Spectral reflectance analysis of tobacco leaves and fungus infection detection

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ABSTRACT

Optical properties of vegetable matter and associated analytical techniques such as VIS and NIR reflectance spectroscopy were used for several decades in the foodstuff industry. By means of these techniques, is possible to study and determine the plant pathology of crops: health or disease conditions, water stress, growth, etc., being a good alternative to traditional chemical or biological tests, generally very invasive and even destructive. The aim of this work is to analyse and quantify the effect of the presence and development of the pathogen *Fusarium* on spectral signatures of Burley tobacco leaves, and to evaluate the potential of radiometric techniques as tools for diagnosis.

1. INTRODUCTION

Optical radiation impinging on a sample of any material is subject to some processes causing part of the energy carried by radiation being transmitted through the sample, part of it being absorbed, and part being reflected (specular or diffuse), in wavebands and proportions that depend on the particular nature of the sample among other factors. Optical properties seems to be able for use as an important tool in identification and classification of plants, in determination of productivity, yield estimation as well as in measuring and analysing different specific parameters of individual plants and crops. Diffuse reflectance of vegetable matter and associated analytical techniques such as VIS and NIR reflectance spectroscopy were used for several decades in the foodstuff industry (Cozzolino et al. 2003, Norris et al. 1976, Murray 1986). By means of these techniques, is possible to study and determine biophysical and physiological status of individual plants and crops: health or disease conditions, water stress, growth stage, etc. (Hamid and Larsolle 2003, Hamid 2005), being a good alternative to traditional chemical or biological tests, generally very invasive and even destructive. The organized array of reflectance in terms of wavelength, ranging generally from VIS to NIR, is called a "spectral signature" of the given specie. The spectral signature could be modifyed according to biological condition of the sample under analysis, for example its health condition, the presence of diseases, plant growth or maturity, nutritional condition, moisture or nutrient content, etc.

Tobacco is a crop of great socio-economical importance at the north-west region of Argentina. It can be affected by several pathogens in different stages of growing; among these pathogens, the fungus Fusarium oxysporum can make the plant to get withered (Ramallo 2005), causing economical and agricultural damages (yield losses, increased chemical products to control the affection, environmental impact). The aim of this work is to analyse

and quantify the effect of the presence and development of the pathogen *Fusarium* on spectral signatures of Burley tobacco leaves, and to evaluate the potential of radiometric techniques as tools for diagnosis.

2. MATERIALS AND METHODS

Measurements of spectral reflectance were performed, ranging from 310 to 1900 nm. The data correspond to two groups: leaves coming from healthy tobacco plants, labelled T; and leaves from plants inoculated with fungus, named F. In the main experiment, samples of each group were taken at 1, 2, 3 and 4 weeks from inoculation. Spectral measurements were performed within 24 hours from the extraction of samples.

Reflectance curves –spectral signatures– were measured by means of a spectroradiometer with double monochromator Optronic OL 750, ranging from 300 nm to 1100 nm by 5 nm steps, and from 1100 nm to 1900 nm by 10 nm steps, on the front of the leaf and without specular component . From these spectral data the chromatic coordinates CIE 1931 (x, y), coordinates UCS 1976 (u', v') and values of (a*, b*) for type A illuminate were obtained.

A pilot experiment was performed taking measurements in three stages (1, 2, and 3 weeks). On each stage, a control sample (T) and inoculated sample were measured. After that, a main experiment was carried on having four stages (1, 2, 3, and 4 weeks). At the last stage, the fungi infection of the inoculated plants was easily visually detectable. For all stages, the spectral signature for 5 samples of each condition (T and F) was determined. Following are presented the obtained spectral signatures, (a*, b*) values and some results obtained applying Principal Component Analysis to the reflectance data of the main experiment.

3. RESULTS

Results of the pilot experiment showed differences in spectral signatures and in chromatic characteristics associated to the healthy (T) and inoculated (F) samples. Those differences were increased on time. In the first stage (a: week 1) there is no significant differences (see Figure 1).

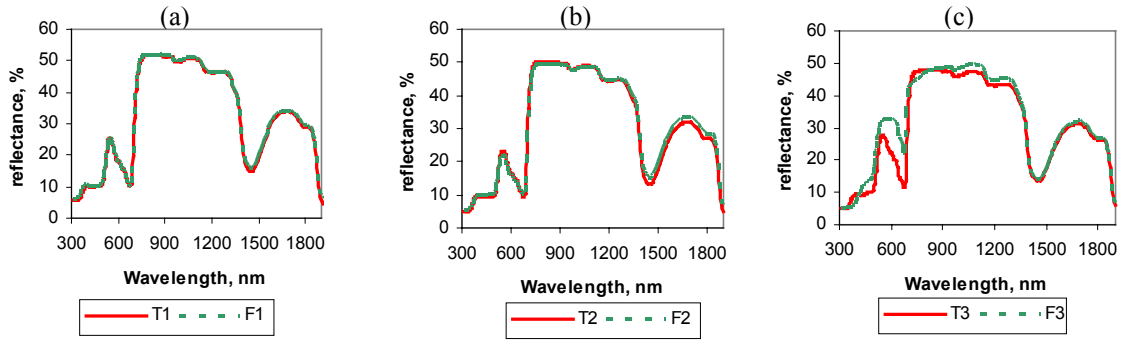

Figure 1. Resulting spectral signatures from pilot experiment: (a) week 1; (b) week 2; (c) week 3.

Differences between samples T's and F's are noticeably incremented as can be seen in Figure 2, that shows the corresponding values (a*, b*).

Figures 3 and 4 show results obtained in the main experiment in which several samples for each condition were measured. Figure 3 shows average spectral signatures corres-ponding to each stage. Averaged values of (a*, b*) for each condition are plotted on Figure 4.

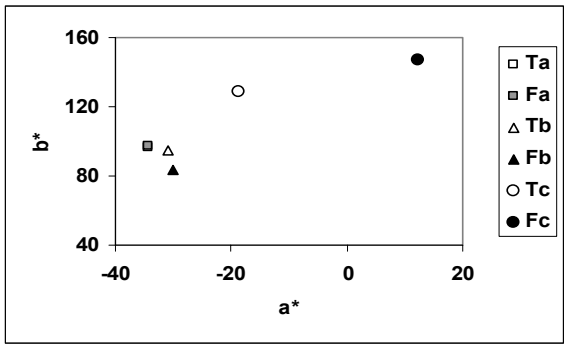

Figure 2. Chromatic values (a, b*) from the pilot experiment: (a) week 1; (b) week 2; (c) week 3.*

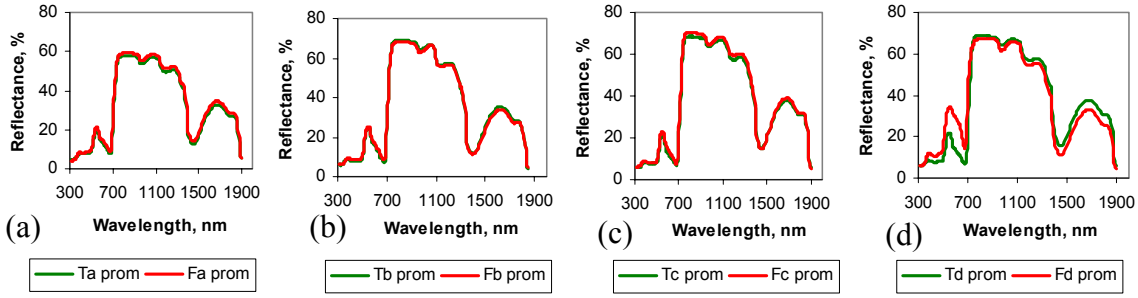

Figure 3. Second experiment: Resulting spectral signatures from the training phase: (a) one week; (b) two weeks; (c) tree weeks, (d) four weeks.

As does the pilot experiment, the second also shows increased differences between samples T and F through successive stages, despite the variability showed by samples at the same condition which in some cases could masks the differences, particularly on weeks 2 and 3, the most important period of time to make the diagnostic effective. Differences in values (a*, b*) for averaged measurements for samples T and F are also markedly increased, as can be seen in Figure 4.

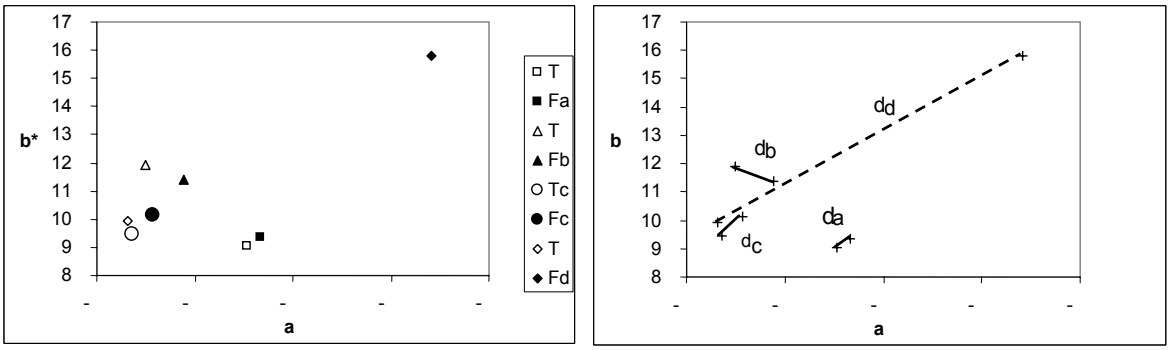

Figure 4. Second experiment: *Left: values (a*, b*) for (a) week 1; (b) week 2; (c)week 3; (d) week 4. Rigth: distance in (a*, b*) space between samples T and F for each stage.*

PCA was performed on the spectral data for samples in each condition (T y F) to analyze the existence of any segregation on the basis of effect of the pathogen *Fusarium* on spectral signatures. Separation between healthy (T) and inoculated (F) simples, showed in Figure 5, right, is sharply noticeable at the week 4, whilst in previous stages there was a trend of the groups to separate, but they were not clearly distinguishable (what is our main interest), as can be seen in Figure 5, left.

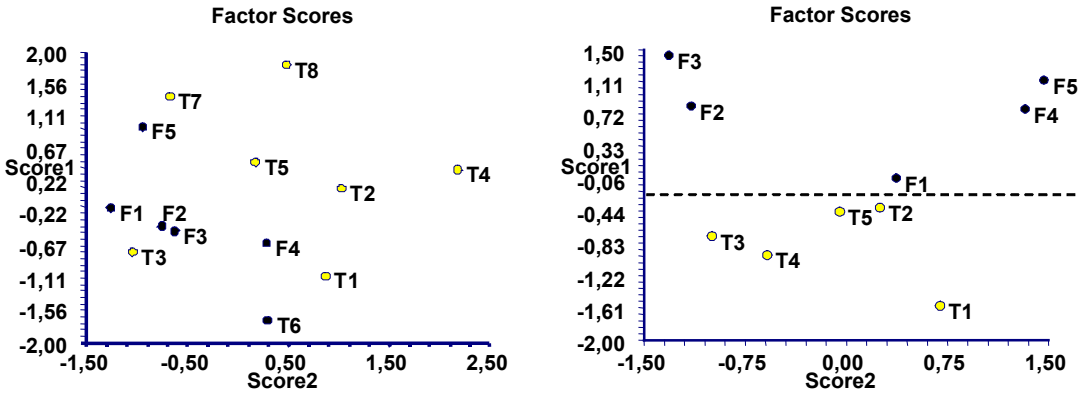

Figure 5. Principal Component Scores plot, for second experiment data. Left: results for week 2, Right: results for week 4.

4. CONCLUSION

The analysis of the obtained results by means of a chromatic values (a*, b*) and Principal Component Analysis, shows that spectral signatures of tobacco leaves are noticeably modified, in the visible region –associated to colour characteristics– as well as in the NIR region, when the fungus was present. This fact seems to indicate that the application of this analytical technique to the measured spectral signatures could give us important information and criteria to predict when the plant (or crop) could be considered infected with this pathogen and take suitable actions on time to avoid serious losses. However, the intrinsic variability founded in reflectance of samples at any condition make the differentiation between healthy and infected plants harder. Much work have to be done in order to overcome such difficulties, specially in the selection of samples to be analysed, increasing the amount of data suitable to the application of PCA.

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