Application of the laser-induced fluorescence effect to remote studies of the photosynthetic mechanisms in plants

N.A. Vorob'eva, A.I. Grishin,* A.P. Zotikova,** G.G. Matvienko,* O.A. Romanovskii,* and O.V. Kharchenko***

* *Institute of Atmospheric Optics, Siberian Branch of the Russian Academy of Sciences, Tomsk*
** *Tomsk Branch of the Forestry Institute, Siberian Branch of the Russian Academy of Sciences, Tomsk*

Received March 23, 2000

Comparative research of chlorophyll content for a number of arboreous plants is carried out using traditional spectrophotometric and fluorescent laser techniques. Seasonal changes are analyzed in the net chlorophyll content during the spring-summer period for coniferous and deciduous plants. The changes in the chlorophyll content due to pine-needles and leaves fading are observed. The experimental results are obtained using the spectrophotometric and lidar techniques to study the seasonal dynamics of chlorophyll and pigment complex destruction in the course of pine needles and leaves fading. Analysis has shown that the results obtained by different methods are identical.

In recent years the lidar sensing techniques, which enable one to make remote contactless measurements, have been widely used in different fields of science. Lidars show certain promise in determining the plant physiological state under natural conditions.[1] Among spectroscopic effects, attractive from the viewpoint of following up the physiological functions of plants and suitable for implementing in lidar systems, we consider the methods based on analyzing the fluorescence of plant tissues.[2–5] Fluorescence of chlorophyll is widely used to analyze the photosynthetic mechanisms in plants.

The photosynthetic function of a leaf and its pigment complex are characterized by strong variability. These variations are connected with the regularities of ontogenesis of a leaf, arboreous organism, and the effect of the extreme factor of a medium, which considerably change the size and rhythm of the plant growth processes, intensity of photosynthesis, and pigment system characteristics.[6]

The pigment system of plants, in particular the chlorophyll content, being a criterion of the vegetation evaluation, simultaneously characterizes the state of the environment. This is indicative of the potential needs in using this criterion as a reference one. However, the currently used techniques of biochemical determination of quantitative chlorophyll content are rather laborious and require considerable time. Besides, in determining the chlorophyll content in different solvents the native structure of the leaf pigment complex is disarranged that produces difficulties in the interpretation of the obtained results. This fact favors the necessity of the development of new techniques for investigating quantitatively the content of pigments and organization of the pigment system of plants. At present the work has been undertaken in order to develop the fluorescent techniques of investigations, in particular, with the use of lasers.

The goal of this paper was to make a comparative study of the chlorophyll content in a number of arboreous plants using traditional spectrophotometric and fluorescent laser techniques, in particular:

1. The investigation of seasonal variations of the sum of chlorophylls during the spring-summer period for coniferous and deciduous plants.

2. The observations of variations in the chlorophyll content due to pine-needles and leaves fading.

In the experiments we used branches from the middle part of the crown cut from one and the same preliminary selected trees over a period from May to September. The objects of research are pine, cedar, birch, and aspen. For spectroscopic determination of chlorophyll content the preset amounts of leaves and pine-needles were powdered in 100% ethyl alcohol and centrifuged during 20 minutes at 8000 revolutions/min. The pigment content was determined with a "Specord–V–VIS" using alcohol extracts with the subsequent calculation by the formulas from Ref. 7. The pigment content in the leaves and pine needles of these branches was also determined over the same period using the remote laser technique.

The results of spectrophotometric determination of the pigment content are given in Tables 1 and 2. From the tables we can see that both the chlorophyll content in different trees and its variation during the observation period are not the same. Thus, two years old needles of pine and cedar are characterized by more or less constant chlorophyll content as compared with the leaves of a birch and an aspen (see Table 1). The same changes were observed in studies the leaves using the remote laser technique.

0235-6880/00/05 502-04 $02.00

Table 1. Seasonal chlorophyll dynamics in the leaves of arboreous plants, mg/g of damp mass

Date of picking up	Pine (two years old needles)	Cedar (two years old needles)	Birch	Aspen
04.20.99	0.83 ± 0.03	–	–	–
05.14	0.97 ± 0.02	1.60 ± 0.02	1.14 ± 0.01	–
06.2	0.96 ± 0.05	1.29 ± 0.03	1.65 ± 0.02	2.51 ± 0.01
06.25	1.11 ± 0.04	1.62 ± 0.01	2.01 ± 0.02	2.76 ± 0.03
07.1	1.02 ± 0.02	1.71 ± 0.02	2.57 ± 0.01	2.53 ± 0.04
09.23	1.64 ± 0.03	1.60 ± 0.04	0.70 ± 0.08	1.25 ± 0.01

Table 2. Variation of chlorophyll content with fading leaves and needles of arboreous plants mg/g of dry mass

Version	Date of sampling	Duration of test, days	Pine	Cedar	Birch	Aspen
Control	14.05	–	2.06 ± 0.04	3.81 ± 0.04	3.8 ± 0.02	–
Test	2.06	19	1.07 ± 0.10	1.38 ± 0.02	1.36 ± 0.08	–
Control	2.06	–	2.04 ± 0.11	3.07 ± 0.07	5.50 ± 0.05	8.96 ± 0.03
Test	1.07	29	0.90 ± 0.12	1.30 ± 0.15	0.85 ± 0.08	0.97 ± 0.09

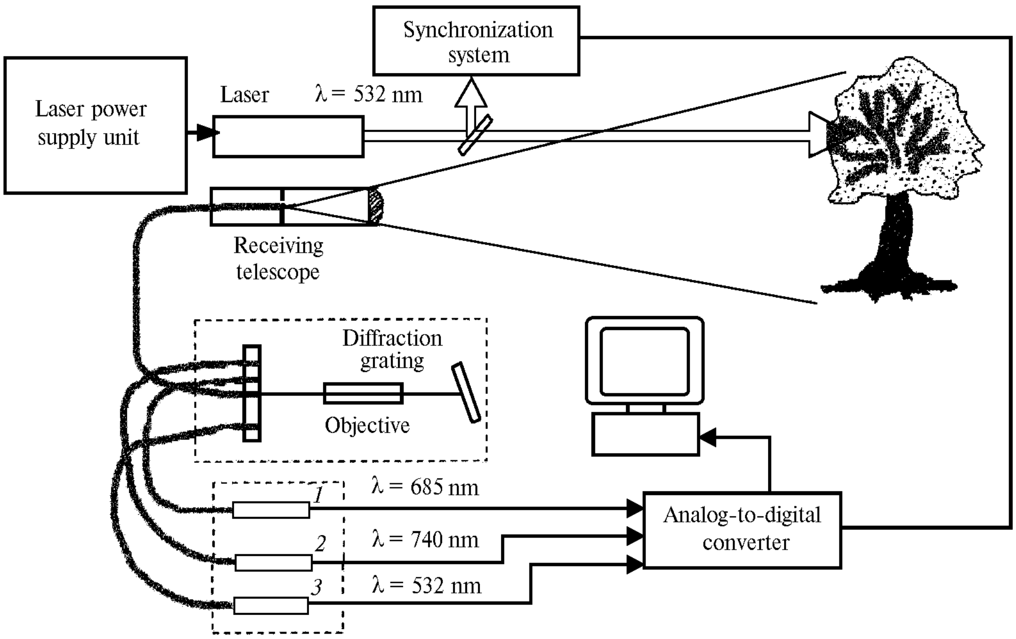

Fig. 1.

With fading of the leaf apparatus, gradual destruction of its pigment complex was observed. The pigment system in pine was most stable, while the pigment system in aspen was destructed most rapidly (see Table 2).

Along with the spectrophotometric measurements, we performed field investigations of the plants based on the study of the laser-induced fluorescence. Previously we measured the laser-induced chlorophyll fluorescence by recording the lidar returns at two wavelengths, 532 and 685 nm.[8,9]

The measurements were based on an updated lidar recording system that uses the radiation of chlorophyll fluorescence at the three wavelengths of 685 nm, 740 nm, and 532 nm. In this case, use of a 12-bit analog-to-digital converter enables us to extend considerably the dynamic range of a lidar return and to increase the measurement accuracy.

Figure 1 shows the optical arrangement of the lidar observations of the plant fluorescence using the three sounding wavelengths.

The lidar operation is as follows. A radiation pulse is emitted by the laser at the wavelength of 532 nm along the direction of an object sounded. The fluorescence is excited in the Stokes region under the action of incident light. A fraction of radiation collected by a receiving telescope is directed by an optical fiber waveguide to the spectrophotometer, assembled following the autocollimation optical arrangement. The radiation intensity is measured at three wavelengths: 685 nm, 740 nm, and 532 nm. The first two wavelengths are caused by the chlorophyll fluorescence of *a* and *b* type, respectively. The third wavelength is necessary for normalizing the received fluorescence radiation. From the spectrophotometer exit the radiation is directed through optical fibers to the block of photomultipliers (*1*, *2*, *3*) used as photodetectors. Then three electric signals are entered in the analog-to-digital converter providing the discretization at the 40-MHz frequency. A synchronization pulse formed from the sounding pulse, when it is delivered into the atmosphere, triggers the analog-to-digital converter. From the analog-to-digital converter output the digital information is entered into a computer where preliminary processing of data is performed and the return signals are stored.

The lidar measurement technique has also been modified. In this cycle, the lidar sensing was made using the same plant samples, which were used in the spectrophotometric investigations. The samples were placed in the pots filled with water at a distance of 70 m from the lidar and those were irradiated by lidar two times a week at nighttime. In this case, we observed the variations of fluorescence characteristics of chlorophyll starting from the time of sampling until the stage corresponding to a total fading of plants. All in all, 35 cycles of measurements were carried out, and the samples were replaced 6 times over a period from April 20 to September 23, 1999.

The time behavior of the fluorescence characteristics for cedar, pine, birch, and aspen is shown in Fig. 2 in relative units (*a* – the wavelength of 685 nm, *b* – 740 nm). The zero value corresponds to measurements on April 20, 1999. Vertical lines show the time of sample replacement.

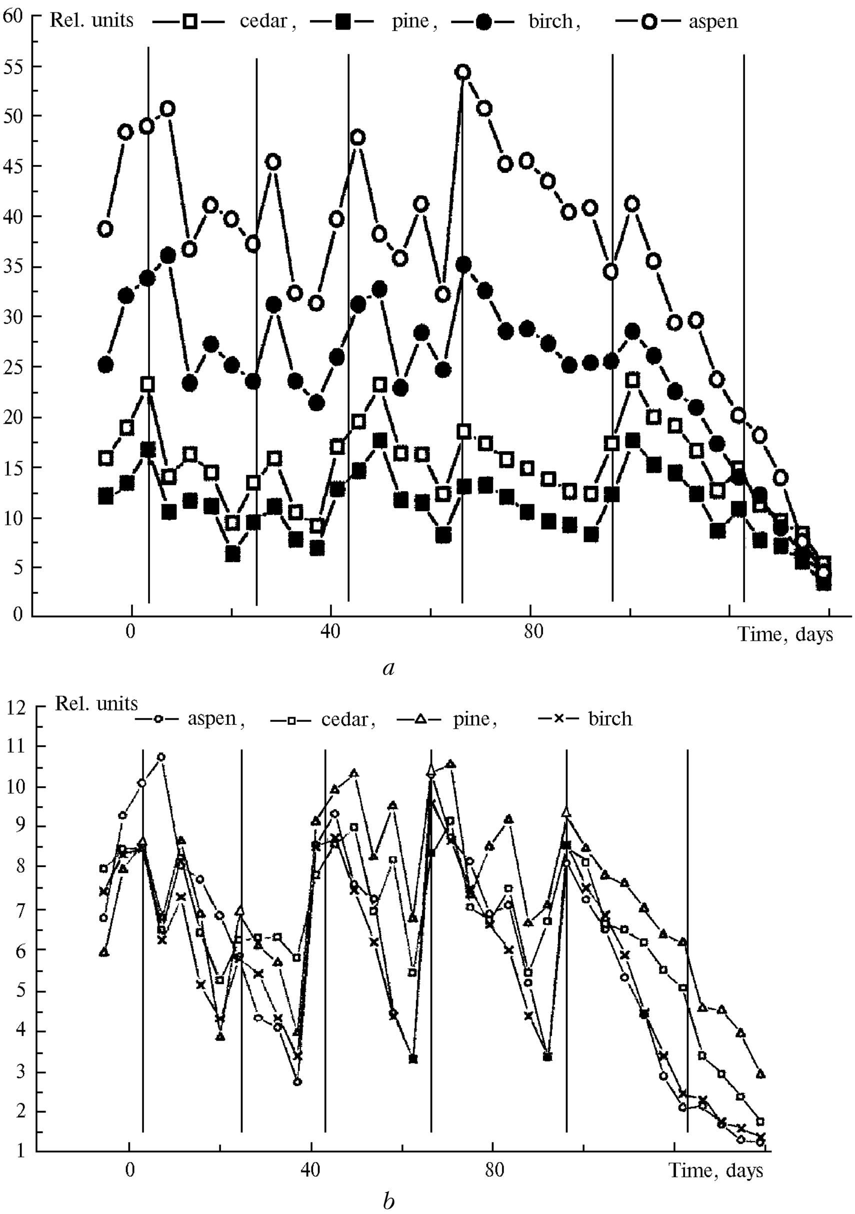

Fig. 2.

Analysis of data presented in Fig. 2 shows that the intensity of fluorescence at the wavelengths of 685 and 740 nm is strongly different, especially in the case of aspen. The ratio of the fluorescence intensities for aspen varies within 4 – 5 times. The least ratios are typical for conifers, for which these values vary within 1.5 – 2 times. These measurements confirm the conclusion drawn previously that the highest intensity of fluorescence is observed for broad-leaved trees during the spring-summer period.

Figure 2 shows that the time behavior of the fluorescence intensity is subject to cyclic variations, whose period coincides with the intervals between the replacement of studied samples. Figure 2 shows that each of these intervals is characterized by a decrease in the fluorescence level. This regularity can easily be observed for broad-leaved trees. It manifests itself in all the intervals for the two spectral ranges. It should be noted that the data acquired at a wavelength of 740 nm changed slightly as compared with the data at 685 nm wavelength. In this case it should be mentioned that the data at 740 nm refer to the chlorophyll type *a*.

A comparison of the results obtained based on the direct measurements of chlorophyll content (spectrophotometric method) and lidar measurements shows that there is certain relation between these results. In any case, the ranges of variability of chlorophyll content coincide based on the data of the spectrophotometric and lidar measurements. It should be noted that this coincidence refers to all types of trees studied in this experiment.

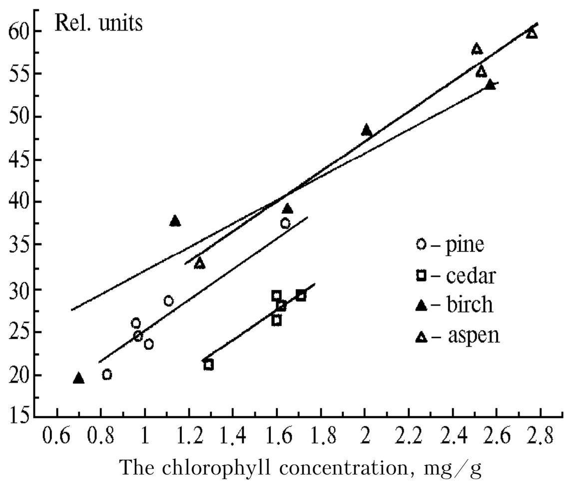

Fig. 3.

Figure 3 shows the calibration characteristics of lidar system connecting the net chlorophyll content in plants with the fluorescence signals. In principle, these data enable us to assess directly the state of plants using this lidar. However, when passing to another measuring system, the calibration should be repeated. Therefore in the subsequent work we plan to determine the interconnection between the chlorophyll concentration and the quantum yield of the fluorescence that would make it possible to improve the quality of measurements.

Thus, we have obtained the experimental data on seasonal dynamics of chlorophyll and destruction of pigment complex in the course of fading of needles and leaves of arboreous plants using the spectrophotometric and lidar techniques.

Analysis showed the identity of the results obtained using different techniques.

Acknowledgments

The research work has been performed under a support from the Russian Foundation for Basic Research, Grants No. 98–04–03068, 98–04–03099, and 99–04–49085.

References

1. Yu.L. Lukin, V.S. Agishev, A.D. Bernikov, et al., Atm. Opt. **2**, No. 5, 414–418 (1989).
2. N.A. Gaevskii and V.N. Morgun, Fiziologiya Rastenii **40**, No. 1, 136–145 (1993).
3. T.V. Nesterenko and F.Ya. Sid'ko, Fiziologiya Rastenii **40**, No. 1, 10–15 (1993).
4. K.Ya. Kondratiev and V.I. Kanevskii, *Laser Remote Sensing of Vegetation* (Gidrometeoizdat, Leningrad, 1987), 168 pp.
5. N.N. Vygodskaya and I.I. Gorshkova, *Theory and Experiment in the Remote Sensing of Vegetation* (Gidrometeoizdat, Leningrad, 1987), 178 pp.
6. A.T. Mokronosov, *Ontogenetic Aspect of Photosynthesis* (Nauka, Moscow, 1981), 333 pp.
7. A.A. Shlyk, *Biochemical Methods in Physiology of Plants* (Nauka, Moscow, 1971), pp. 91–154.
8. A.I. Grishin, G.G. Matvienko, O.V. Kharchenko, and V.I. Timofeev, Atmos. Oceanic Opt. **10**, No. 7, 503–506 (1997).
9. A.I. Grishin, G.G. Matvienko, O.V. Kharchenko, et al., Atmos. Oceanic Opt. **12**, No. 4, 320–323 (1999).