Selection of emission detection ranges for the laser method of plant stress revealing at a fluorescence excitation wavelength of 355 nm

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Abstract. The paper considers the development of a laser fluorescent method for the detection of plant stress conditions. The results of experimental studies of laser-induced fluorescence spectra of plants in normal and various stress conditions caused by various pollutants in the soil are presented for the laser wavelength of fluorescence excitation of 355 nm. A comparative analysis of various options has been carried out for choosing the spectral ranges of laser-induced fluorescent radiation plant registration. It is shown that for the task of monitoring the state of plants, the most effective (from the point of view of reliability of correct detection of stress conditions) ranges of fluorescent radiation registration are spectral ranges with central wavelengths of 685 and 740 nm.

1. Introduction
The methods of laser remote sensing are most efficient for the operative control of the natural environment [1].

A most promising development in the use of laser methods is fluorescent monitoring of the vegetation (see, for example, [2–9]). An excess or lack of water, soil pollutants, plant diseases, nutrient deficiencies and other factors make it impossible for the plants to develop normally (stressful conditions of vegetation). Effective methods for vegetation stress conditions detecting are laser fluorescent methods. The physical basis of most methods is a change in the fluorescence spectrum of plants under stress.

A prospective option for a device to monitor the state of vegetation is a laser fluorimeter that receives fluorescent radiation in two narrow spectral ranges (and uses the ratio of fluorescence intensities in these spectral ranges as an information parameter).

From the point of view of laser energy characteristics and eye safety, the third harmonic of a yttrium aluminum garnet with a wavelength of 355 nm represents the greatest interest for creating a laser sensing equipment.

However, the choice of the most effective spectral ranges in which the registration of fluorescent radiation of plants remains unclear.

2. Laboratory facility for conducting experiments
A laboratory facility was created to study the spectra of laser-induced fluorescence. The block diagram of the facility is shown in figure 1.
The third harmonic of the YAG: Nd laser was used as the source of the fluorescence radiation excitation. The main parameters of the installation are shown in table 1.

| Parameter                                      | Value     |
|------------------------------------------------|-----------|
| Laser pulse energy (MJ)                        | 0.8       |
| Wave length (nm)                               | 355       |
| Pulse Width (ns)                               | <8        |
| Repetition Frequency (Hz)                      | up to 500 |
| Diameter of receiving lens (mm)                | 15        |
| The distance from the laser source to the plant (m) | ~ 1.4  |
| The diameter of the laser spot in the plane of the sample (mm) | 20 |

The radiation detection system was built on the basis of a polychromator and a highly sensitive matrix detector (ICCD) with a brightness amplifier based on image intensifier tube. It allowed to record spectra in the range of 295-750 nm with a resolution of 5 nm.

The calibration of the equipment included: calibration of the polychromator by the wavelength and calibration of the receiving system for detecting radiation by sensitivity. The output power of the laser was monitored and the measurement results were normalized to this value. The calibration of the equipment was additionally controlled by the Raman scattering spectrum of distilled water.

To manage the laboratory facility, a specialized software was developed in the LabView programming environment.

3. Experimental results

Experimental researches of fluorescence spectra were carried out for different plant species that were in both normal and stressful states. Universal soil was used for planting.

As a result of experimental measurements using laboratory facility for a wavelength of 355 nm fluorescence, spectra of laser-induced fluorescence of mustard, maize, alfalfa, and moss were obtained in a normal state and under stress conditions caused by putting pollutants into the soil (various petroleum products — diesel, gasoline; coolant for cars).

Figure 2 below shows examples of plant fluorescence spectra under normal and stress conditions for a fluorescence excitation wavelength of 355 nm. The fluorescence spectra of peat moss are shown in a normal state (figure 2, a) and under stress caused by diesel pollution (diesel fuel from the Samara refinery) (figure 2, b) one hour after pollution.
The peak at the wavelength of 532 nm in the fluorescence spectra corresponds to the second harmonic of the radiation of a yttrium-aluminum garnet laser.

Figure 2 shows that the spectrum of laser-induced fluorescence is distorted for a plant under stress (caused, in particular, by anthropogenic pollution of the soil). Thus, the analysis of the laser-induced fluorescence spectra shape upon excitation of fluorescence at an eye-safe excitation wavelength of 355 nm allows us to detect vegetation stress state.

4. Analysis of the results of measured fluorescence spectra processing

In the majority of publications devoted to laser fluorescence monitoring of the plant state for the fluorescence excitation wavelength of 355 nm, the most acceptable choices of two central wavelengths are the following: one wavelength in the range of 680-690 nm, the other one in the range of 730-740 nm; one wavelength in the range of 440-540 nm, the other one in the range of 680-690 nm; one wavelength in the range of 440-540 nm, the other one in the range of 730-740 nm (see, for example, [10-15]).

As an informational sign to detect stressful conditions of plants this paper uses the ratio $R_{\lambda_1}/R_{\lambda_2} = I(\lambda_1)/I(\lambda_2)$ of fluorescence intensities $I(\lambda)$ recorded in the following spectral ranges:

1 - $R_{450/685}$, 2 - $R_{685/740}$, 3 - $R_{675/730}$, 4 - $R_{680/730}$, 5 - $R_{685/730}$, 6 - $R_{685/735}$, 7 - $R_{690/735}$, 8 - $R_{680/740}$, 9 - $R_{690/740}$, 10 - $R_{450/690}$, 11 - $R_{450/680}$, 12 - $R_{445/685}$, 13 - $R_{445/690}$, 14 - $R_{445/680}$, 15 - $R_{445/690}$, 16 - $R_{445/685}$, 17 - $R_{445/680}$ with a spectral bandwidth of 10nm.

Figure 3 shows the results of processing the measured fluorescence spectra. It reveals the relationships $R$ (options $i = 1 - 17$ described above) for mustard.

The curve with diamond shaped markers is the normal condition of plants, the curve with circular markers is a plant under stress caused by the introduction of A-95 gasoline into the soil (20 ml of gasoline was poured into a container of 100 mm x 60 mm x 40 mm; 20 hours after entering the soil pollutant).

Figure 3 demonstrates that the value of the $R$ parameter for plants under stress is greater than the value for plants in a normal state. However, their difference substantially depends on the selected spectral ranges of the registration.
Figure 3. The results of processing the fluorescence spectra of mustard in a normal and stressful state (stress is caused by the introduction of A-95 gasoline into the soil).

As a parameter characterizing the efficiency of the choice of spectral registration ranges, we take the average value (for different plants and different pollutants) of the difference $\Delta R$ between the values $R$ for stress and normal plant conditions. The greater this difference is, the bigger is the reliability of the correct detection of stress conditions.

Analysis of experimental data shows that the largest values $\Delta R$ are implemented for $R_{685/740}$, $R_{680/730}$, $R_{685/735}$, $R_{690/735}$, $R_{690/740}$.

Figure 4 shows the average values $\Delta R$ for them (for variants $n=1, 6, 1 - R_{685/740}, 2 - R_{680/730}, 3 - R_{685/735}, 4 - R_{690/740}, 5 - R_{690/740}, 6 - R_{690/740}$).

Figure 4. Mean values $\Delta R$.

From figure 4 one can see that the ratio of fluorescence intensities $\Delta R$ has the largest value $R_{685/740}$ among various variants. For this best choice of selection of spectral registration channels, figure 5 shows the ratio $R_{685/740}$ for different plants and different types of stress. Here, markers in the form of diamonds are the normal state, markers in the form of circles are the stress state caused by various pollutants.
Figure 5. Relational values $R_{685/740}$ for different plants and different types of pollutants.

Figure 5 shows: (1) is mustard 1 hour after irrigation with petrol A-95; (2) is mustard 20 hours after pouring A-95 gasoline into the soil (20 ml); (3) is corn 4 days after adding coolant to the soil; (4) is corn 1 hour after pouring A-95 gasoline into the soil (20 ml); (5) is corn 1 day after pouring A-95 gasoline into the soil (20 ml); (6) is alfalfa 1 day after adding diesel fuel to the soil (10 ml); (7) is alfalfa 7 days after adding diesel fuel to the soil (10 ml); 8 - alfalfa, 15 days after adding diesel fuel to the soil (10 ml); (9) is alfalfa 7 days after adding diesel fuel to the soil (20 ml); (10) is alfalfa 12 days after adding diesel fuel to the soil (20 ml); (11) is alfalfa 15 days after adding diesel fuel to the soil (20 ml); (12) is alfalfa 18 days after adding diesel fuel to the soil (5 ml); (13) is moss 1 hour after adding diesel fuel to the soil (20 ml).

On the basis of the fluorescence spectra obtained, mathematical modelling was performed to solve the problem of detecting stress states of vegetation. It was assumed that the recorded values of the fluorescence emission intensities are random. The average values of these values were taken from experimental fluorescence spectra. Measurement noise was assumed normal with a zero mean value and with a relative rms value of 1-10%. The spectral width of the registration spectral ranges was assumed to be 10 nm.

The decision to detect the stress state of the plant was made when the condition $R_{685/740} > R_{th}$ is satisfied, where $R_{th}$ is the threshold value, which was chosen in the middle between the parameter values for plants in normal and stress state.

Mathematical modeling results of correct detection probability of $P_c$ stress state of plants and the probability of false alarms $P_f$ with the value of the relative mean-square value of measurement errors $\delta=2\%$ are given in table 2.

The plant numbers in table 2 correspond to the numbers in figure 5.

| k  | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pc (%) | 98.5 | >99.9 | 94.7 | 96.2 | 96.2 | 84.9 | 99.9 | 99.8 | 97.1 | >99.9 | >99.9 | 98.4 | >99.9 |
| Pf (%) | 0.6  | <0.1 | 3.4  | 2.2  | 2.2  | 13.8 | 0.01 | 0.04 | 1.6  | <0.1  | <0.1  | 0.6  | <0.1 |

The results shown in table 2 demonstrate the high reliability of detecting stressful conditions of plants using spectral ranges with central wavelengths of 685 and 740 nm.

5. Conclusion

Thus, for the wavelength of fluorescence excitation of 355 nm, experimental studies of the spectra of laser-induced fluorescence of plants in normal and stressful conditions caused by the presence of
various pollutants in the soil have been carried out. A comparative analysis of various choices of spectral ranges for recording fluorescent radiation shows that, for the task of monitoring plant conditions, the spectral ranges with central wavelengths of 685 and 740 nm are the most effective ones (in terms of the reliability of correct detection of stress conditions). The results of mathematical modelling show that in most situations the laser fluorescent method (using spectral registration ranges with central wavelengths of 685 and 740 nm) allows to detect areas of vegetation that are in stress due to soil contamination, with a probability of correct detection close to 100 percent and the probability of false alarms ~ units and tenths of a percent.

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