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Laser monitoring of stress conditions of plants

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Abstract: Based on our own research and analysis of existing methods for monitoring vegetation cover, the advantages of laser remote sensing have been proven. Preliminary experiments were carried out on such simplified models as extracts of pigments that play a major role in the photographic processes of interest to us. These models were chlorophyll fractions "a" and "b" extracted from plant leaves. These measurements made it possible to state that by selective excitation of fluorescence in preparations from acetone solutions of chlorophyll, it is possible to identify certain effects caused by differences in the groups and configurations of pigments inherent in their native state in the photosynthetic apparatus. Measurements carried out on plants subject to mineral stress showed that cotton grown under conditions without the addition of basic nutrients such as potassium, nitrogen and phosphorus to the substrate shows a lower fluorescence yield compared to control plants grown under normal mineral nutrition. At the same time, the dynamics of changes in the spectral characteristics of plants subject to water stress showed the opposite pattern. On the sixth day after stopping watering, when signs of wilting began to appear, the fluorescence output increased by 30% compared to control plants. As a result, these two competing processes make it difficult to unambiguously diagnose the real state from the integrated fluorescence output. However, the spectral shape turned out to be more informative than the integral fluorescence output. Therefore, to most accurately identify the state of the plant, an analysis of the spectral structure of the fluorescent response was chosen

1 Introduction

To solve the problems of monitoring vegetation cover, traditional methods of analyzing the physical and chemical properties of the soil condition - humidity, mineral composition and other indicators, by direct sampling in the studied areas of land are widely used. However, this technology is quite labor-intensive and does not allow obtaining operational information, especially when surveying fairly large areas. Therefore, non-contact methods have now been developed based on the use of physical laws of interaction of electromagnetic waves with matter, which consists of recording return signals in a wide range of radiation that carry information about the state of the object. The most widely used sensors are those that record back radiation, installed on mobile platforms - tractors, airplanes and artificial Earth satellites.

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It is known that in plants throughout the entire period of ontogenesis, the photosynthetic apparatus (PSA) plays the main role, the functioning of which is closely related to environmental conditions. The main factors determining their physiological state are the quality of the soil - the presence of basic elements of mineral nutrition, humidity, illumination, temperature, etc. Deviations from the norm of any of these factors ultimately lead to both structural changes in the entire plant organism and to changes in the functioning of the PSA.

There are remote methods for sensing vegetation using various optical devices. In particular, photographic tracking cameras installed on artificial Earth satellites and aerial vehicles provide the opportunity for operational monitoring over fairly large areas [1,2].

However, methods based on remote recording of reflected solar radiation do not allow one to clearly distinguish the species of plants and, even more so, accurately diagnose their physiological state. Active sensing methods based on measuring secondary radiation from plant organs in the form of fluorescence under the influence of laser irradiation turned out to be very promising in this regard [3,4].

2 Materials and methods

The phenomenon of fluorescence is the emission by a substance of part of the absorbed light with a shift to a longer wavelength region of the spectrum. It is this circumstance that is very convenient for remote measurements, since it makes it possible to easily separate the signals of reflected and secondary radiation. A particularly remarkable property of fluorescent radiation is the ability to obtain information about the environment surrounding the luminescent object, since photons contain information about the environment in which the molecule was located immediately before emission.

In plants, chlorophyll molecules contained in the leaves serve as optical indicators. The main task of these molecules is to convert light energy into chemical energy to carry out the process of photosynthesis. Under natural conditions, this process includes several stages: absorption of light energy by PSA pigments, energy migration to reaction centers, charge separation and subsequent charge transfer along the electron transport chain. The efficiency of energy absorption and transmission is determined by the state of pigment-protein complexes, which serve as a kind of antennas. As a result of interaction with proteins, chlorophyll changes its optical properties, forming a set of spectral forms with overlapping ranges of absorption spectra. This ensures efficient energy migration from antenna chlorophylls to reaction centers [5]. Deactivation of the excited states of pigments will also be accompanied by a competing process – chlorophyll a fluorescence, the intensity of which is inversely proportional to the intensity of photochemical reactions [6]. Therefore, assessing the state of plants based on the intensity and spectral structure of the fluorescent response can serve as an effective remote diagnostic technique.

3 Results

Since the living leaf of a plant is a rather complex system from the point of view of studying photophysical and photochemical processes, in order to develop basic approaches to real experiments, it was necessary to conduct experiments on such more simplified models as extracts of pigments that play a major role in the photo processes of interest to us. The chlorophyll fractions "a" and "b" extracted from the leaves served as these models.

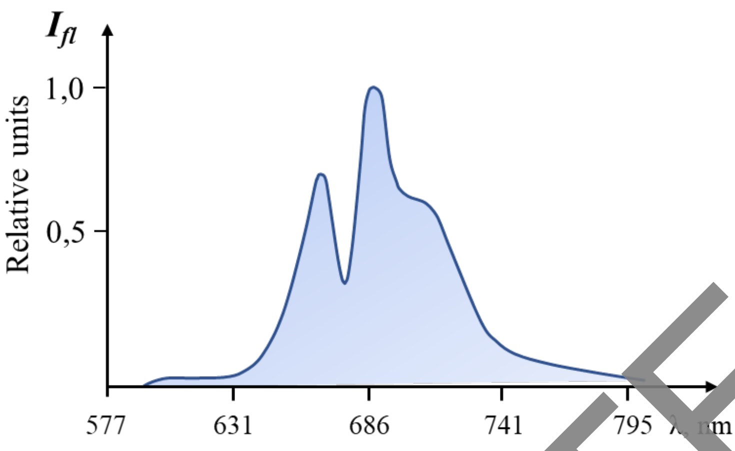

Fig.1 Fluorescence spectrum of chlorophylls *"a"* + *"b"*, "*M*" – forms cotton at excitation λ = 476.5 nm

Fluorescence spectra of acetone extract of pigments from the leaves of the "*M*" form of cotton, at exciting light lengths of 476.5 nm and 514.5 nm show (Fig.1) that the total chlorophyll fraction contains two pronounced fluorescence maxima in the region of 657 and 690 nm, and one, less pronounced, in the region of 720 nm. Apparently, the pronounced maximum in the region of 657 nm and the long-wave hump are associated with the absorption of chlorophyll "*b*", which has a maximum absorption in the region of 465 nm. These results demonstrate that it is possible to choose a wavelength of exciting radiation at which the differences in the shapes of the fluorescence spectra of pigments with similar concentrations of fractions will be greatest. These measurements also allow us to state that by selectively exciting fluorescence in preparations from acetone solutions of chlorophyll, it is possible to identify certain effects due to differences in groups and configurations of pigments inherent in their native state in the photosynthesis apparatus. To provide the necessary statistics, a series of laser-induced fluorescence (LIF) spectra of the same-aged leaves of experimental and control cotton plants were measured. In parallel, the chlorophyll content and assimilation surface were determined on leaves of similar age. The error in determining the concentration of chlorophyll at three measurements did not exceed 8%. Analysis of the chlorophyll content and spectral curves of the leaf of plants shows that cotton grown in conditions without the addition of the main elements of mineral nutrition (nitrogen, phosphorus and potassium) to the substrate differs from the control ones by a lower fluorescence yield. In normalized spectra, this difference manifests itself in the form of an increase in the relative height of the long-wave arm (Fig.2).

The observed results are in qualitative agreement with the results of [6], where it was found that a decrease in potassium from the norm leads to an increase in fluorescence yield. The increase in fluorescence is obviously associated with a decrease in the efficiency of photosynthesis, as a result of which most of the absorbed and unused energy is emitted as radiation. At the same time, nitrogen and phosphorus deficiency weakens the fluorescence intensity by two or three times at 690 and 735 nm, which is due to a decrease in the chlorophyll content in the leaves. As a result, these two competing processes make it difficult to unambiguously diagnose the integrated fluorescence output. In addition, during remote measurements, the fluorescence emission image has a natural scatter depending on the projective vegetation cover and, accordingly, changes in the concentration of chlorophyll along the sounding route. However, the spectral form turned out to be more informative than the integral fluorescence output. Therefore, the analysis of the spectral structure of the fluorescent response was chosen for the most accurate identification of the plant condition.

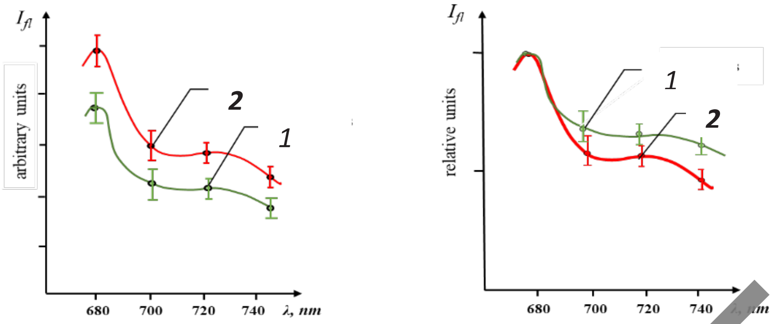

Fig.2 Spectra of cotton leaves depending on its physiological state: *1* - control and *2* - exposed to a lack of mineral nutrition (confidence interval with a confidence probability of 0.95)

During a series of experiments on plants experiencing water stress, the following was found: on the sixth day after the termination of watering, signs of wilting began to appear on the leaves of plants. Spectral measurements showed that the absolute fluorescence yield increased by about 30% compared to the control plants. The shape of the spectrum between the control and withering plants was practically the same.

Similar experiments on measuring the fluorescent spectra of cotton plants artificially infected with the fungus *Verticillium dahlaye (V.D.)* showed the following: within 2 days after infection, no significant differences in the structure and intensity of the fluorescence spectra of healthy and infected plants were observed (Fig.3). The first noticeable differences in the fluorescence spectra were observed on day 3 (after 60 hours after infection). The changes were expressed in a decrease in the relative intensity of the peak at 735 nm and in its monotonous decline over the following days, with the main peak of 685 nm remaining unchanged. At this stage of the latent period of the disease, artificially infected plants did not differ in appearance from healthy ones. Barely noticeable visual signs of verticillous wilting were detected only on the fifth day after infection with the fungus.

Visually, these changes were expressed in chlorosis and yellowing of the leaf tissues along its edges, as well as between the veins in the form of small islands. On the 9th - 10th day after infection of cotton, necrosis of yellowed areas and their drying occurred. These visual signs of cotton disease were in accordance with the indicators of the evaluation of the sections of the stems and the data of microbiological analysis.

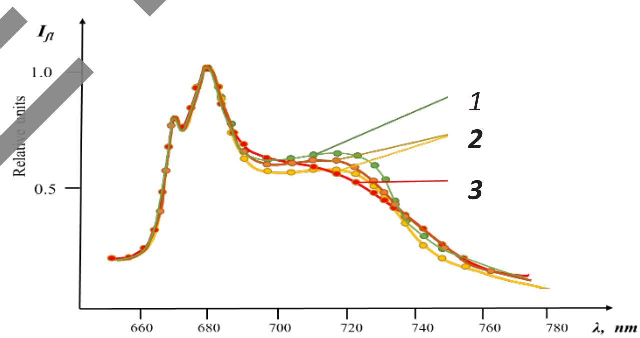

Fig.3 LIF spectra of cotton leaves depending on its physiological state in the absence of visual signs of the disease: *1* – healthy leaves; *2* – leaves of cotton artificially infected with a fungus (after 48 hours after infection); *3* – leaves of cotton artificially infected with a fungus (after 60 hours after infection).

4 Conclusions

Thus, fairly clear differences were found in the structure of the fluorescence spectra corresponding to healthy and infected plants. It is noteworthy that in the fluorescence spectra, the pathological condition of plants manifested several days before the appearance of visual signs of the disease.

It should be noted that the shape of the spectrum corresponding to the same state of the plant will differ somewhat from each other even for leaves located on different tiers of the stem. As a result, the overall spectral picture of the remote data will be somewhat "smeared", although the overall ensemble of spectrograms corresponding to each state has its own individuality. Nevertheless, this circumstance significantly complicates the unambiguous interpretation of remote data. These difficulties were overcome by involving statistical methods based on pattern recognition in the processing of the results. For this purpose, training spectral images were created for various modifications of plants during preliminary laboratory measurements. These images were digitized intensity values in selected discrete sections of the spectrum. Based on these data, n –dimensional discriminant functions corresponding to a certain type of plant are constructed, where n is the number of selected. In the present tests, the choice of 12 spectral channels in the range of 670 – 750 nm provided a fairly accurate identification of remote data [7,8].

Thus, laser sensing can become one of the tools of precision agriculture (PA), which ensures the achievement of maximum crop yields with minimal use of resources [9]. Along with the rapid development of big data cloud computing and unmanned aerial vehicle technologies, such research is becoming especially relevant [10].

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