INSTRUMENTS AND METHODS OF MEASUREMENT

LASER METHOD FOR VEGETATION MONITORING

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The paper considers a laser fluorescence method for vegetation monitoring. It presents the results of an experimental study of plants' laser-induced fluorescence spectra under various stress conditions caused by the presence of soil pollutants, excess water or mechanical damages. At the fluorescence excitation wavelength of 532 nm, the impact of different stress factors proves to manifest itself in both increasing the laser-induced fluorescence intensity and changing the form of the fluorescence spectrum, according to stress types and various vegetation types. Fluorescence intensities ratio at two wavelengths of 680 and 740 nm can be regarded as an identifying factor characterizing the form change of the laser-induced fluorescence spectrum. Measurement of both the intensity and spectrum form of plants' laser-induced fluorescence can be the basis of the laser method for detecting plants' stress conditions.

***Keywords*:** laser method, fluorescence, vegetation, detection of stress conditions.

Methods based on the analysis of the laser-induced fluorescence spectra are widely used in science and technology. The development of high-power pulsed lasers has made it possible to use the methods of fluorescence analysis in remote sensing. The vegetation monitoring can be considered as one of the promising field of laser fluorescence analysis application [1–16].

Stress conditions in plants can be caused by many reasons such as lack or excess of soil dampness; mechanical damages; diseases; low or high temperatures; lack of nutrients; lack of light; soil salinization; soil contamination by heavy metals or petroleum products; excessive soil acidity; the use of pesticides, herbicides, insecticides, etc.

Such stress conditions are difficult to identify at the early stages judging by the appearance of plants. However, the fluorescence analysis can detect plants' potentially stress conditions based on the spectrum distortion of laser-induced fluorescence.

The operating principle of the laser fluorometer used for plants' condition monitoring is based on vegetation laser irradiation in the forms of either ultraviolet or visible light (for fluorescence excitation), as well as on the registration and analysis of fluorescent radiation.

The most important informative fluorescence indicator is a form of vegetation fluorescence spectra.

Nowadays there are some experimental data on fluorescence spectra of various kinds of both healthy vegetation and vegetation in various stressful situations. These experimental data have been obtained by various authors using different equipment at different excitation wavelengths (266, 275, 280, 300–400, 308, 325, 327, 337, 340, 355, 360, 380, 395, 396,397, 400, 400–450, 404, 405, 408, 422, 425, 428, 436, 440, 450, 452, 460, 470, 440–500, 480, 488, 500, 515, 525, 532, 535, 550, 590, 600, 627, 630, 633, 635 nm).

The greatest number of the experiments is devoted to the study of plant fluorescence using lasers (for fluorescence excitation) at wavelengths of 337 (nitrogen laser), 355 and 532 nm (the third and second harmonics of Nd-YAG laser). A solid-state pulsed laser at a wavelength of 532 nm is the most promising laser source for designing on-board equipment. It has the advantage (when developing the equipment for remote sensing) both over a nitrogen laser at the wavelength of 337 nm (it is better to exploit solid-state lasers in the on-board equipment), and a laser at the wavelength of 355 nm (the pulse energy of YAG laser third harmonic is lower than of the second one) .

It should be also noted, although during the radiation at the wavelength of 532 nm there is a small chlorophyll absorption in solutions and isolated chloroplasts, this radiation is efficiently absorbed by plant leaves due to a complex leaf structure (by virtue of it the optical light paths are significantly increased as compared with the geometric thickness of the sheet) [17]. In [18] it is shown that despite the low chlorophyll specific absorption coefficients in this spectral region, the green light is efficiently absorbed intothe leaf tissue (approximately 80 % of the absorption in either red or blue spectral regions).

Therefore, it is not unusual that there are many publications presenting the results of the researches into healthy vegetation fluorescence spectra at the excitation wavelength of 532 nm, is quite large [5–9, 12, 19–22].

However, there are few publications focusing on the research into plants' fluorescence spectra in stress situations at the excitation wavelength of 532 nm [10, 12, 16, 23] (and publications of the same authors based on the same experimental material in other books).

This article describes the research into a laser method of monitoring plants' stress conditions at the fluorescence excitation wavelength of 532 nm. This problem is of pragmatic interest for monitoring some plant conditions (such as cultivated plants) according to the results of remote sensing.

Experiment. In order to measure spectra of laser-induced fluorescence,a laboratory setup was designed, a block diagram of which is shown in Fig. 1.

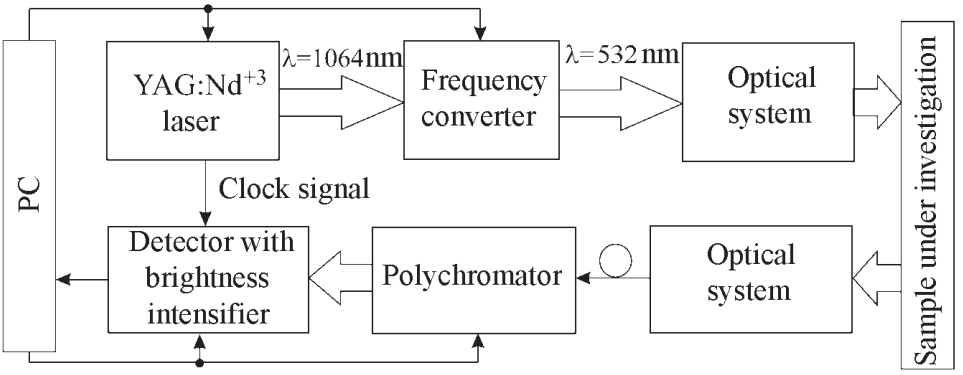

Fig. 1. Block diagram of the laboratory setup

The second harmonic of YAG:Nd laser is used as the source of excitation of fluorescence radiation. Registration subsystem of the fluorescence radiation is designed on the basis of both the polychromator and highly sensitive matrix detector with amplified brightness.

Plants' fluorescence spectra were measured on the setup in the range of 595. . .800 nm. Both the fluorescence spectrum and reflected laser radiation intensity were registered simultaneously at the wavelength of 532 nm.

The main parameters of the laboratory setup and the laser source are shown below.

The main parameters of the laboratory facility

The spectrum registration range, nm	595–800
The diameter of the receiver lens, mm	15
The distance to the plant, m	~ 1

The main parameters of the laser

The energy of the laser pulse, mJ	2.1
Pulse duration, ns	< 7
Wavelength, nm	532
Pulse rate, Hz	up to 500
Mode composition	TEM00
Beam divergence, mrad	< 3
Beam diameter, mm	0.8
Stability of the pulse energy, RMS,	< 1
Cooling	Air
Dimensions, mm:	
transmitter unit	$164 \times 274 \times 93$
power supply unit	$340 \times 365 \times 290$
Power consumption, W	< 300

Experimental study of fluorescence spectra included a polychromator wavelength calibration as a preliminary step using standard methods and

the calibration light source based on a mercury-argon lamp with a line spectrum; as well as a registration system sensitivity calibration in the range of 250 to 750 nm according to the standard procedure using the calibration light source DH2000-CAL. The detector background noise was also registered based on the brightness intensifier. The obtained background noise distribution was recorded in a file and was subsequently subtracted from the measured spectra. It allowed reducing their influence partially, thereby increasing the signal-to-noise ratio.

The polychromator slit width of 200 mkm was chosen in order to measure the fluorescence spectra. It provides spectral resolution of 5 nm. At the same time, for registering the reflected laser beam intensity the entrance slit width was set to be equal to 4 mm, which allowed both reducing the luminous flux entering the polychromator input and receiving signals without saturation.

Experimental studies were carried out using fast-growing and unpretentious species of plant, i.e. different types of lettuce, cucumbers (as an example of cultivated plants), grass.

Discussion of the results. Fig. 2 shows some typical examples of plants' measured fluorescence spectra in good conditions. There are

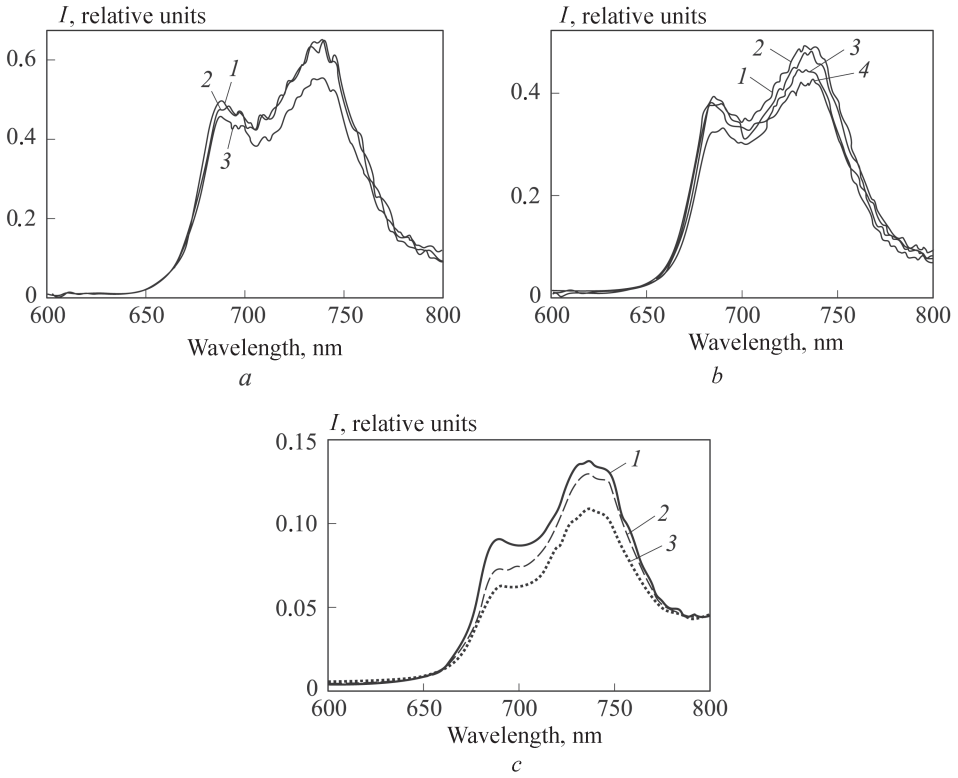

Fig. 2. Fluorescence spectra of plants in good condition

the fluorescence spectra of april cucumber (Fig. 2, *a*, curves *1*...*3* — spectra during different measurements), fluorescence spectra of watercress (Fig. 2, *b*, curves *1*...*4* — spectra during different measurements) and grass from Decor Aros turf lawn mixture (Fig. 2, *c*, curves *1*... *3* — spectra during different measurements).

In Fig. 2, *a*... *c* it can be clearly seen that plants' fluorescence spectra in the normal state have two maxima at about 680 nm (for some plants the maxima are weakly expressed — see. Fig. 2, *c*) and at about 740 nm. For most plants in normal states, the ratio of fluorescence intensities $R_{680/740}$ at the wavelengths of 680 and 740 nm is less than 0.8 (at the fluorescence excitation wavelength in either green or blue-green regions of the spectrum) [15].

When a plant is under stress, its fluorescence spectrum changes.

Fig. 3...5 show specific examples of plants' measured fluorescence spectra under various stress conditions.

Fig. 3 shows the spectra of laser-induced grass fluorescence (grown from a mixture of Decor Aros lawn mixture) under normal (curves *1*, *2*) and stress (curve *3*) conditions caused by introduction to the soil copper sulphate $CuSO_4$ (5 g, diluted in 200 ml of water for 3 pots ($9 \times 9 \times 10$ cm) with grass).

Curve *1* corresponds to the measurement of the laser-induced fluorescence spectrum made in a month after the first grass shoots, and curve *2* — two weeks later, just before the introduction of the soil pollutant. Curve *3* corresponds to the fluorescence spectrum of grass under stress, measured two weeks after introducing copper sulphate to the soil.

In Fig. 3 it is seen that the influence of the stress factor (in this case caused by introducing copper sulphate) may result in changing the fluorescence level. The shape of the fluorescence spectrum changes little.

This effect is clear, as the first phase of the plant stress is the primary inductive stress response [24]. This stage is characterized by a decrease in the rate of photosynthesis, which is accompanied by a significant increase in the chlorophyll fluorescence intensity. In this case, the increase in fluorescence quantum yield is due to a decrease in the efficiency of primary processes of photosynthesis. The absorbed light energy is not used in photosynthesis, so the fluorescence intensity increases.

Fig. 4 illustrates a different change pattern in the fluorescence spectra of a stressed plant. Fig. 4 shows the spectra of the laser-induced fluorescence of watercress under normal (curves *1*...*3*) and stress (curves *4*...*7*) conditions caused by mechanical damage to plants, i.e. salad trampling. Different curves correspond to different measurements in time (before the mechanical damage and in the range of 20 to 40 minutes after the mechanical damage).

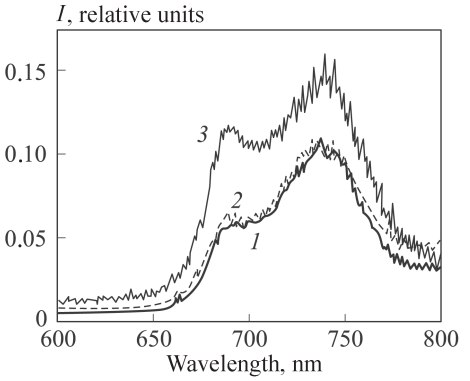

Fig. 3. The fluorescence spectra of grass under stress caused by introduction of copper sulfate to the soil

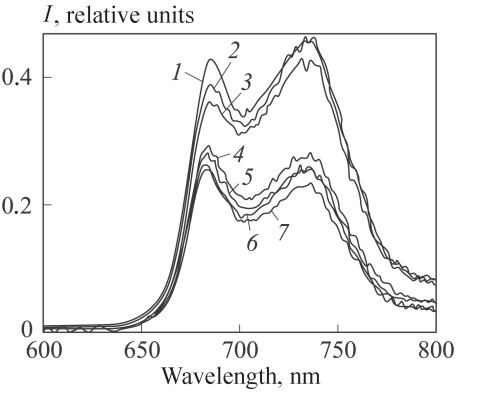

Fig. 4. The fluorescence spectra of watercress under stress caused by mechanical damage to plant

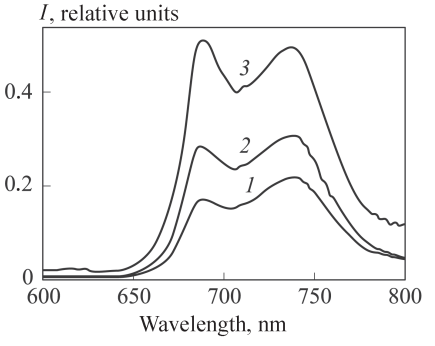

Рис. 5. The fluorescence spectra of watercress under stress caused by excess watering

Fig. 4 clearly shows that the influence of the stress factors (in this case caused by the mechanical damage) can manifest itself in changing the form of the fluorescence spectrum. The ratio of fluorescence intensities $R_{680/740}$ at wavelengths of 680 and 740 nm for the plant under stress is greater than unity, and the value of $R_{680/740}$ for the plant in the normal state is less than unity. The fluorescence level of the plant under stress even slightly less than the fluorescence level of the plant in a good condition.

Experts in plant physiology associate the effect of changing the form of the fluorescence spectrum of plants under stress with fluctuations in the activity of photosystem II, which result in changing the ratio of the fluorescence intensities in both the red (680 nm) and far-red (740 nm) regions [25].

Fig. 5 illustrates another possible kind of change in the fluorescence spectrum of the stressed plant.

Fig. 5 shows the spectra of laser-induced fluorescence of watercress in the normal (curve *1*) and stressed (curve *3*) conditions caused by excess plant watering. Curve *3* corresponds to the average (using different samples) fluorescence spectrum under excess irrigation for 24 days. Curve *2* shows an intermediate change of the condition (between the exactly normal and the exactly stressed) and corresponds to the averaged (using different samples) fluorescence spectrum under excess irrigation for 17 days.

In Fig. 5 it can be seen that the impact of the stress factor (in this case caused by the excessive amount of water when watering) gradually accumulates, with increasing the time of incorrect plant watering. It can manifest itself both in changing the spectrum form and increasing the fluorescence level simultaneously. In the figure, the measured fluorescence levels of the plants under stress (curve *3*) are significantly above the fluorescence level of the plants in good conditions. Moreover, the ratio of fluorescence intensities $R_{680/740}$ at the wavelengths of 680 and 740 nm for the plant under stress is greater than unity, and the value $R_{680/740}$ for the plant in a normal condition is less than unity.

The experimental results show the application perspectiveness of the laser fluorescence method for monitoring the plant conditions using the fluorescence excitation wavelength of 532 nm. Such monitoring can be implemented using an UAV and airborne laser fluorometer. Objective information that may be remotely obtained by the onboard laser fluorometer is a form of the fluorescence spectrum and the relative fluorescence intensity of the test site (for example, cultivated plant fields). Since the fluorescence of soil is much less than the fluorescence of vegetation (it can be seen from the data experimentally obtained both by the authors of this paper and by other authors), the measured fluorescence spectrumform and fluorescence intensity will specifically characterize the vegetation.

Conclusions. Thus, the experimental studies of plants' laser-induced fluorescence at the excitation wavelength of 532 nm indicate that the impact of the stress factors on plants caused by the presence of contaminants in the soil, an excess amount of water or mechanical damage, significantly distorts the plant fluorescence spectra. The influence of the stress factor can manifest itself in either a modified form of the fluorescence spectrum (the ratio of fluorescence intensities at the two wavelengths of 680 and 740 nm is the identifying factor), or a change in the fluorescence level that can be used as the basis of the laser method of monitoring the plant conditions.

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