FLUORESCENCE IMAGING SPECTROSCOPY APPLIED TO CITRUS DISEASES

C. WETTERICH, J. BELASQUE JUNIOR*, L. G. MARCASSA

Instituto de Física de São Carlos
Universidade de São Paulo,
Cx.Postal 369, São Carlos, 13560970, SP, Brazil
** Departamento Científico – Fundecitrus*
Av. Dr. Adhemar P. de Barros, Araraquara, 20114 807-040, SP, Brazil

ABSTRACT

Diseases are one of the most serious threats for citrus production worldwide. Sao Paulo, Brazil, and Florida, USA, are the most important citrus producers and, both, are making efforts for citrus diseases control. Citrus canker is one of the serious diseases, caused by the *Xanthomonas citri* subsp. *citri* bacteria (synonym. *Xanthomonas axonopodis* pv. *citri*), which infects citrus plants and relatives, causing large economic losses in citrus producing areas. Another important disease affecting the citrus production worldwide is the Huanglongbing (HLB, Greening), present too in Sao Paulo and Florida. Both bacterial pathogens are, mainly, under suppression control, applied by eradication of symptomatic and no-symptomatic plants. In this way, the detection and diagnostic of the related symptoms and, consequently, the eradication of the citrus trees are essential for higher economical losses prevention. However this task is time and finance consuming because it requires frequent field inspections followed by laboratory diagnostic. Our goal is to develop a new optical technique, applied in field conditions, to detect citrus diseases using a portable fluorescence imaging unit.

Keywords: Citrus Canker, Huanglongbing, fluorescence imaging spectroscopy, plant disease diagnosis.

INTRODUCTION

Presently, there is an interest to develop early stress detection techniques for agricultural crops. This interest is because such techniques have the potential to avoid economic losses in the field and to reduce the ecological impact of agriculture on the environment. We should point out that among several potential technologies; biophotonics is the one with the highest potential use in precision agriculture. This is because there was a fast development and miniaturization of new light sources and detectors in the last decade, allowing the use of cutting edge technology directly on the field.

Our research group has been applying biophotonics to detect citrus diseases using optical fiber laser-induced fluorescence spectroscopy for several years [Lins et al, 2005; Marcassa et al., 2006; Belasque et al, 2008; Lins et al, 2009; Lins et al, 2010]. However, recently we have concluded that such technique is not the best solution for this application [Lins et al, 2010]. Our results have shown a large variation on disease discrimination, which we believe is due to the fact that the optical fiber is placed in only a very small part of the diseased leaf. Until now we have placed it between the apparently healthy tissue (green appearance) and the necrotic or yellow parts of the leaf, therefore the optical fiber only collects light from a small portion of the leaf, since its area is smaller than 1 mm^2. It is possible that this is not the best part of the leaf to be analyzed. For this reason we believe that a more viable solution is fluorescence spectroscopy imaging, in order to obtain a more accurate diagnostic.

In this work, we describe the construction of our fluorescence spectroscopy imaging system and the first test with Citrus canker, Citrus scab, Citrus variegated chlorosis (CVC) and Huanglongbing (HLB, Greening). In the sequence, we present the materials and methods, followed by the first results and discussions. Finally, we present our conclusions.

MATERIALS AND METHODS

Fluorescence Imaging Spectroscopy System

Our fluorescence spectroscopy imaging system is a portable unit, which is composed of: i) a standard laptop computer; ii) a monochromatic charged couple device camera (CCD) (model mvBlueFOX120a, Matrix Vision, Germany), which uses a USB communication port; iii) a filter wheel (model CFW-1-8, Finger Lakes Instrumentation, USA), which holds up to eight optical filters and uses a USB communication port; iv) four pass band optical filters (models FB570-10, FB610-10, FB690-10 and FB740-10, Thorlabs, USA); v) an objective lens; vi) and high power light emitting diodes (LED's) at different wavelengths (365, 405, 470 and 530 nm) as excitation sources. The CCD and filter wheel are computer controlled by a Labview software. The system is quite portable and can be run on car batteries.

Fluorescence Spectrum Imaging Processing

So far we have used in our optical fiber laser-induced fluorescence spectroscopy studies [Lins et al, 2005; Marcassa et al., 2006; Belasque et al, 2008; Lins et al, 2009; Lins et al, 2010] the figure of merit approach (FM), which is defined as:

$$FM = \frac{\int_{680}^{712} I(\lambda)d\lambda}{\int_{712}^{750} I(\lambda)d\lambda} \qquad (1)$$

where the FM is the ratio of two integration of the spectrum I((λ) at different wavelength ranges (680-712nm by 712-750nm). Since the fluorescence

spectroscopy imaging system uses optical filters, it does not have the spectral resolution to obtain FM. Nevertheless, the filters have a bandwidth, which maybe interpreted as an integral over the transmission curve of the filter. Therefore, the images at 690 nm (IM_1) and at 740 nm (IM_2) maybe define respectively as:

$$IM_1 = \int_{filter\ 690\ nm} I(\lambda)d\lambda \quad , \quad IM_2 = \int_{filter\ 740\ nm} I(\lambda)d\lambda \tag{2}$$

And finally, we can define the figure of merit image (FMI) as:

$$FMI = \frac{IM_1}{IM_2} \tag{3}$$

Experiment

In the experiment, 80 citrus trees were evaluated in six farms from four municipalities in São Paulo State, Brazil. The leaf samples come from plants of *Citrus reticulata* (Blanco), *Citrus sinensis* (L. Osbeck), *Citrus aurantifolia* (Swingle), *Citrus latifolia* (Tanaka), *Citrus limonia* (L. Osbeck), and *C. sinensis* x *C. reticulata* hybrid; which presented diseases symptoms of Citrus canker, Citrus scab, CVC, and HLB. The images were collected from five leaves with visual symptoms of disease and from one healthy leaves per plant. A total group of 400 symptomatic leaves and 80 healthy leaves were collected. We should point out that in order to avoid any detachment time effect the LIF measurements were performed in the first five minutes after the leaves were collected from each plant [Lins et al, 2010]. After the measurements onsite, the leaves were transported in closed styrofoam boxes to laboratory for diseases diagnostic using traditional tests [Schaad et al, 2001; Golmohammadi et al, 2007; Cubero et al, 2001; Mavrodieva et al, 2004]. Citrus canker and Citrus scab diseased samples were differentiated by isolation of *Xanthomonas* bacterium pathogen in nutrient agar (NA) media [Schaad et al, 2001]. CVC and HLB diseased samples were diagnostic by PCR protocols.

RESULTS AND DISCUSSION

Initially, we present our results on the healthy leaf images. Fig. 1 shows the image of a healthy leaf at 690nm (a), at 740 nm (b) and FMI (c). It is convenient to define a region of interest (ROI) within the leaf, as shown in fig.1c. It is important to point out that such ROI may vary from leaf to leaf, since the leaf size varies as well. Nevertheless, we have observed that the average value inside the ROI, considering all healthy samples, is about 2.0 ±0.4 at using the excitation light at 470 nm. This large dispersion may be due to different environmental and physiological conditions of each plant, and it has been observed by us previously [Lins et al, 2010]. However, the standard deviation in each leaf is about 10% only. Fig. 1d shows the histogram of a healthy leaf FMI.

Fig. 2 shows typical FMI for diseased leaves contaminated with Citrus canker, Citrus scab, CVC, and HLB using all excitation sources (365, 405, 470 and 530 nm). We also show digital photos of the different leaves. From the images of figure 2 we can make some observations that may lead to discrimination of various diseases. Only the Citrus canker has high values of FMI for excitation at 365 nm. The sample of CVC has the highest values of FMI for excitation at 470 nm. The sample of Citrus scab does not have high values of FMI for 405 nm, despite the samples of Citrus canker and CVC still present high levels. The greening has intermediate values for FMI for excitation at 470 nm, but there is not well defined lesion.

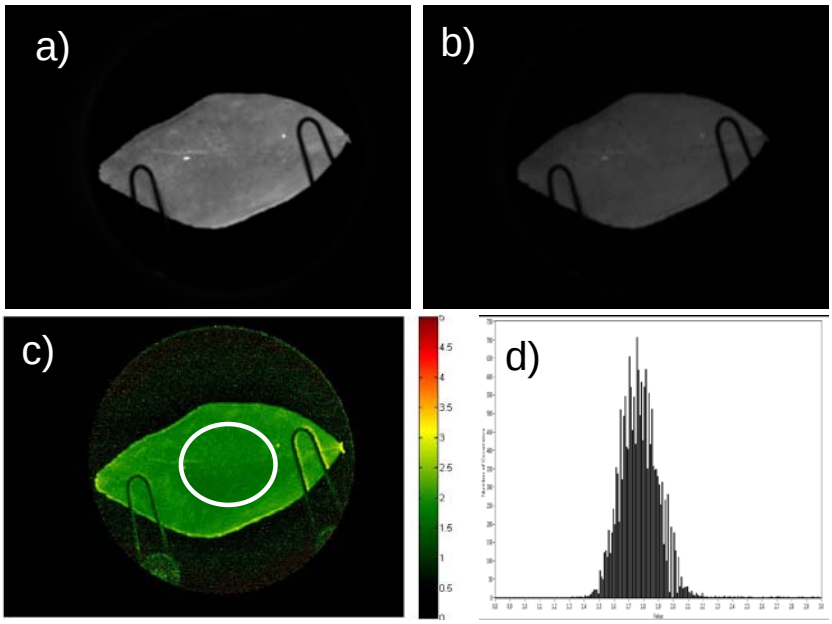

Fig. 1 – Fluorescence Image of a healthy leaf at (a) 690 nm and (b) 740 nm. (c) Figure of merit image (FMI), which shows a region of interest (ROI) in white. d) Histogram of a healthy leaf FMI.

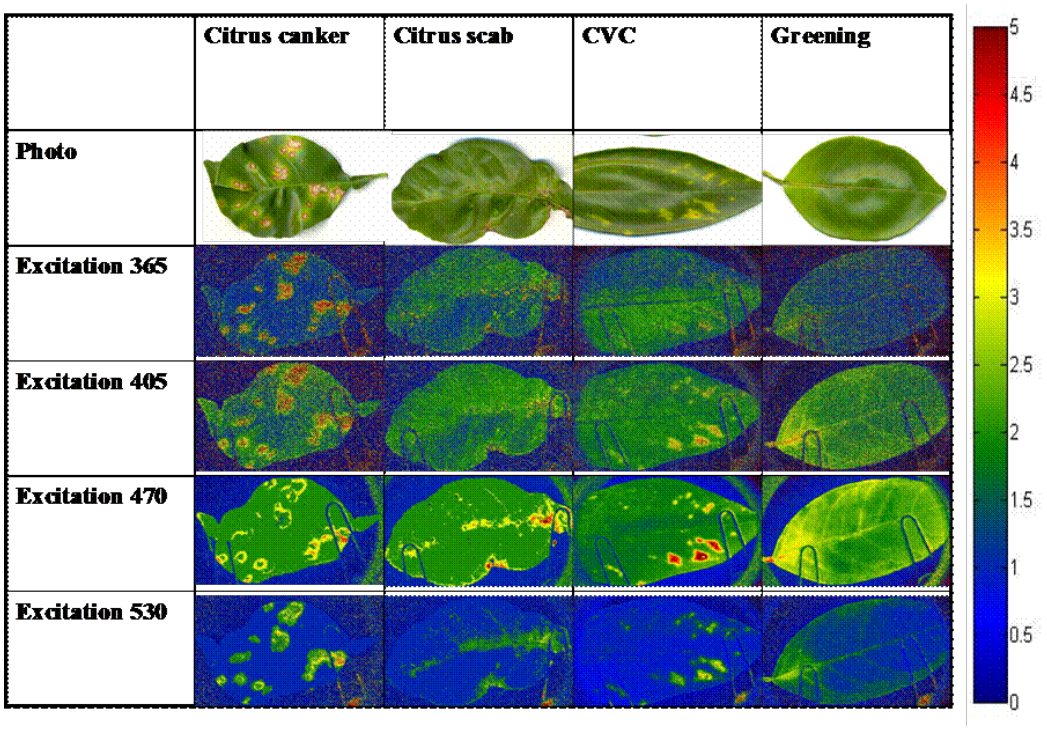

Fig. 2 – FMI for Citrus canker, Citrus scab, CVC, and HLB using all excitation sources (365, 405, 470 and 530 nm). Digital photos are also shown.

CONCLUSION

In this work we have applied fluorescence imaging spectroscopy to investigate the spectral response caused by Citrus canker, Citrus scab, CVC, and HLB using four excitation sources (365, 405, 470 and 530 nm). The preliminary results have shown the fluorescence imaging spectroscopy has the potential to discriminate all the citrus diseases studied in this work. We believe that imaging processing techniques will be necessary to create accurate and precise, automated field applied, detection systems for the major citrus diseases under suppression or quarantine regulatory efforts worldwide.

ACKNOWLEDGEMENTS

We thanks Fundação de Amparo a Pesquisa do Estado de São Paulo-FAPESP and Conselho Nacional de Desenvolvimento Científico e tecnológico-CNPq for financial support.

REFERENCES

Belasque Jr J., Gasparoto M.C.G. and Marcassa L.G., 2008, Detection of mechanical and disease stresses in citrus plants by fluorescence spectroscopy, Appl. Opt. 47, 1922.

Cubero J., Graham J.H., and Gottwald T.R., 2001, Quantitative PCR Method for Diagnosis of Citrus Bacterial Canker, Appl. Environ. Microbiol. 67, 2849.

Golmohammadi M., Cubero J., Penalver J., Quesada J.M., M. Lopez M.M., and Llop P., 2007, Diagnosis of *Xanthomonas axonopodis* pv. *citri*, causal agent of citrus canker, in commercial fruits by isolation and PCR-based methods, Journal of applied microbiology, 103, 2309.

Lins E.C., Dias Nunes F., Gasparato M.C.G., Belasque Jr. J., Bagnato V.S. and Marcassa L.G, 2005, Fluorescence spectroscopy to detect water stress in orange trees, in *Proceedings of IEEE Conference on Microwave and Optoelectronics* (Institute of Electrical and Electronics Engineers, New York), pp. 534-537.

Lins E.C, Belasque Jr. J. and Marcassa L.G, 2009, Detection of citrus canker in citrus plants using laser induced fluorescence spectroscopy, Precision Agriculture, 10, 319.

Lins E.C, Belasque Jr. J. and Marcassa L.G, 2010, Optical fiber laser induced fluorescence spectroscopy as a citrus canker diagnostic, Appl. Opt. 49, 663.

Marcassa L.G., Gasparoto M.C.G., Belasque Jr J., Lins E.C, Dias Nunes F., and Bagnato V.S., 2006, Fluorescence Spectroscopy Applied to Orange Trees, Laser Physics, 16, 884.

Mavrodieva V., Levy L., and Gabriel D.W., 2004, Improved sampling methods for real-time polymerase chain reaction diagnosis of citrus canker from field samples,Phytopathology 94, 61.

Schaad N.W., Jones J.B. and W. Chun, 2001, Laboratory guide for identification of plant pathogenic bacteria, 2$^{o.}$ Edition, APS Press, Saint Paul, Minnesota.