements, we should consider the possible spread of f caused by the humidity anomaly, external illumination, nonuniform chlorophyll and fluorescence distribution over plant crowns, surrounding temperature, pressure, and so on. Peculiarities of distribution of f over the crown of investigated trees are analyzed below. Quantitative contribution of other factors cannot be estimated now.

The experimental measurements were conducted twice a week in the evening and night time in August – September 1996. This makes it possible to investigate the plant fluorescence characteristics when the crown color changed from green in August to yellow in September and when deciduous trees lost their leaves. As investigated objects, we have chosen: birch-tree (*Betula verucosa L*), aspen-tree (*Populus tremula L*), and pine-tree (*Pinus silvestris*) 25–45 years old.

EXPERIMENTAL RESULTS

The results of two-month measurements of the fluorescence intensity are shown in Fig. 2. It is seen from the figure that irrespective of the plant types (deciduous or coniferous), the ratio f typically decreased with the approach of fall and winter. In this case, the largest range of variation was observed for the deciduous trees. For birch-tree and aspen-tree, f during this period changed 20–25 times, whereas f for the pine- tree changed only 4 times. For the deciduous trees, the smallest fluorescence intensity was observed at the end of September, whereas for the coniferous trees (pine- tree) – approximately on August 20–30. These peculiarities of fluorescence of coniferous and deciduous plants are well known.[4] They can be explained by the change

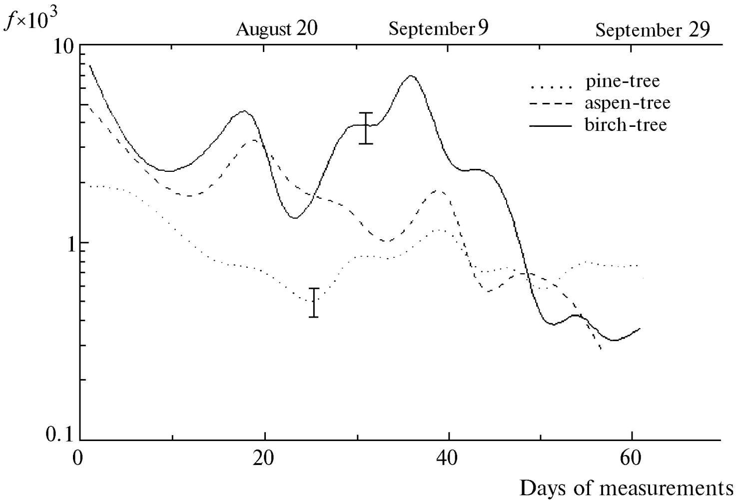

FIG. 2. Temporal behavior of the ratio f for three types of trees – pine-tree, aspen-tree, and birch-tree. Vertical bars show a probability level of 0.95.

of chlorophyll content in plant tissue and may provide the methodical basis for remote monitoring of chlorophyll concentration in green plants.

The entire period of observations may be tentatively divided into three intervals: "summer" (from August 1 to 30), "transient period" (from August 30 to September 10), and "fall" (from September 10 to 30), each characterized by specific behavior of the parameter f. Summer is characterized by the largest values of the quantum yield of fluorescence for all types of plants at the beginning and by its smooth decrease at the end. This period is interesting due to the presence of the second maximum of the parameter f for the deciduous trees observed on August 15–20 and absence of this maximum for the coniferous trees. In the transient period, we have the region of the increase of the fluorescence characteristics. In this case, the maxima for birch and aspen coincide in time with the appearance of the first yellow leaves on these trees. Additional measurements conducted in this period showed that the fluorescence of the yellow leaves of birch and aspen was even higher than that of the green leaves.

Fall period is characterized by the deficiency of chlorophyll and fall of the leaf manifested through the sharp decrease of fluorescence characteristics of the deciduous trees and relative constancy of these characteristics for the coniferous trees.

The absolute values of the ratio f in all periods of observation showed the specific dependence. Intensity of birch fluorescence exceeds the fluorescence intensity of other types of trees practically for the entire period of observations, except the fall of the leaf when the fluorescence of the deciduous trees became weaker than the fluorescence of the coniferous (pine) trees. The smallest values of the ratio f were obtained in sensing of the pine. Fluorescence intensity of aspen was intermediate between those of birch and pine.

Speaking about the identity of chlorophyll and photosystem cycles, as a whole, together with the decrease of the fluorescence intensity by the fall, the measurement curves have local peculiarities (maxima and minima), which are repeated for the deciduous trees (maxima on August 20 and September 5–10).

As sensing of various parts of tree crowns showed, the intensity of fluorescence had the spread. For the deciduous trees this spread of the parameter f was smallest in summer and was 10–20%. For the pine, this period was characterized by the largest variability of the ratio f of the crown, which could be as large as 30%. In transient period and in fall variation of pine fluorescence intensity on the crown was decreased and did not exceed 8%. For the deciduous trees, transient period and fall were accompanied by the appearance of yellow leaves, which differed noticeably by the fluorescent characteristics from the green leaves. As a result, the degree of inhomogeneity of the ratio f on the crown increased and reached 35% for aspen and 45–85% for birch.

As a whole, the experiment has shown the following:

1. We can determine with confidence the specific category of trees as well as the state of their deciduous and coniferous cover by the methods of lidar sensing.
2. The deciduous trees possess the largest range of variation of the parameter f.
3. Immediately before the fall of the leaf, when the leaves became yellow, we observed the increase of the parameter f for the trees.

It also should be noted that this technique can be used effectively for airborne sensing of the Earth's surface.

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