4.15 Visible/near-infrared spectroscopy for discrimination of HLB-infected citrus leaves from healthy leaves

Poole G.[1], Windham W.[2], Heitschmidt G.[2], Park B.[2], and Gottwald T.[1]

[2] USDA ARS, STPP, Fort Pierce, FL, 34945, [1] USDA ARS,QSARU, Athens, GA 30605

Introduction:

Researchers have used various hyperspectral systems, covering several areas of the electromagnetic spectrum to investigate all types of disease/plant interactions. Delalieux et al. (1) used a spectrophotometer to investigate apple scab disease. They found that they could differentiate between infected and non-infected leaves at 10 days using several different statistical models, using data from 1350-2500 nm. After 26 days of inoculation, the visible portion of the electromagnetic spectrum (400-700 nm) was also useful for discrimination purposes, which they speculated to be due to chlorosis in the leaves. Liu et al. (2), using a spectrophotometer measuring between 350-2500 nm, investigated brown spot disease in rice. By using large portions of the spectrum for their analyses, they were able to determine disease severity with a RMSE of 2%.

The purpose of this research was to investigate using visible and near-infrared (400 – 1100 nm) spectroscopy to differentiate HLB infected citrus leaves from uninfected leaves.

Materials and methods:

Fifty-five leaves were collected from various citrus plants on the United States Horticultural Research Laboratory's research farm that showed the various symptoms of HLB infection (green islands, blotchiness, heavy chlorosis, etc). Leaves (N=22) were also collected from citrus trees in a controlled greenhouse and were used as a "negative" control. All leaves were collected on the same day. A 38mm diameter section was cut from the center of the leaf and placed into a cylindrical sample cell (38-mm I.D.) with an optical quartz surface and foam core backing (Figure 1). The leaf samples were analyzed using an NIRSystems 6500 monochromator (NIRSystems, Silver Spring, MD). Spectra were recorded from 400 nm to 2500 nm in 2-nm intervals and analyzed from 400 nm to 950 nm. A commercial spectral analysis program (NIRS3, Infrasoft International, Inc., Port Matilda, PA) was used to analyze the spectra and for partial least squares (PLS) regression. PLS regression was used to relate the spectra to an arbitrary HLB critical threshold (CT) variable of 20 for HLB-infected (positive) leaves and 40 for HLB-uninfected leaves (negative). The spectral data set was transformed with multiplicative scatter correction and smoothed by a 9-point running average.

Figure 1: Sample cups for the FOSS, with leaf samples already placed.

Results and Discussion:

A PLS calibration was obtained for prediction of HLB CT values. The model contained 6 PLS components with a standard error of prediction of 5.4 and a multiple coefficient of determination of 0.66, from full one out cross-validation. The first four PLS components accounted for 68% of the total spectral variation with components 1, 2 and 4 expressing 41, 15, and 6% of the

variation, respectively. The PLS loadings contain peaks in the red (600-700 nm) regions of the spectrum for photosynthesis.

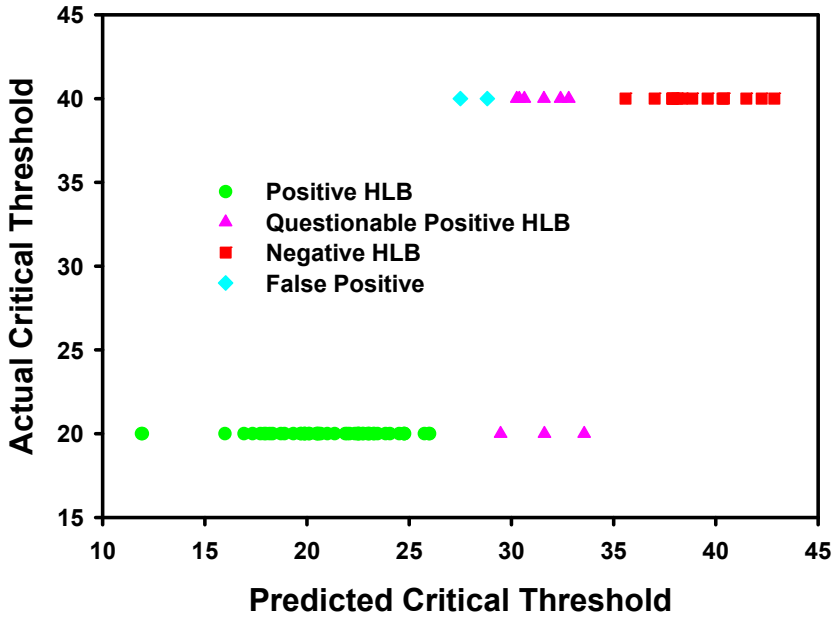

Figure 2. Predicted CT values of HLB positive and negative citrus leaves.

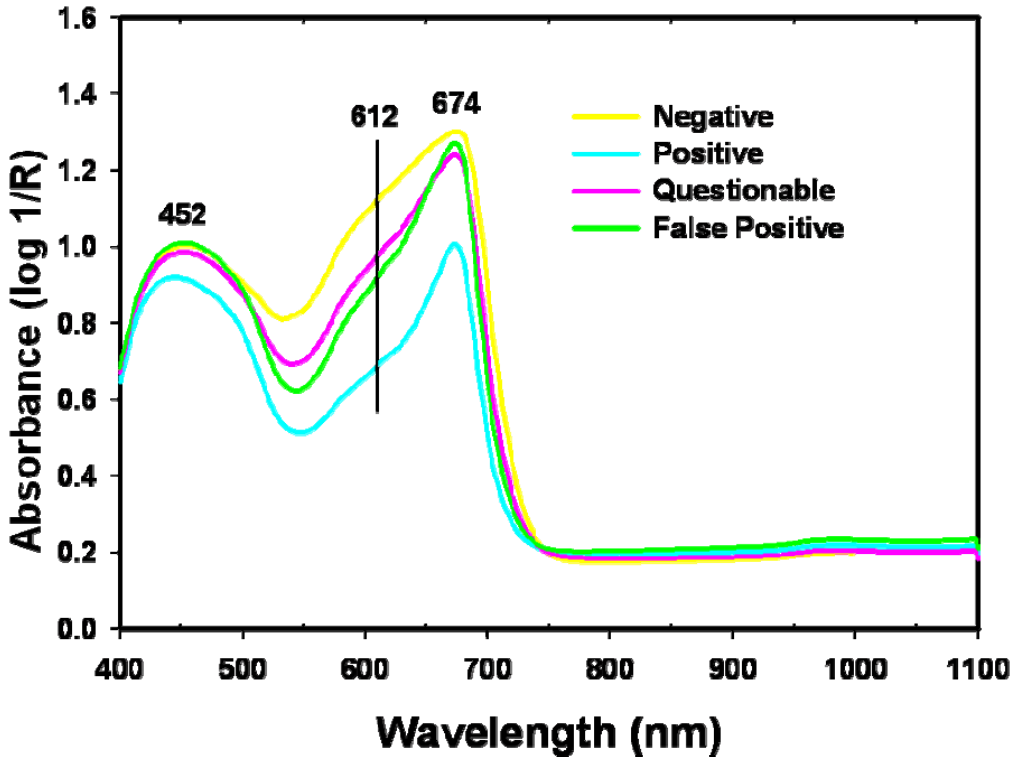

Figure 3: Spectral curves for negative, positive, questionable, and false positive leaves, infected with Huanglongbing.

The scatter plot of predicted CT values from full one out cross-validation is shown in Fig 2. Ninety-five percent of the positive HLB leaves were accurately predicted with an average CT of 21, and 64% of the negative HLB leaves were accurately predicted with an average CT of 39.2. However, three positive HLB leaves and 6 negative HLB leaves had an average value 31.4 which placed them in the "questionable category" of infection. Two negative HLB leaves were incorrectly predicted as HLB false positives having an average CT of 28.2.
Spectra within each of the four groups of predicted samples shown in Figure 2 were averaged (Figure 3). Average spectra for the negative HLB leaves showed the expected spectral curve that all healthy plants show (3). There was a peak in the blue (400-500 nm) and red (600-700 nm) regions of the spectrum resulting from photosynthesis (3).

The primary difference between the HLB negative and the HLB positive leaves was that these peaks associated with chlorophyll absorption decreased for the infected leaves. PLS loading factor 1 had a large absorption band at 612 nm which appears to be a measure of the degree of "greenness" in the leaves.
A reduction in chlorophyll absorption is commonly seen when a plant is stressed by a biostressor. Chlorophyll absorption in a healthy plant is weak around 690-700 nm (4). As the plant becomes stressed (in this case, by the HLB infection) there is an expected decrease in chlorophyll production, which would lead to a corresponding increase in reflectance at this part of the spectrum (5).
Being that most plant stressors will induce this change in chlorophyll absorption due to a decrease in chlorophyll production, it is unlikely that this visible near-infrared (400 to 1000 nm) portion of the electromagnetic spectrum alone will be able to differentiate HLB infected plants from plants that are compromised by some other biostressor.

Conclusion:

A PLS model correctly predicted 95% and 64% of the leaves that were positive and negative for HLB, respectively. The PLS loadings indicated absorption bands related to chlorophyll, and bands associated with chlorophyll absorption decreased in infected leaves. However, some HLB negative leaves also showed decreased chlorophyll absorption which resulted in an inaccurate prediction. Based on the results from this study, it is unlikely that the visible near-infrared (400 to 1000 nm) portion of the electromagnetic spectrum alone will be able to differentiate HLB infected plants from plants that are compromised by some other biostressor.

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