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CITRUS GREENING DISEASE INVESTIGATION USING MICRO SYNCHROTRON RADIATION X-RAY FLUORESCENCE IN ASSOCIATION WITH SOFT INDEPENDENT MODELLING OF CLASS ANALOGY (SIMCA)

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This study describes the use of micro synchrotron radiation X-ray fluorescence (μSR-XRF) to investigate citrus greening disease in sweet orange (Citrus sinensis) plants. An experiment using healthy plants as control and plants of the same variety infected with Candidatus Liberibacter asiaticus (CLas) was performed to verify variations of the mineral composition of citrus leaves. A μSR-XRF system using the D09B X-ray fluorescence beam line at the Brazilian Synchrotron Light Source (LNLS, Campinas, São Paulo State) was employed for this purpose. The data were analyzed using a chemometric tool called soft independent modelling of class analogy (SIMCA). The promising results from SIMCA models reinforce the evidence that plants infected by citrus greening (both asymptomatic and symptomatic) undergo alterations in their micro- and macronutrient compositions.

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The detection of citrus greening or *huanglongbing* (HLB) in plants prior to the onset of visual symptoms is a challenging task. Expeditious and reliable methods are urgently needed because citrus greening contamination occurs very rapidly[1,2]. This spread of disease leads to the progressive reduction of orchards, resulting in economic damage to citrus producers and related industries[3,4]. Currently, the development of methods for early diagnosis would result in important tools for citrus greening management and control.

Nowadays, visual inspection is one of the most applied methods to diagnose citrus greening; however, visual inspection is highly influenced by subjective interpretation, and diagnostic errors can be higher than 30%[5]. Additionally, plants may be infected for up to several months (between 6 and 36 months) before visual symptoms are detected[6]. Another common method for diagnosis is to use polymerase chain reaction (PCR) to test for the bacterial deoxyribonucleic acid (DNA).[7] The main drawback to PCR assays is the fact that these methods are time consuming and expensive[8].

The purpose of this study was to investigate the mineral composition of citrus leaves infected by citrus greening using spectra obtained by micro synchrotron radiation X-ray fluorescence (μSR-XRF); due to the ability of this technique to scan samples in different positions with adequate sensitivity, repeatability and reproducibility, without pre-treatment of the samples.

The measurements were performed with the D09B X-ray fluorescence beam line at the Brazilian Synchrotron Light Source (LNLS, Campinas, São Paulo State). The setup configuration used a monochromatic beam with a 200 *x* 200 μm cross section. The multilayer monochromator consisted of 75 d-periods of W/C layers. The maximum photon energy was 12 keV, and the tests were executed in an air atmosphere. The active total area of the detector was 30 mm^2. The 4 mm circular collimator was used in the detector to improve the signal-to-noise ratio of the XRF spectra and to avoid decreasing of resolution at higher count rates. The distances from the sample (citrus leaf) to the source and to the detector were 120 and 20 mm, respectively. An Si(Li) detector with an 8 μm Be window was used; this detector has an energy resolution of 165 eV for the Mn Kα line. The samples were positioned at an angle of 45° with respect to both the incident beam and the detector. The measurement positions on the leaves were marked with computer-controlled X, Y, Z, θ_z stages and an optical microscope. The scanning of the leaves was divided into three regions: midrib and two lateral sides, where nine spectra were obtained per leaf. The vertical and horizontal distances between points were 1 mm. The irradiation time per point was 100 s and the dead time was approximately 2%. The diagram of μSR-XRF configuration setup was shown in Figure 1.

The evaluation of the mineral composition using μSR-XRF and soft independent modelling of class analogy (SIMCA) was suitable for understanding the effects of the citrus greening disease and proposing

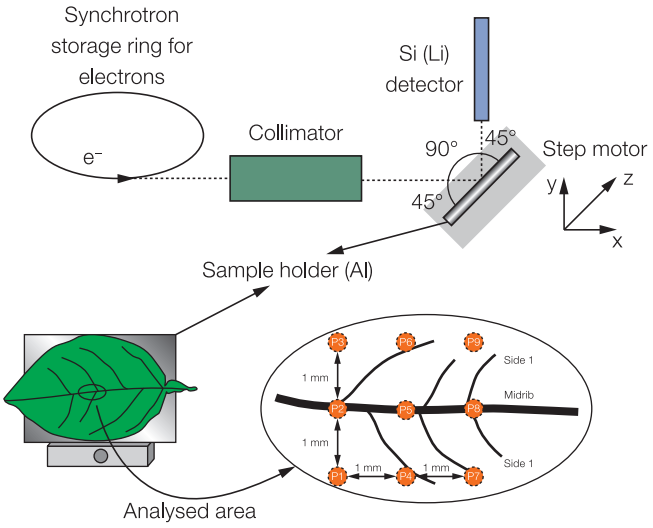

Figure 1. Diagram of micro synchrotron radiation X-ray fluorescence (μSR-XRF) configuration setup for measurements of citrus leaves.

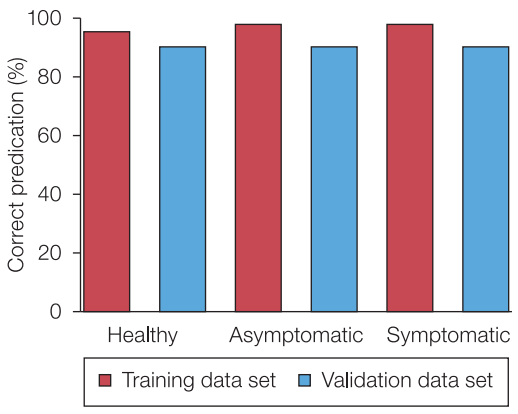

Figure 2. Values of correct predictions obtained from *soft independent modelling of class analogy* (SIMCA) models.

the classification models, as shown in Figure 2. The elements K, Ca, Fe, Cu and Zn and the region of coherent and incoherent scatterings were very important for the differentiation of these three conditions.

The monitoring of the mineral compositions of the plants was qualitative but could generate responses about the infected samples, mainly those in the asymptomatic stage.

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