AN INVESTIGATION OF THE SPECTRAL KINETIC CHARACTERISTICS OF LASER-INDUCED FLUORESCENCE OF VEGETATION

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Laboratory remote-sensing studies of the fluorescence of vegetation induced by a He-Ne laser were conducted in order to shed light on problems connected with the identifications of their species-membership and possible ontogenetic changes and the evaluation of the action of various stress factors.

It was shown that an analysis of the spectral-kinetic characteristics of the laser-induced fluorescence enables an early diagnosis of the physiological state of the vegetation.

Non-contact, objective remote methods of investigation and control continue to acquire an ever-growing importance now In various branches of industry and agriculture. Thus, today there already exists a real basis for the use of Iidar systems to evaluate the physiological state of vegetation[1–3]. The idea of the photosynthesis in the green leaf as a dynamic system of pigments trapping and utilizing the electromagnetic energy of the ultraviolet and visible regions of the spectrum provides the basis of this application.

The efficiency of the photosynthetic apparatus is determined first of all by the efficiency of resonance energy migration between the pigments comprising the photosynthetic unit. Variation of the efficiency of energy migration may be caused by a variety of factors, viz. , nutrition element deficit, water regime disturbance, fungal infections, herbicides, temperature, soil *pH*, etc.

The decisive role played by the resonance energy migration efficiency means that every pigment of photosynthetic unit is a potential fluorophor, so the disturbance or natural conformational rearrangement of any link in the conjugate pigment chain will inevitably result in changes of the fluorescence energetically associated with the pigment (or pigment system) of the sector where the corresponding transformation has occurred. Therefore it is quite natural to try and use a fluorescent lidar for early noninvasive diagnostics of the physiological state of the plant.

The present paper is aimed at an evaluation of the information content of the spectral kinetic characteristics of the laser-induced fluorescence (LIF) of plants with respect to the characteristic features of the species, age transformations, as well sis stress factors such as defoliant treatment and water deficit. A block-diagram of the experimental setup used for investigating the laser-induced plant fluorescence is shown in Fig. 1. The LG-52 helium-neon laser (power 8 mV) was used as the radiation source. The fluorescence radiation was collected with a 120 mm-diameter telescope, the spectral signal selection of the LIF was carried out using an MUM grating monochromator, and the output radiation was recorded with a FEU-79 photomultiplier. The spectral scanning speed was 1 nm/s. All measurements were made at a distance of 4 m from the leaf surface to the receiving mirror under conditions of full laboratory illumination.

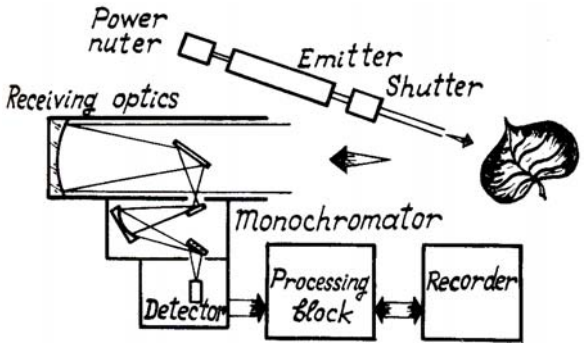

FIG. 1. Block-diagram of the setup used for investigating the LIF of plants.

A specific feature of plant fluorescence is its time-dependence which is due to the existence of a dynamic system of light and dark stages of photosynthesis. Therefore, the results of spectral and kinetic studies of the LIF are presented by the two mutually complementary functional-informational data sets.

The kinetics of the LIF was recorded for two minutes after the irradiation has been switched on (after this time the kinetic curve of the LIF has practically reached steady state) at the wavelengths of 685 and 725 nm which correspond to the minimum emission regions of photosystems 2 and 1 (PS2 and PS1), respectively.

Formalization of the kinetic 'curves of the LIF is performed by computing the parameters τ and n of the approximating function

$$I_{fl}=\exp\left[-\frac{t}{\tau}\right]^n.$$

To avoid kinetic distortions we waited three minutes after the irradiation was switched on before recording the LIF spectra.

To reveal the fine structure of the LIF spectra the differentiation method was used, and the selection of informative regions of the LIF spectra was made by correlation analysis.

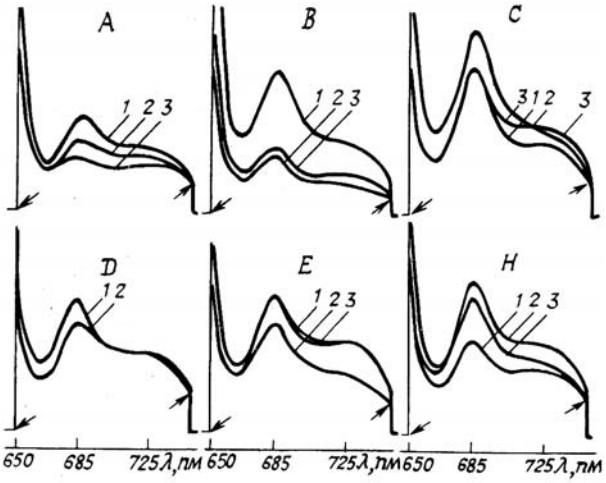

FIG. 2. LIF spectra of various plant species.
A. Family of gymnosperms (Gymnospermae)
1) juniper (Juniperus communis)
2) juniper (Juniperus zeravschanica)
3) Pine (Pinus sylvestris)
B–H. Family of anglosperms (Angiospermae)
B. Class of monocotyledons (Monocotyledonae)
1) palm (Trachycarpus excessus)
2) stipa splertdens (Zasiagrostis splendens)
3) palm grass (Selaria verticallata)
C–H. Class of dicotyledons (Dicotyledonae)
C. 1) wormwood (Artemisia cina)
2) dandelion (Taraxacum officinale)
3) peony (Paeonia lactiflora)
D. Order of Leguminosae (Fabales)
1) lucerne (Medicago saliva)
2) acacia (Cassia griffIthii)
E. Order of Rosaceae (Rosal)
1) plum (Prunus domestica)
2) blackberry (Rubus caesius)
3) quince (Cydonia oblonga)
H. Order of Malvaceae (Malvales)
1) cotton (Gossypium hirsutum)
2) hibiscus (Hibiscus esculentus)
3) Mallow (Halva silvestris)

Figure 2 shows the LIF spectra of leaves of the two families of higher plants: gymnosperms (A) and angiosperms (B–H). The latter falls into two classes-monocotyledons (B) and dicotyledons (C–H) of various orders.

The correlation analysis enables us to establish that in the case of excitation by a helium-neon laser the spectral regions of the LIF informative to the species-related characteristic features of higher plants are In the vicinity of 650, 685 and 715–740 nm, fluorescence in the two latter regions usually being treated as emissions from PS2 and PS1, respectively[3,4].

The signals recorded at 650 nm are characterized, first of all, by the maximum species-related differences and, second, by an almost complete lack of kinetic changes. In addition, an increase in the angle of incidence of the exciting radiation results in a much faster decrease of the radiation intensity at 650 nm than in the emission regions of PS1 and PS2 emissions where their decrease takes place symbatically, with conservation of maximum intensity ratios. One may suppose, therefore, that the received signal at 650 nm is informative as a result of the morphological peculiarities of the reflecting surface of the leaf.

Among all of the representatives of the higher plants under study, the lowest intensity of the LIF signals in the PS2 and PS1 emission regions is characteristic of the gymnosperms (A) and the highest intensity is characteristic of the monocotyledons (B) (in all cases the sensitivity of the recording system was constant, with the exception of the spectral data in Fig. 2b, which were recorded with a twofold lower sensitivity).

It is characteristic of the representatives of the gymnosperms under study that the ratio of $PS2_{(max)}$ to $PS1_{(max)}$ is almost constant while the representatives of the angiosperms are characterized by a wide variability of this ratio.

The wavelengths of the PS2 fluorescence maximum vary strongly, with the longest one typical of the gymnosperms, and the shortest one typical of the monocotyledons.

Our studies of the kinetic characteristics of the LIF in the PS2 and PS1 emission regions have not revealed any peculiarities associated with species differences.

The use of a heliun-neon laser as the radiation source allowed only observations of tetrapyrrhole pigments to be made. Therefore, we think it is reasonable, for the purpose of identifying the plant species, to use a shorter-wavelength source, which will make it possible to investigate fluorescence of other pigments (carotinoids, quinones, flavinoids) whose qualitative and quantitative composition is more specific[5,6].

To study age transformations, the LIF of the first true leaf of a cotton plant was investigated for three weeks after its appearance. Analysis of the derivatives of the spectral contour of the LIF (Fig. 3) shows that as the leaf develops, the degree of structurization increases in the region 715 to 740 nm. This increase coincides with the period of maximum decrease of the kinetic curve parameters of the LIF (Table 1). The changes observed are evidently due to the growth of the PS1 pigment complex taking place after formation of photosystem 2.

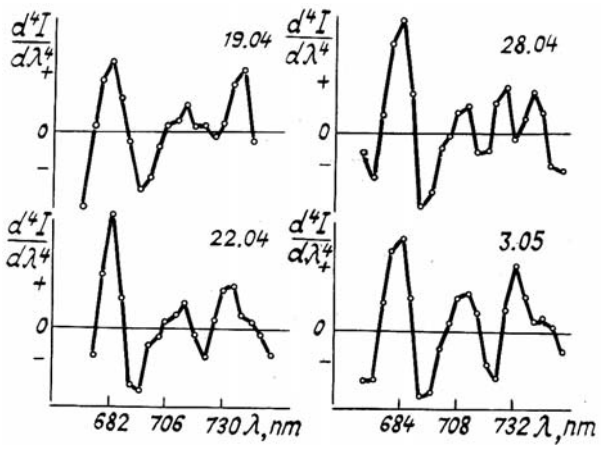

FIG. 3. Derivatives of the spectral contour of the LIF of the leaves of a cotton plant at various stages of growth.

At the same time, as the leaf develops, the main maximum of the LIF is shifted from 682 to 685 nm, which is apparently a reflection of conformational rearrangements in the heterogeneous pigment complex PS2, aimed at the consistent functioning of photosystems in the process of ontogenesis.

Figure 4 shows the spectral changes of the LIF of cotton plaint leaves two hours after being treated with defoliants (dropp, butyphos).

It has been established that defoliant treatment leads to a definite increase of the fluorescence intensity ratio $PS2_{max}/PS1_{(max)}$ equal to 2.29 and 2.34 for butyphos and dropp, respectively, compared against a reference ratio of 1.93. This increase is caused, to a greater extent, by the emission changes in the main fluorescence maximum, which is apparently accounted for by a greater level of dysfunction of the photosynthetic apparatus on the side of PS2 as a result of blocking of the electron-transport chain caused by the action of the defoliant[7], and also a decrease of the efficiency of reabsorption of the light-collecting complexes of PS1.

TABLE 1.

t, hour	Kinetic curve parameters of LIF						
	water stress				age transformations		
	λ=685nm		λ=725nm		date	λ=685nm	
	τ	n	τ	n		τ	n
0	132	0.50	186	0.55	19.04.1988	83	0.86
24	168	0.45	215	0.58	22.04.1988	83	0.66
48	111	0.92	130	1.09	28.04.1988	70	0.72
72	238	0.92	283	0.98	03.05.1988	87	0.89
96	15381	0.51	739	0.76	———	—	—

Water stress was produced by ceasing the watering of the cotton plant, which was being grown in a reenhouse. The LIF of the plant leaves was observed every 24 hours.

On the other hand, the increase in the LIF in the PS1 emission region may result from breaking the native mutual bonding of the chlorophyll-albumen complexes, which provide the efficient light collection at the chlorophyll of the PS1 reactive center, which is indirectly confirmed by the changes in the degree of spectral contour structurization in this region (Fig. 4).

It has been established that as water stress develops the main spectral changes occur in the emission region of PS1 (Fig. 5). It should be noted that the changes occurring in the leaf during the first 72 hours have no visual signs of withering and their state is completely reversible after the resumption of watering, and it is on the fourth day that there appear visual signs of withering with simultaneous abrupt degradation of the spectral contour of the LIF throughout the entire wavelength range under study.

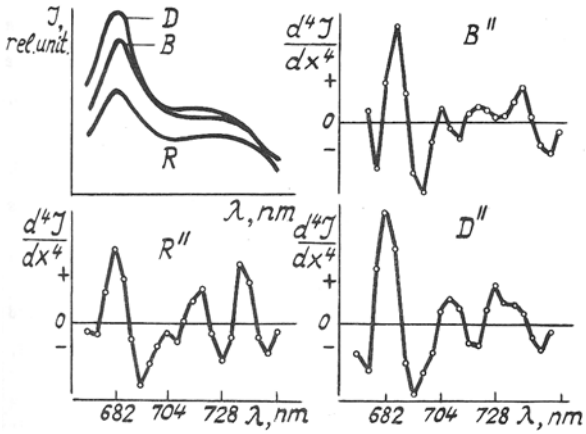

FIG. 4. The effect of treatment with defoliants on the spectra and their derivatives of the LIF of the leaves of a cotton plant. R—leaves of reference plants; B—leaves of plants treated with butyphos (10^{-3} m); D—leaves of plants treated with dropp (10^{-3} m).

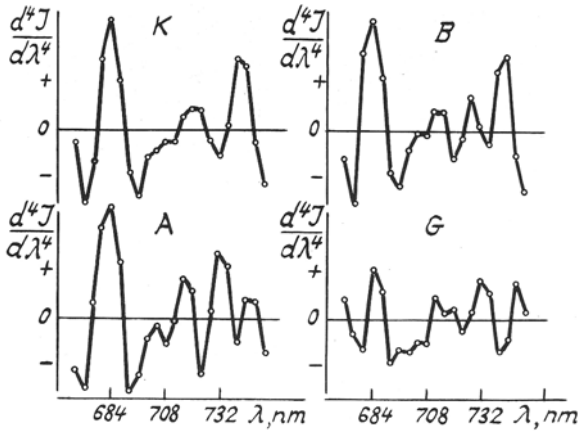

FIG. 5. Derivatives of the spectral contour of the LIF of leaves of a cotton plant under conditions of water stress. R-leaves of reference plants; A, B, C—leaves of plants without watering for 48, 72 and 96 hours, respectively.

The most distinct stages of plant response to the cessation of watering are displayed in the change of the character of the LIF kinetic curves of both PS1 and PS2 (Fig. 6, Table 1). It is characteristic of all the reversible stages that the Initially exponential kinetic curves become more linear-like, as the dehydration continues. Then, with the appearance of visible signs of drought, the LIF completely loses its kinetic character (Fig. 6).

On the other hand, as the drought progresses, three stages of spectral contour structurization of the LIF of PS1 in the cotton plant are clearly seen (Fig. 5). The first stage (0 to 48 hours) is an increase in structurization which has, in our opinion, a compensatory and adaptive character in response to the impact of stress. A point in favor of this is the abrupt change of the characteristic parameters of the LIF kinetics (Table 1). The second stage (48–72 hours) of initial destructurization is apparently indicative of the exhaustion of the plant's self-protective abilities. And, finally, the third stage is a progressing degradation of the functionally essential dynamic interaction of the chlorophyll aggregates which constitute the photosynthetic unit PS1, which takes place along with the appearance of visible signs of withering and the almost complete lack of any kinetics of the LIF.

Thus, the above experimental data demonstrate the fundamental possibility of using the LIF method both for the purposes of remote identification of species membership of the vegetation and for remote diagnosis of the physiological state of plants under varying environmental conditions.

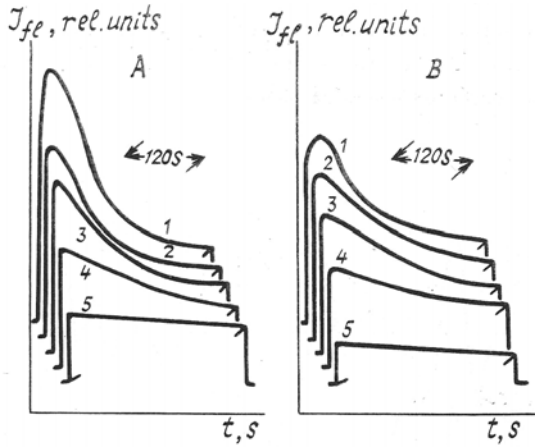

FIG. 6. Kinetic curves of the LIF of PS1 (B) and PS2 (A) of leaves of a cotton plant under conditions of water stress; 1—Leaves of reference plants; 2, 3, 4, 5—leaves of plants without watering for 24, 48, 72 and 96 hours, respectively.

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