Spectral Evaluations for Developing Optical Methods of Huanglongbing (HLB) Detection in Citrus Orchards

SINDHUJA SANKARAN AND REZA EHSANI*

University of Florida, IFAS, Citrus Research and Education Center, Precision Agriculture, 700 Experiment Station Road, Lake Alfred, FL 33850

*Corresponding author; phone: (863) 956-1151, ext. 1228; email: ehsani@ufl.edu

Additional index words. optical sensors, disease detection, SVC spectroradiometer, mid-infrared spectrometer

Huanglongbing (HLB) is a devastating citrus disease that threatens the citrus industry in Florida. Several efforts are ongoing to control and contain this disease to protect the citrus industry. Among different approaches, detection of HLB is one of the critical steps in HLB management and control. At the Citrus Research and Education Center, Lake Alfred, we are working on multiple approaches to develop an effective and accurate mobile sensor system that can be used to detect HLB under field conditions. This research presents some of our ongoing work on HLB detection. A SVC HR-1024 spectroradiometer (350–2,500 nm) and a portable InfraSpec VFA-IR spectrometer (5,150–10,720 nm) were used to collect data from the healthy and HLB-infected citrus leaves to evaluate the applicability of the optical sensors. The reflectance data were collected in the visible, near-infrared, and mid-infrared regions of the electromagnetic spectra. The reflectance data were analyzed using statistical methods to classify HLB-infected citrus leaves from that of healthy ones. Preliminary results showed the potential of these methods in detecting HLB-infected citrus leaves with good accuracy.

Huanglongbing (HLB) or citrus greening is a devastating citrus disease that threatens the economics of citrus production in Florida and other parts of world. Researchers, citrus industries, and other stakeholders are working together to control and eliminate the disease for sustainable citrus production. Disease detection is a vital step in citrus management. Currently, visual scouting by trained personnel is the most widely used technique for the identification of disease symptoms in the leaves of the HLB-infected trees. Once the trees are identified as diseased by the scouting crew, the leaves are collected from these trees and sent for polymerase chain reaction (PCR) analysis for confirmation. After the samples are found to be HLB-positive, the trees are removed as they act as a possible source of inoculum and can result in further spread of the disease through vectors (psyllids). As the costs associated with HLB disease management are high (Muraro and Morris, 2009) and with challenges to attain a high scouting efficiency and accuracy (Futch et al., 2009), there is a need for accurate field-based sensors for HLB detection in citrus leaves.

Portable and rugged optical sensors are being explored for their real-time, non-destructive, and rapid field-based detection of citrus diseases. The reflectance (from the tree canopy) in the visible and infrared regions of the electromagnetic spectra is known to provide information on the physiological stress levels in plants (Muhammed, 2005; Sankaran et al., 2010a; Xu et al., 2007). Some of these wavebands specific to a disease/stress condition can be used to identify plant diseases and nutrient deficiencies.

In this study, visible-near infrared and mid-infrared spectrometers have been investigated as possible sensing technologies for HLB detection in citrus leaves. The present work evaluates the potential of SVC HR-1024 spectroradiometer (visible-near infrared range, 350–2,500 nm) and InfraSpec spectrometer (mid-infrared range, 5.15–10.72 µm) in classifying the HLB infected leaves from that of the healthy ones.

Materials and Methods

Visible-near infrared spectrometry. The spectral reflectance data from HLB-infected symptomatic and healthy leaves (Devil’s Garden, Southern Gardens citrus grove, Clewiston, FL) were collected using a high-resolution field-portable spectroradiometer, SVC HR-1024 (Spectra Vista Corp., Poughkeepsie, NY) interfaced with a computer in the spectral range 350 nm to 2,500 nm under field conditions. The spectroradiometer was fixed to an agricultural vehicle during data collection (Fig. 1a).

The HLB-infected samples were pre-marked and the samples were confirmed using PCR technique. The number of healthy and HLB-infected samples was about 100 each. Five spectra from each sample were collected and averaged for analysis. During the data collection, the distance between the spectroradiometer and the leaf sample was maintained about 0.5–0.6 m. Additional light generated using two 500-W portable halogen lamps was utilized for data collection. The spectroradiometer was calibrated before use.

Mid-infrared spectrometry. Citrus leaves of different varieties (‘Hamlin’, ‘Valencia’, sweet orange and grapefruit) were collected from trees (44 healthy and 54 HLB-infected) located at the Citrus Research and Education Center groves, Lake Alfred, FL. The HLB-infected samples (PCR confirmed) consisted of...
symptomatic leaves with blotchy mottle or some yellowing. The samples for data collection were prepared by grinding the leaves into fine powder. Though the present technique involves a simple sample preparation method, in future the process can be automated through incorporation of mechanical grinding for field-based sensing application. Presently, in this research, efforts were made to assess the applicability of mid-infrared spectroscopy in HLB detection.

A InfraSpec VFA-IR spectrometer (Wilks Enterprise Inc., East Norwalk, CT), a portable MIR instrument, was used to collect the mid-infrared absorbance spectra in the range 5.15 to 10.72 µm (1941–932 cm⁻¹). This portable instrument (Fig. 1b) was interfaced with a computer and operated in attenuated total reflection (ATR) mode. The features of the spectrometer were controlled using Igor Pro 6.01, a program provided by the manufacturer. The healthy and HLB-infected leaf samples were placed in the ATR crystal window (50 × 16 mm) of the spectrometer and five replicate spectra were collected. Background was considered as a blank crystal (without any sample).

**Data analysis.** Raw data from both spectrometers were preprocessed before further analysis. The raw data from the spectroradiometer were normalized (Euclidean normalization), while the data from mid-infrared spectrometer was baseline corrected using Igor Pro 6.01 for the range 5.15 to 10.72 µm. Following the normalization, the visible-near infrared reflectance data were binned for every 25-nm wavelength to reduce the data points from 989 to 86 spectral reflectance features. The baseline-corrected mid-infrared data consisted of 128 spectral absorbance features. After normalization/baseline correction, first and second derivatives from both datasets were estimated using a Savitzky-Golay filter having a window size of seven and polynomial order of two (quadratic).

The raw preprocessed spectral data were combined with first and second derivatives, resulting in total numbers of 246 and 370 spectral features from visible-near infrared and mid-infrared spectrometers, respectively. Principal component analysis (PCA) was performed to reduce the number of spectral features such that the principal components (PCs) accounted for 99% variance. The PC scores were used as input features in the classification algorithms. The dataset was divided into 80% training dataset and 20% test dataset after randomization. Two classification algorithms, k-nearest neighbor (kNN) and quadratic discriminant analysis (QDA), were developed using the training dataset and the developed algorithm was then tested using the test dataset. The algorithms were tested three times and the average overall and individual class (healthy and HLB) classification accuracies were determined.

**Results and Discussion**

Figure 2 (a and b) presents typical reflectance spectra of healthy and HLB-infected samples collected using a SVC spectroradiometer and InfraSpec spectrometer, respectively. The PCA plots of both datasets indicated a good separation of PC scores between healthy and HLB class (Fig. 3 depicts scores of 3 PCs).

The number of PCs representing 99% of the variation in the entire visible-near infrared and mid-infrared datasets was found to be 24 and 54, respectively. The selected PC scores were used as input features in the classification algorithm. The classification of the visible-near infrared spectral test dataset resulted in an overall average classification accuracy of about 93% and 86% using QDA-based and kNN-based algorithm, respectively (Fig. 4a). Similarly, the corresponding classification accuracy achieved during the classification of mid-infrared spectral test data was about 86% and 97%, respectively (Fig. 4b). The “k” in kNN-based algorithm was optimized from 1 to 15 based on the k value that yields highest overall as well as individual class classification accuracy. It was found that k = 7 resulted in higher classification accuracies than other values for visible-near infrared spectral data. However, k = 1 or 2 was found to be optimum while classifying the mid-infrared spectral data.

Comparing the performance of QDA and kNN, the QDA-based algorithm performed better than kNN-based algorithm while classifying visible-near infrared spectral data yielding a high HLB class classification accuracy (fewer false negatives, HLB classified as healthy). On the other hand, during mid-infrared spectral analysis, the kNN-based algorithm was found to be better than QDA-based algorithm in terms of both overall as well as individual class classification accuracy. Thus, for visible-near infrared spectral analysis, QDA was found to be the more suitable algorithm, while for mid-infrared spectral analysis, kNN (k = 1 or 2) was found to be more applicable, resulting in higher classification accuracies.

Starch is known to accumulate in HLB-infected leaves in comparison to healthy leaves (Etxeberria et al., 2007, 2009; Kim et al., 2009; Onuki et al., 2002; Taba et al., 2006). The difference in starch content in the healthy and HLB-infected leaves could have lead to a change in spectral signature in both visible and infrared regions. One of the peaks in the mid-infrared region (9–10.5 µm) could be attributed to the starch present in the leaves (Hawkins et al., 2010; Sankaran et al., 2010b).

![Figure 2. Few representative sample spectra of (a) normalized visible-near infrared spectra and (b) baseline corrected mid-infrared spectra.](image-url)
In this study, both visible-near infrared and mid-infrared spectrometry showed good potential in HLB detection. The average classification accuracies of both the algorithms were found to be >85%. The QDA- and kNN-based algorithm resulted in fewer false negatives in visible-near infrared and mid-infrared spectrometry, respectively. Though this study demonstrates the potential of optical methods for HLB detection in citrus, there are many challenges that need to be addressed in future studies. Some of the aspects that need further attention are the detection of non-symptomatic HLB-infected leaves and identification of nutrient-deficiencies in leaves showing similar symptoms as HLB.

**Literature Cited**


