Fluorescence Spectroscopy to Detect Water Stress in Orange Trees

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Abstract **— The laser-induced fluorescence spectroscopy was used to investigate water stress in orange trees (Citrus aurantium L.). In our experiments six three were under water stress conditions and four in regular conditions, as control. The fluorescence spectroscopy was investigated by using a 442-nm 15mW He-Cd gas multimode discharge laser and a 532-nm 10mW Nd^{3+}:YAG laser for fourteen days. The stress manifestation can be detected by variation of fluorescence ratios of the leaves. The fluorescence ratios present a significant variation. The results show that it is possible to observe water stress by analyzing fluorescence spectra of orange leaves. They also the possibility to develop a tool to detect the water stress in the field.**

Index Terms **— Fluorescence spectroscopy, laser applications, spectrum analysis+.**

I. Introduction

Chlorophyll fluorescence has been used successfully as a nondestructive and no intrusive signal in plant biochemistry, physiology and ecology. Such technique is easy to use for many purposes in laboratory and fieldwork [5]-[6].

To detect water and mineral deficiency in plants and trees became necessary to improve the production of fruits and foods around the world.

Fluorescence analysis is a successfully technique that has been used to detect water stress in maple leaves [8], and olive leaves [1].

The orange production is important in world due the high consumption of orange juice and high concentration of vitamins.

In this work, we present a technique to detect water stress in orange trees (Citrus aurantium L.) in laboratory using an optoelectronic device. The results suggest that it is possible to detect it with good precision; stimulating the development of a portable device to be used in the field.

II. Materials and Methods

A. Plants, leaves and water stress model

The plants used in this report were orange trees (Citrus aurantium L.). Ten leaves were selected from each plant for a diary fluorescence measurement.

We have used a very simple water stress model, in which the plants under water stress did not receive water supply for fourteen days. The control plants continue to receive one liter of water per day. All plants were placed in a room with low natural luminescence and without direct contact with environment. They were fertilized with the same organic material and placed in identical vases to guarantee identical conditions.

B. Chlorophyll fluorescence and fluorescence ratio

Light in the green region excites the chlorophyll fluorescence directly while light in the UV-blue region excites the fluorescence of chlorophyll and other pigments [7]. Chlorophyll fluorescence is an accurate and non-destructive probe of photosynthetic efficiency which can reflect the impacts of environmental changes on a plant. According to Cerovic et al. [2], the fluorescence of leaves in blue-green region and red-far red region change independently in response to different physiological and environmental factors and these changes can be detected accurately by fluorescence ratios.

In this report the fluorescence ratios used are:

1) Red to far-red (RF/FRF): This parameter is defined by the ratio between the fluorescence intensity at 685 nm and the fluorescence intensity at 735 nm. It depends only the chlorophyll content of the leaf and it is used to measure water stress in plants [3]-[4], [8].

2) Blue to red (BF/RF): It is defined by the ratio between the fluorescence intensity at 452nm and the fluorescence intensity at 685nm.

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3) Blue to far-red (BF/FRF): It is defined by the ratio between the fluorescence intensity at 452nm and the fluorescence intensity at 735nm.

The BF/RF and the BF/FRF together compose the blue to chlorophyll fluorescence ratio (BF/ChlF). This fluorescence ratio is more sensitive to detect stress in plants and environmental changes than RF/FRF ratio because the blue fluorescence and the chlorophyll fluorescence have distinct origins [2].

The laser-induced fluorescence at 532nm is limited to supply only the RF/FRF ratio; the correlation between BF/ChlF induced at 532nm and environmental factors has been investigated.

C. The measurement system

The system is composed of a spectrometer (440-850 nm) with 5nm resolution, a seven fibers Y-type catheter which delivers the laser light through the central fiber and collects the fluorescence from the target tissue using six fibers distributed around the center one (Spectr-Cluster, Cluster Ltd, Moscow, Russia) and two excitation sources. The excitation sources used are a 10 mW second harmonic of the Nd^{3+}:YAG laser at 532 nm and a15 mW He-Cd gas multimode discharge laser at 442 nm. The system is computer controlled using a software (LightView) to record and analyze the fluorescence spectra.

D . Measurement Procedures

The measurement was done keeping the catheter probe at a typical distance of 2 mm from the leaf to prevent background noise and fluorescence limitations as atmospheric scattering, leaf geometry and low power light capture. In each tree, three spectra were obtained from 10 different leaves performing thirty fluorescence curves. From these spectra were obtained the fluorescence ratios RF/FRF and BF/ChlF by averaging the values of the curves. In these analyses the values are measured from the spectrum line base, which in general have a non null value. Other procedure is to normalize the spectra values by using the back scattering peak value.

III. RESULTS

In Fig.1 typical fluorescence spectra of orange tree leaves using the measurement system described above for two different wavelength radiations (532nm and 442nm) are shown. It is easy to observe that the overall shape of the fluorescence spectrum depends on the wavelength of laser light source. Therefore, in order to extract some general information about the stress process, we have used two different ratios.

A. RF/FRF ratio

In Fig.2, we show the RF/FRF ratio as a function of time for the water stress and control trees using a 442nm laser as light source.

The variation may be caused by water stress because chlorophyll participates directly in the photosynthesis process. According to the results obtained by Dahn and coworkers in maize [4] the FR/FRF ratio decreased under water stress. However these results disagree with the results in maple leaves [8], where the RF/FRF increases between 0.8-1.1.

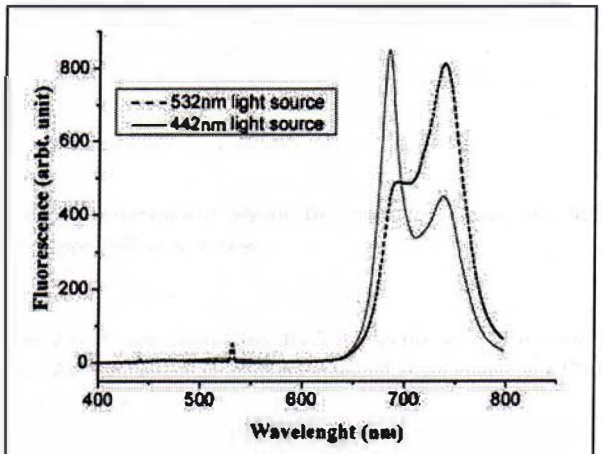

Fig. 1. Spectrum of orange tree leaf fluorescence using 442nm and 532 nm lasers as light source.

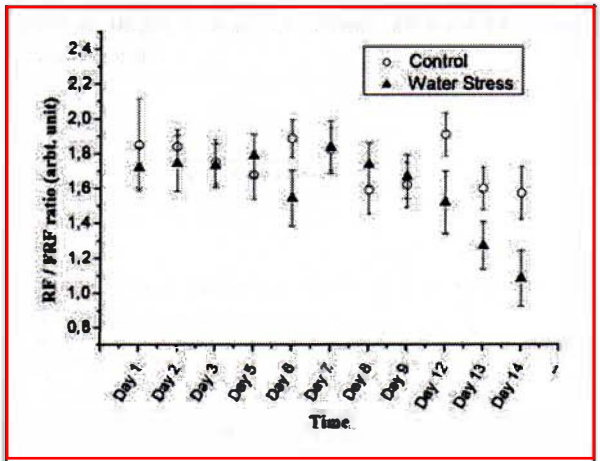

Fig.2. RF/FRF ratio for orange leaves using a light source emitting in 442nm.

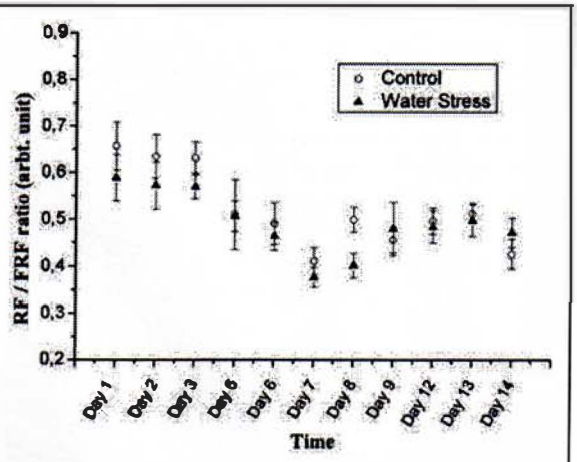

Fig.3. RF/FRF ratio for orange leaves using a light source emitting in 532nm.

The Fig.3 presents the RF/FRF using 532nm as light source, as the graphical behavior for both plants are identical it is not possible detect the response to stress.

B. BF/ChlF ratio

The Fig.4 shows the variation of the BF/ChlF using emission in 442nm. In their review Cerovic et al. presents the use of BF/ChlF ratio as tool to detect mineral deficiencies in plants. Typical N-deficient plants show large increase in the BF/ChlF compared to control plants while K-deficient present a large decrease in BF/ChlF [2]. The model chosen in this work do not prevent the control of minerals in plants, therefore conclude that any changes in the BF/ChlF is owning to water stress without mineral controls is incorrect. Even so, the BF/RF and BF/FRF behavior for stressed plants is approximately identical to control plants.

VII. Conclusion

The use of RF/FRF ratio as parameter of water stress detection was successfully for de model chosen and using a UV-blue emission laser as light source.

Others measurements of the fluorescence spectrum and environmental factors, as daily temperature and relative humidity, seeking correlations between both might confirm the water stress presence.

The model chosen have limitations to detect water stress using chlorophyll ratios due the mineral reductions. This is a progress report; other measurements will be carrying out to understand the plants response of environmental effects and development of other techniques of water stress detection.

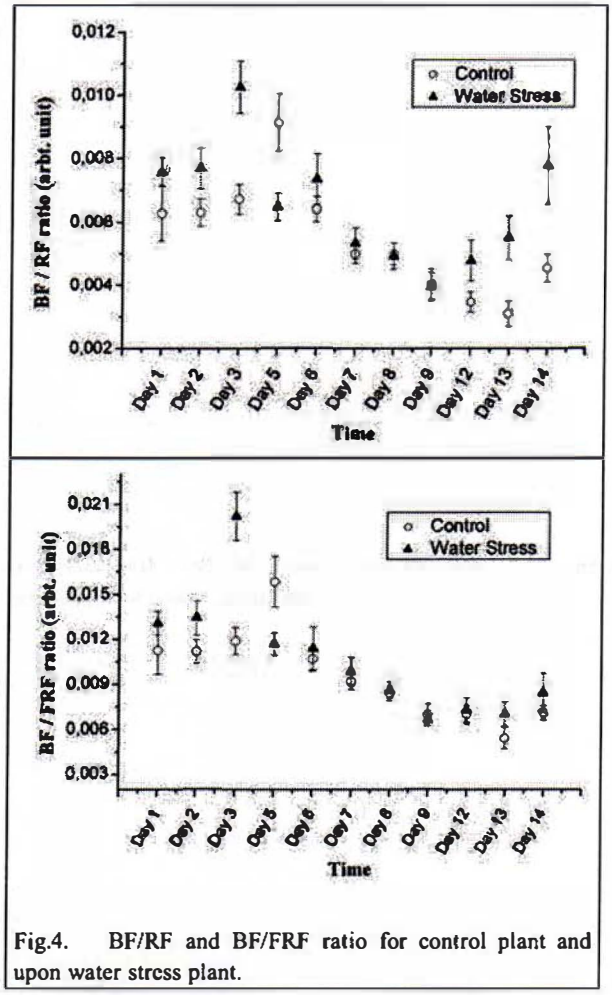

Fig.4. BF/RF and BF/FRF ratio for control plant and upon water stress plant.

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