Remote evaluation of the state of photosynthetic mechanism in plants by the method of laser–induced fluorescence

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Laser-induced fluorescence in plants that are under normal and stress conditions is studied. Fluorescent properties of some plants upon the exposure to Xe–Cl-laser radiation at $\lambda = 308$ nm are analyzed under laboratory conditions. A stable relation between the recorded fluorescence signals and the chlorophyll content is found. Results of remote measurements of fluorescence signals from some trees under natural conditions are presented. Lidar sensing methods are shown to be applicable to reliable determination of species, as well as of the state of foliage or conifer of the trees.

Introduction

Study of plant chloroplast pigments, as a basic structure-functional chain of the photosynthesis process, dominates among numerous methods for evaluation of the state and injury of biological objects. However, it is not easy to analyze trees having large size and a complex morphological structure. Therefore, the search for new methods to study the photosynthetic mechanism in plants, which would supplement the traditional approaches, is very urgent now. The sought methods should provide sufficiently bulky and reliable information about properties of organisms under study. The luminescence methods, which allow nondestructive monitoring of the processes running in a cell at the membrane and molecular levels, acquire a great significance.[1] Most interesting are remote methods of plant state diagnostics, among which the lidar sensing method plays a great part.

Among optical methods applicable to study the photosynthetic mechanism in plants, the methods analyzing fluorescence of chlorophyll upon exposure to sensing laser beam deserve special attention.[1,2] In this case, the greatest effect can be achieved in complex studies combining laboratory and *in situ* measurements. The laboratory measurements in our case provide the study of fluorescence spectra in a wide spectral range (300–750 nm) for plants under normal and stress conditions. The relation between the fluorescence signals and the chlorophyll content can also be found from the laboratory studies of leaf samples of some plants. The *in situ* measurements, in their turn, allow one to follow up the dynamics of the state of the photosynthetic mechanism under natural conditions. To evaluate this state, we used the fluorescence of *a* chlorophyll at 685 nm wavelength.

Laboratory studies

The fluorescence properties of some plant structures upon exposure to Xe–Cl-laser radiation at $\lambda = 308$ nm were studied under laboratory conditions. The Xe–Cl type of a laser source has been chosen because it allows most complete fluorescence spectra to be obtained.

To study fluorescent properties of the cuticle and mesophyll *in vivo*, leaves of such trees as birch, aspen, bird cherry, and rowan-tree were sampled during the spring-summer vegetation period. The samples corresponded to the middle layer, that is, the trees' age ranged from 10 to 30 years. The time from leaf disengagement to recording its fluorescence spectrum lasted 5 – 30 minutes. A leaf was disengaged in daytime and was in light during the whole experiment. Since luminescent indicators are very sensitive to changes in the physiological state of plants, we used a cut leaf plate in addition to an intact leaf.

The recorded fluorescence spectra of the intact (*1*) and cut (*2*) sections of a birch leaf are shown in Fig. 1. It is seen from Fig. 1 that fluorescence spectra have characteristic peaks at 440, 685, and 740 nm. Experiments have shown that the intensity of fluorescence of the cut birch leaf is several times higher than that of the intact leaf. Thus, the obtained results support the well-known fact that enhanced synthesis of the fluorescing compounds in cells is indicative of the plant injury or sickness.[3] When membranes are damaged, these compounds leave cells.[3]

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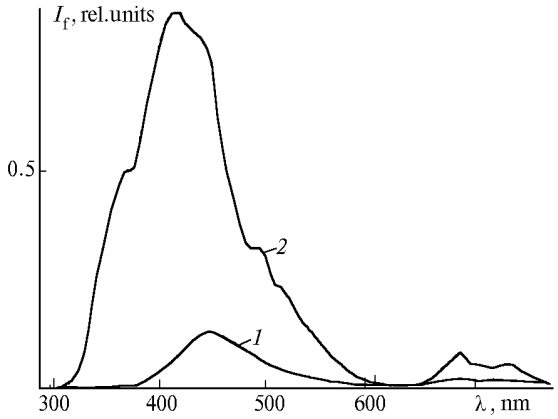

Fig. 1. The recorded fluorescence spectra of the intact (*1*) and cut (*2*) sections of a birch leaf.

In this paper, special study has been conducted of the relation between the integral intensity of chlorophyll fluorescence in living plant tissue and the chlorophyll content. Toward this end, the fluorescence spectra of chlorophyll contained in birch leaves have been studied. Simultaneously, the content of pigments in leaf samples has been analyzed by the standard spectrophotometric method. Figure 2 shows the dependence of the integral intensity of the fluorescence band on the chlorophyll content in the analyzed samples. As is seen, it is the direct proportion.

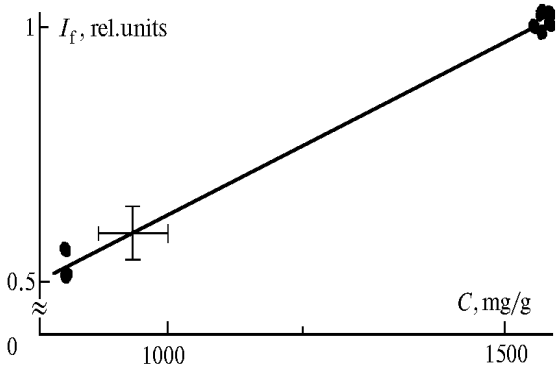

Fig. 2. Integral intensity of the chlorophyll fluorescence band vs. chlorophyll content. The vertical and horizontal bars show the 95-% confidence intervals.

The maximum fluorescence signal is proportional to the chlorophyll content in an object at a maximum yield of the fluorescence. This takes place when the chain of energy transfer from light quanta into the power system of the leaf is disrupted due to the effect of some chemical substances, for example, diuron (herbicide), or laser radiation.[4–6] In our experiments, the plants were exposed to a high-power (power density of 100 kW/cm^2) laser beam.

Thus, the laboratory experiments, simulating the remote laser sensing of plants, have shown that recorded values of the fluorescence signals are proportional to the chlorophyll content.

In situ measurements

To estimate remotely the chlorophyll content in plants under natural conditions by the method of laser-induced fluorescence, we have used second-harmonic radiation of a Nd:YAG laser at 532 nm. This spectral region has been chosen because the green radiation is absorbed more weakly by the cell membranes and therefore is more informative in determining the chlorophyll content.[7] The pulsed character of the sensing radiation and its power (1–3 kW/cm^2) provide for analysis of short-lived fluorescence of the nanosecond duration range.[1] At the same time, they give the possibility of operating within the range of linear interaction of optical radiation with the plant structures.

We have studied *in situ* the fluorescence intensity near 685 nm that corresponds to the fluorescence of *a* chlorophyll molecules of the photosystem 2 (PhS2) in a living leaf. The information promises of this range has earlier been supported by numerous experiments.[8,9]

The lidar used in the *in situ* measurements has been described in detail in Ref. 10, where its structure, operation, the basic relationships, and the accuracy characteristics can also be found. The parameter measured in experiments is the parameter *f*, equal to the ratio of signals from plants at 685 and 532 nm. It is shown in Ref. 10 that this parameter is proportional to the quantum yield of the fluorescence and can characterize it accurate to a constant factor.

The experimental measurements have been conducted since August of 1996 to June of 1997 two times a week in evening and at night time. As objects of the study, we have selected birch (Betula verucosa L.), aspen (Populas tremula L.), and pine tree (Pinus silvestris) aging from 25 to 45 years. The results of the fluorescence intensity measurements are shown in Fig. 3.

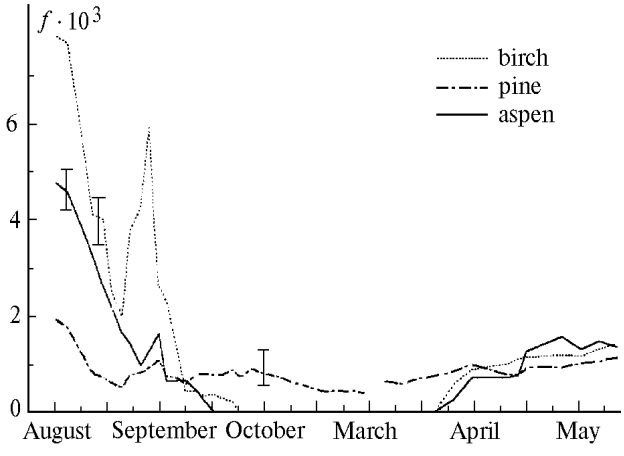

Fig. 3. Time behavior of the ratio *f* for three tree species. The vertical bars show the 95-% confidence intervals.

Peculiarities of the fluorescence of coniferous and deciduous trees are well known.[7] They are caused by different chlorophyll content in plant tissue and can serve as a methodical basis for remote monitoring of the chlorophyll concentration in green plants.

The whole period of observations can be divided into several intervals: summer-fall, winter, and spring. For the summer-fall period, the quantum yield typically peaks at the beginning of the period and then gradually falls off by the end of season for all the tree species. This period is interesting due to the presence of second peak of the parameter f for the deciduous trees, which falls on the early September. A slight increase in the fluorescence intensity for coniferous trees in this period is within the 95-% confidence interval (vertical bars), therefore it is not considered here as a peak.

It should be noted that the period of increase in the fluorescence intensity for deciduous trees coincides with the time of appearance of first yellow leaves on these trees. The regularity found can be explained by the destruction of pigment and chlorophyll-protein complexes of tilacoid membranes and, likely, the energy redistribution between the PhS1 and PhS2 pigment systems in the defoliation period and the increasing part of PhS2, which provides for fluorescence at 685 nm (Ref. 9). Additional measurements conducted at the end of this period have shown that fluorescence of yellow birch and aspen leaves is more intense than that of green leaves.

The winter period of observations is characterized by minimal variability of the ratio f for the coniferous trees. A smooth increase of the quantum yield of fluorescence is observed during the spring period for all trees, what can be explained quite naturally.

The absolute values of the ratio f in all the observation periods depend on a species. The fluorescence intensity of birches exceeds that of other trees practically all over the period of observations. The only exception is the defoliation period, when fluorescence of the deciduous trees becomes weaker than the fluorescence of coniferous trees (pine). The least value of the ratio f is observed in sensing of pine trees. The fluorescence intensity of the aspen has the intermediate value between those of the birch and pine. The different fluorescence intensity for different species is due to different chlorophyll content, as well as different ways of absorbed energy re-distribution among the chlorophyll-protein complexes and reaction centers in both of the photosystems.

Sensing of different parts of a top of trees has shown some scatter in values. For the deciduous trees, this scatter is minimal in summer-fall period reaching about 10 to 20%. For the pine, this period is characterized by the largest variability of the ratio f over the top; this variability may achieve 30%. In other periods, variability of the fluorescence intensity of the pine over the top decreases down to less than 8%. For the deciduous trees, the fall period is accompanied by the appearance of yellow leaves, which differ significantly in their fluorescence properties from the green ones. As a result, the ratio f starts to vary more widely over the top of a tree; and it can reach 35% for an aspen and 45 to 85% for a birch tree.

The fluorescence intensity variability over the top of a tree can be explained by different quality of leaves on the tree and in a tree stand.[11] (The leaf quality is closely related to its illumination conditions.) A leaf adapts to maximum absorption at the level of changes in the electron-transport chain of photosynthesis, pigments, and other parameters.

Of some interest is the analysis of influence of the background illumination on the quantum yield of fluorescence. Figure 4 shows the time behavior of the parameter f under different illumination conditions.

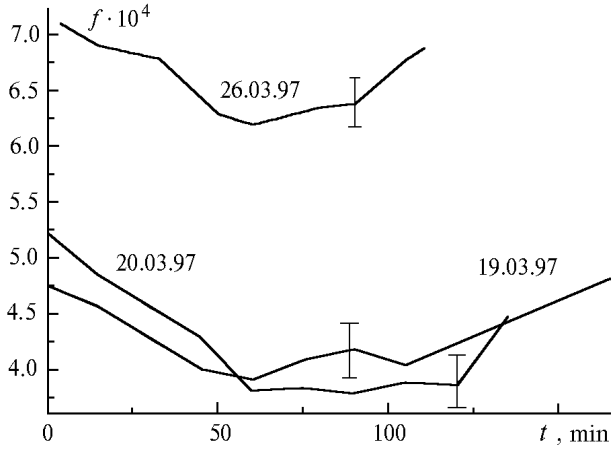

Fig. 4. Temporal behavior of the ratio f for a pine tree under different conditions of illumination.

The origin of coordinates in Fig. 4 corresponds to the sunset time. During the measurements, the illumination varied by about 50 times. It is seen from the data presented that immediately after sunset the fluorescence intensity starts to decrease slowly during 50 to 80 minutes. Then it returns to its initial value for almost the same time. The variability of f is 20 to 28%, what is within the measurement error. It should be noted that such a behavior of f cannot be explained within the framework of the existing models.

Conclusions

1. The directly proportional relation has been established between the integral intensity of the fluorescence band and the chlorophyll content in the analyzed samples.

2. The lidar sensing methods are suitable for a reliable determination of trees' species, as well as of the state of foliage or coniferous cover.

3. The parameter f is most variable in the deciduous trees.

4. Just before the defoliation, when leaves turn yellow, the ratio f grows for the deciduous trees.

It should also be noted that this method can be efficiently used, when sensing natural resources from onboard an aircraft.

Acknowledgments

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