EARLY DETECTION OF CITRUS GREENING (HLB) USING GROUND BASED HYPERSPECTRAL IMAGING AND SPECTROSCOPY

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2010
To my parents my loving wife and daughter
ACKNOWLEDGMENTS

I would like to bestow my sincere gratitude to advisor and dissertation chair Dr. Reza Ehsani, Assistant Professor of Agricultural and Biological Engineering, Citrus Research and Education Center (CREC), University of Florida (UFL) for his consistent guidance, encouragement and support throughout this research work at UF. His thorough and thoughtful coaching was unselfishly tireless, and his enthusiasm has left me an everlasting impression.

I am greatly indebted to my supervisory committee Dr. WonSuk “Danial” Lee, Associate Professor of Agricultural and Biological Engineering, UFL, Dr. Masoud Salyani, Professor of Agricultural and Biological Engineering, CREC, UFL, Dr. John Schueller, Professor of Mechanical and Aerospace Engineering, UFL and Dr. Amr Abd-Elrahman, Assistant Professor of Geomatics, UFL for their guidance and suggestions to complete this work. Their ideas, wisdom, and suggestions have helped me sail smoothly through the graduate studies. Dr. Gene Albrigo, Emeritus Professor, CREC helped with numerous and detailed criticisms of parts of the dissertation.

The field experiment would not have successful without the help of Sherrie Buchanon, Dr. Joe M Maja, Raghav Panchapakesan, Sajith Udumala, Bhargav Prasad, Andre Colaco and John Pilkey. In addition, I would like to thank Dr. Davood Karimi and Dr. Sindhuja Sankaran and for their guidance in my data analysis. Cecile Robertson at CREC deserves special thanks for her assistance in greenhouse study and PCR testing. I also thankful to Jennifer Dawson and Kathy Snyder at CREC library for providing me literature and other lab support whenever I required.

I am greatly thankful to the staff of the Agricultural and Biological Engineering department and CREC family for sharing their insights into getting through graduate
research. I would like to thank all of those not explicitly mentioned here who have aided my intellectual and social growth throughout my academic career. Finally, I would like to extend my special thanks to my parents and my wife Ekta for their continued moral support, love and care throughout this milestone of my life. I bow my head before the Almighty, for all the blessing showered on me during the entire course of this work.
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<td>ADAR</td>
<td>Airborne Data acquisition and registration</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>ANN</td>
<td>Artificial Neural Network</td>
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<td>BP-NN</td>
<td>Back Propagation Neural Network</td>
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<td>CART</td>
<td>Classification And Regression Tree</td>
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<td>CO$_2$</td>
<td>Carbon di Oxide</td>
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<tr>
<td>DPLS</td>
<td>Discriminant Partial Least Squares</td>
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<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
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<td>FASS</td>
<td>Florida Agricultural Statistics Survey</td>
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<tr>
<td>HLB</td>
<td>Huanglongbing or greening</td>
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<td>G</td>
<td>Greenness Index</td>
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<td>GAE</td>
<td>Gallic Acid Equivalent</td>
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<td>IFOV</td>
<td>Instantaneous Field of View</td>
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<td>IF</td>
<td>Immunofluorescence</td>
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<td>KNN</td>
<td>K Nearest Neighbors</td>
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<tr>
<td>LDA</td>
<td>Linear Discriminant Analysis</td>
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<tr>
<td>LR</td>
<td>Logistic Regression</td>
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<tr>
<td>LIF</td>
<td>Laser Induced Fluorescence</td>
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<td>LVQ</td>
<td>Learning Vector Quantization</td>
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<td>mPLS</td>
<td>Modified Partial Least Squares</td>
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<tr>
<td>MCARI</td>
<td>Modified Chlorophyll Absorption in Reflectance Index</td>
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<td>MNF</td>
<td>Minimum Noise Fraction</td>
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<td>MTVI</td>
<td>Modified Triangular Vegetation Index</td>
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<tr>
<td>NASS</td>
<td>National Agriculture Statistics Survey</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>NDVI</td>
<td>Normalized Difference Vegetation Index</td>
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<td>NIR</td>
<td>Near Infrared</td>
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<td>nm</td>
<td>Nanometer</td>
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<td>PCA</td>
<td>Principal Component Analysis</td>
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<td>PCR</td>
<td>Polymer Chain Reaction</td>
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<td>PDA</td>
<td>Procrustes Discriminant Analysis</td>
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<td>PLS</td>
<td>Partial Least Squares</td>
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<td>PNN</td>
<td>Probabilistic Neural Network (PNN)</td>
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<td>QDA</td>
<td>Quadratic Discriminant Analysis</td>
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<td>R</td>
<td>Reflectance</td>
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<tr>
<td>RDVI</td>
<td>Renormalized Difference Vegetation Index</td>
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<tr>
<td>SAM</td>
<td>Spectral Angle Mapping</td>
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<td>SFF</td>
<td>Spectral Feature Fitting</td>
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<td>SIMCA</td>
<td>Soft Independent Modeling of Class Analogy</td>
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<tr>
<td>SIPI</td>
<td>Structure Intensive Pigment Index</td>
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<tr>
<td>SOM</td>
<td>Self Organizing Maps</td>
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<td>SR</td>
<td>Simple Ratio Index</td>
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<td>SVM</td>
<td>Support Vector Machine</td>
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<td>TVI</td>
<td>Triangular Vegetation Index</td>
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<tr>
<td>UV</td>
<td>Ultra Violet</td>
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<td>VIS</td>
<td>Visible</td>
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Citrus greening, also known as Huanglongbing or HLB, is a major threat to the U.S. citrus industry. Currently, scouting and visual inspection are used for screening infected trees. However, this is a time-consuming and expensive method for HLB disease detection. Moreover, as it is subjective, the current method may involve high detection error rates. The objective of this research was to evaluate the optical sensors for the detection of HLB, other diseases and nutrient deficiencies in citrus.

This dissertation describes the status of citrus in Florida, current HLB status in FL, various advanced techniques for plant disease detection. It further reviews various diseases and nutrient deficiencies in citrus that may be confused with HLB.

Initially, the spectral characteristics of healthy and HLB infected tree canopies were investigated. A FieldSpec spectroradiometer (350-2500 nm) was used to detect HLB-infected trees. Discriminability, spectral derivative analysis and spectral ratio analysis were used to distinguish HLB. It was found that the spectral bands of green to red and near infrared have the ability to discriminate HLB-infected trees from healthy
trees. These wavelength regions include green peak wavelengths at around 530-564 nm, 710-715 nm (red edge), and near infrared wavelengths of 1041 nm and 2014 nm.

In the next step partial least squares (PLS) and discriminant statistical analyses were used to identify and discriminate spectral characteristics of HLB infections in citrus trees. Results suggest that both techniques have the potential to discriminate HLB for different varieties of citrus. Overall, the full range of data gave more accurate results compared to a narrower range of reflectance data with both statistical techniques. However, the narrower, visible, range (400 nm to 900 nm) data produced better results with PLS modeling. In contrast, discriminant analysis produced better overall results with the full reflectance range.

Machine learning techniques like k-nearest neighbors (KNN), logistic regression, and support vector machines (SVM) were applied for classifying the HLB data. Analysis showed that with one spectral measurement, none of the classification methods was successful in discriminating healthy from infected trees, because of the large variability in the spectral measurements. When five spectra from the same tree were used for classification, SVM and weighted KNN methods classified spectra with 3.0 and 6.5 percent error, respectively. The results from this study indicated that the canopy visible and near infrared (VIS-NIR) spectral reflectance can be used for detecting HLB infected citrus trees. However, high classification accuracy (> 90%) requires multiple measurements from a single tree.

Since ASD and SVC spectroradiometers are very expensive and difficult for growers to use in field data collection, a rugged, low-cost, multi-band active optic sensor was used to identify the HLB infected trees from the healthy trees. The sensor consisted
of four bands: two visible bands at 570 nm and 670 nm, and two NIR bands at 870 nm and 970 nm. Extensive field measurements were conducted using this sensor. Analysis of the data showed that due to the large variability in the data, it was not possible to discriminate healthy and infected trees based only on a single measurement from a tree. Using multiple measurements from a tree, however, it was possible to achieve high classification accuracy. With five measurements from a tree, classification methods such as k-nearest neighbors, support vector machines, and decision trees achieved classification errors of less than 5 percent. The results demonstrated the potential of a multi-band active optic sensor for detecting HLB-infected citrus trees under field conditions.

This research further investigated the application of hyperspectral camera for HLB detection. Hyperspectral images of HLB infected trees and healthy trees were collected with a Specim hyperspectral camera (Autovision Inc., Los Angeles, CA, USA) having a spectral range from 306.5 nm to 1067.1 nm with 2.7 nm spectral resolution. These images were processed in ENVI 4.5 (ITT Visual Information Solutions, Boulder, Colorado). Various vegetation indices were estimated. ANOVA was used to compare the mean vegetation indices of healthy and HLB trees. Results showed that hyperspectral imaging have a potential to discriminate HLB from healthy samples.
CHAPTER 1
GENERAL INTRODUCTION

Introduction

Florida produces 20 percent of the world’s oranges with about 10 percent of the world’s orange acreage. Florida accounts for 71 percent of total U.S. Citrus production (NASS, 2009). Florida’s orange production (162 million boxes) has decreased 5 percent since 2007-08. Grapefruit production in Florida (21.7 million boxes) has also reduced 18 percent from last season. One of the possible reasons for reduced citrus production could be attributed to exotic diseases. Huanglongbing (HLB), also known as greening, is a systemic bacterial disease transmitted by a psyllid insect, is considered one of the most devastating citrus diseases in the world. HLB has been translated loosely as yellow shoot disease because of the characteristic yellow shoots caused by the disease. It is caused by a phloem-limited bacterium, Candidatus Liberibacter asiaticus. HLB was first detected in Florida in August of 2005 (Chung and Brlansky, 2005). Since this is a relatively new disease in USA, very little published information is available on the dynamics, epidemiology, and molecular characteristics of this disease.

Dissertation Organization

This dissertation consists of a review of the literature and six chapters prepared for partial fulfillment of the requirement for the degree, Doctor of Philosophy. The author of the dissertation is Ashish R Mishra. Dr. Reza Ehsani served as a major advisor provided all the facilities and constructive suggestions to conduct this research. The first chapter serves as a detailed literature review on the Huanglongbing, interaction of light with vegetation, soil and water, various advance techniques used in plant disease detection specially hyperspectral spectroscopy and imaging. The second chapter
reviews various diseases, nutrient deficiencies that could affect citrus production. Special focus was given on those diseases and deficiencies whose symptoms could be confused with HLB symptoms. The third chapter demonstrates the spectral characteristics of citrus leaves and identifies critical wavelength for HLB detection. The fourth chapter describes partial least squares (PLS) technique and discriminant analysis for the HLB identification. The fifth chapter is the manuscript, submitted in transactions of ASABE journal, reports the feasibility of hyperspectral spectroscopy and several machine learning techniques in HLB detection. The sixth chapter is the manuscript, submitted in biosystems engineering journal, reports the application of multispectral sensor to detect HLB in field condition. The seventh chapter discusses the application of hyperspectral imaging and various vegetation indices used in HLB discussion. In the last chapter summary and future directions are included.

**Literature Review**

**Huanglongbing or Citrus Greening**

Huang means yellow, long means dragon and bing refers to disease. Therefore, Huanglongbing refers as ‘yellow shoot disease’, and it has been reported under different names such as greening in many countries. HLB has destroyed an estimated 60 million trees in Africa and Asia (Bove, 2006).

Huanglongbing (HLB) is caused by the gram-negative bacterium *Candidatus Liberibacter asiaticus* (Garnier et al., 2000). Asian citrus psyllid (*Diaphorina citri*) is the vector of citrus greening or HLB. The bacteria are restricted to the sieve tubes of infected plants, and are acquired and transmitted by nymphs and adults of Asian citrus psyllid during feeding (Garnier and Bové, 1983). Psyllids prefer feeding and breeding on
younger leaves (Halbert and Manjunath, 2004) resulting younger trees at a higher risk of infection as they produce newer leaves and flushes throughout the year.

Figure 1-1. Symptoms of HLB in citrus leaves

Figure 1-2. HLB infected citrus fruits

Symptoms of HLB infected citrus include a blotchy mottle or asymmetrical chlorosis (Figure 1-1), and yellowing of leaf veins due to inefficient production of chlorophyll (Brlansky et al., 2007). The angular blotching has been considered specific for the disease and consists of blotches of yellow on dark greenish-grey leaves. On the same tree, some branches may not be infected by HLB. Fruits of HLB infected trees are affected in various ways. Some of them, when they reach an inch or more in diameter
become lopsided (Figure 1-2). Fruit matures only on one side with the immature side remaining green when the fruit ripens, hence the name "greening". Other normal-shaped fruits that attain full size may fail to color properly and remain lusterless, greenish yellow, and many of them fall before harvest. Infected fruits also lose their expected taste. Seeds are also affected from HLB-infected trees. Since the outer seed coat does not develop sufficiently to cover the inner brown coat, partially developed seeds have the brown color of the inner seed coat.

**Interaction of Light with Vegetation, Soil and Water**

Plants absorb the ultraviolet and the visible regions of the spectrum very efficiently. The reflectance and transmittance of plant leaves increases dramatically in near-infrared region, resulting in the absorbance falling to a very low value. There are two regions of the spectrum where relatively less absorption occurs. At wavelengths longer than 1200 nm water vapor absorption rises very steeply, whereas in the red and the blue regions of visible spectrum, pigment absorption is very strong. The presence of pigments other than chlorophyll tends to broaden the domain of absorption throughout the visible region. Scattering is also caused by structures within the leaf. Such structure may include mitochondria, ribosomes, nuclei, starch grains, and other plastids. The visible absorbance substantially increases from the lighter to the darker leaves and the NIR absorbance is the highest for the thinner leaf. The most striking feature in the near infrared is the fact that the transmittance of the thinner leaves is greater than the reflectance.

A lack of chlorophyll pigmentation can reduce drastically the absorption of the visible light by a leaf. The white leaf exhibits very little absorption through the green and red spectral regions and only increases in the blue due to absorption by
protochlorophyll. The carotenoid and chlorophyll pigments are partially lacking in the white leaf.

Gates et al. (1965) considered that near infrared reflectance is a function of the cell shape and size as well as the amount of intercellular space. Initially, the mesophyll of a very young leaf contains spongy parenchyma with considerable air spaces. It favors the mechanism of internal reflection. When the leaf matures, the cell enlarges, crowding together, reducing the intercellular space and reducing the reflectance. It would then appear that during final maturing the cell structure and intercellular space relationship becomes favorable for increased reflectance.

![Figure 1-3. Spectral reflectance of soil, green and dry vegetation](image)

Reflectance is characterized by a relative maximum in the green band at 550 nm and minimum at 400 and 670 nm caused by absorption of radiation by chlorophyll
(Figure 1-3). Reflectance within the 690-700 nm range is particularly sensitive to early stress-induced decreases in leaf chlorophyll content (Carter, 1993) and represent the shift of red edge that are closely related to chlorophyll content. Increased leaf reflectance near 530 nm has indicated pigment transformations and changes in thylakoid processes (Gamon et al., 1990). Reddy et al. (2001) found that chlorophyll concentration of maize, groundnut and soybean crops mainly affected leaf spectral reflectance at 450-520 nm and 620 nm region. However, Zhao et al., (2003) reported that the chlorophyll correlated most strongly with the reflectance at 554 nm and 712 nm. Reflectance in this region is relatively high and mostly constant. This was related to the low absorption by the leaf. Minor relative minima were at 950 nm and 1160 nm due to selective absorption by the presence of water. After 1300 nm, the absorbance by water plays a dominant role with maxima at 1450 and 1950 nm.

Fouche (1999) suggested that reflectance at the 779 nm wavelength may provide the best detection of N deficiency in cotton, tobacco and wheat. Carter and Estep (2002) reported that a simple linear relationship existed between leaf nitrogen (%) and reflectance at 721 nm in corn. Zhao et al. (2003) concluded that on the basis of leaf area chlorophyll a, chlorophyll b, carotenoid, and chlorophyll a+b concentration could be estimated using reflectance ratios in the near infrared region of 712 nm to 1088, 1097, 809 nm, respectively.

Table 1-1 revealed that there are 42 minor absorption features in fresh leaves that would probably have dampened by the five major absorption features. These absorption features are the result of the bending and stretching of the oxygen and hydrogen bond
(O-H), bond between carbon and nitrogen (C-N) and single and double bonds between carbon and hydrogen (C-H, C=H) in different chemicals.
Table 1-1. Absorption features in visible and near-Infrared wavebands that have been related to particular foliar chemical concentration (Curran, 1989). *(Chemicals in italics have a wavelength of stronger absorption)*

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Electronic transition or bond vibration</th>
<th>Chemical(s)</th>
<th>Remote Sensing Consideration</th>
</tr>
</thead>
<tbody>
<tr>
<td>430</td>
<td>Electron transition</td>
<td><em>Chlorophyll a</em></td>
<td></td>
</tr>
<tr>
<td>460</td>
<td>Electron transition</td>
<td><em>Chlorophyll b</em></td>
<td></td>
</tr>
<tr>
<td>640</td>
<td>Electron transition</td>
<td><em>Chlorophyll b</em></td>
<td></td>
</tr>
<tr>
<td>660</td>
<td>Electron transition</td>
<td><em>Chlorophyll a</em></td>
<td></td>
</tr>
<tr>
<td>910</td>
<td>C-H stretch</td>
<td>Protein</td>
<td></td>
</tr>
<tr>
<td>930</td>
<td>C-H stretch</td>
<td>Oil</td>
<td></td>
</tr>
<tr>
<td>970</td>
<td>O-H stretch</td>
<td><em>Water, starch</em></td>
<td>Atmospheric scattering</td>
</tr>
<tr>
<td>990</td>
<td>O-H stretch</td>
<td>starch</td>
<td></td>
</tr>
<tr>
<td>1020</td>
<td>N-H stretch</td>
<td>Protein</td>
<td></td>
</tr>
<tr>
<td>1040</td>
<td>C-H stretch, C-H deformation</td>
<td>Oil</td>
<td></td>
</tr>
<tr>
<td>1120</td>
<td>O-H stretch, C-H stretch, C-H deformation</td>
<td>Lignin</td>
<td></td>
</tr>
<tr>
<td>1200</td>
<td>O-H bend</td>
<td><em>Water, cellulose, starch, lignin</em></td>
<td></td>
</tr>
<tr>
<td>1400</td>
<td>O-H bend</td>
<td><em>Water</em></td>
<td></td>
</tr>
<tr>
<td>1420</td>
<td>C-H stretch, C-H deformation</td>
<td>Lignin</td>
<td></td>
</tr>
<tr>
<td>1450</td>
<td>O-H stretch, C-H stretch, C-H deformation</td>
<td>Starch, sugar, lignin, water</td>
<td>Atmospheric absorption</td>
</tr>
<tr>
<td>1490</td>
<td>O-H stretch</td>
<td>Cellulose, sugar</td>
<td></td>
</tr>
<tr>
<td>1510</td>
<td>N-H stretch</td>
<td><em>Protein, nitrogen</em></td>
<td></td>
</tr>
<tr>
<td>1530</td>
<td>O-H stretch</td>
<td>Starch</td>
<td></td>
</tr>
<tr>
<td>1540</td>
<td>O-H stretch</td>
<td>Starch, Cellulose</td>
<td></td>
</tr>
<tr>
<td>1580</td>
<td>O-H stretch</td>
<td>Starch, sugar</td>
<td></td>
</tr>
<tr>
<td>1690</td>
<td>C-H stretch</td>
<td><em>Lignin, starch, protein, nitrogen</em></td>
<td></td>
</tr>
<tr>
<td>1780</td>
<td>C-H stretch, O-H stretch, H-O-H deformation</td>
<td><em>Cellulose, sugar, starch</em></td>
<td></td>
</tr>
<tr>
<td>1820</td>
<td>O-H stretch, C-O stretch</td>
<td>Cellulose</td>
<td></td>
</tr>
<tr>
<td>1900</td>
<td>O-H stretch, O-H deformation</td>
<td>Starch</td>
<td></td>
</tr>
<tr>
<td>1960</td>
<td>O-H stretch, O-H bend</td>
<td>Sugar, starch</td>
<td></td>
</tr>
<tr>
<td>Wavelength (nm)</td>
<td>Electronic transition or bond vibration</td>
<td>Chemical(s)</td>
<td>Remote Sensing Consideration</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------------------------------</td>
<td>-------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>1940</td>
<td>O-H stretch, O-H deformation</td>
<td>Water, lignin, protein, nitrogen, starch, cellulose</td>
<td>Rapid decrease in signal to noise ratio of sensors</td>
</tr>
<tr>
<td>1960</td>
<td>O-H stretch, O-H bend</td>
<td>Sugar, starch</td>
<td></td>
</tr>
<tr>
<td>1980</td>
<td>N-H asymmetry</td>
<td>Protein</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>O-H deformation, C-O deformation</td>
<td>Starch</td>
<td></td>
</tr>
<tr>
<td>2060</td>
<td>N=H bend, N-H stretch</td>
<td>Protein, nitrogen</td>
<td></td>
</tr>
<tr>
<td>2080</td>
<td>O-H stretch, O-H deformation</td>
<td>Sugar, starch</td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>O=H bend, C-O stretch, C-O stretch</td>
<td>Starch, cellulose</td>
<td></td>
</tr>
<tr>
<td>2130</td>
<td>N-H stretch</td>
<td>Protein</td>
<td></td>
</tr>
<tr>
<td>2180</td>
<td>N-H bend, C-H stretch/C-O stretch/C-N stretch</td>
<td>Protein, nitrogen</td>
<td></td>
</tr>
<tr>
<td>2240</td>
<td>C-H stretch</td>
<td>Protein</td>
<td></td>
</tr>
<tr>
<td>2250</td>
<td>O-H stretch, C-H deformation</td>
<td>Starch</td>
<td></td>
</tr>
<tr>
<td>2270</td>
<td>C-H stretch/O-H stretch, CH\textsubscript{2} bend/CH\textsubscript{2} stretch</td>
<td>Cellulose, sugar, starch</td>
<td></td>
</tr>
<tr>
<td>2280</td>
<td>C-H stretch/ CH\textsubscript{2} deformation</td>
<td>Cellulose, starch</td>
<td></td>
</tr>
<tr>
<td>2300</td>
<td>N-H stretch, C=O stretch, C-H bend</td>
<td>Protein, nitrogen</td>
<td></td>
</tr>
<tr>
<td>2310</td>
<td>C-H bend</td>
<td>Oil</td>
<td></td>
</tr>
<tr>
<td>2320</td>
<td>C-H stretch/ CH\textsubscript{2} deformation</td>
<td>Starch</td>
<td></td>
</tr>
<tr>
<td>2340</td>
<td>C-H stretch/ O-H deformation/ C-H deformation/ O-H stretch</td>
<td>Cellulose</td>
<td></td>
</tr>
<tr>
<td>2350</td>
<td>CH\textsubscript{2} bend, C-H deformation</td>
<td>Cellulose, protein, nitrogen</td>
<td></td>
</tr>
</tbody>
</table>
Advance Techniques for Detecting Plant Disease

Plants can exhibit a host of symptoms reflecting various disorders that can adversely influence their health, vigor and productivity to varying degrees. Identifying disease symptoms is essential as inappropriate actions may sometimes prove to be costly and detrimental to the yield. Proper disease control actions or remedial measures can be undertaken if the symptoms are identified early. Sankaran et al. (2010) categorized disease detection techniques into direct and indirect method. Direct methods include serological methods (Enzyme-linked immunosorbent assay (ELISA), immunofluorescence (IF) and flow cyrometry) and molecular methods (Polymerase chain reaction (PCR), DNA arrays). Biomarker-based disease detection (Gaseous metabolite profiling, plant metabolite profiling) and plant properties/stress based detection (imaging techniques, spectroscopic methods) can be classified under indirect method. In the current research, techniques related to plant properties/stress detection are relevant. Therefore, these techniques will be discussed in detail.

Spectroscopic and Imaging Techniques

In the recent years, spectroscopic and imaging techniques are very popular in plant diseases detection (Graeff et al., 2006; Huang and Apan, 2006; Moshou et al. 2006), and food quality control (Sundaram et. al., 2009; Sighicelli et al. 2009; Shackelford et al., 2004). In the modern era, plant disease detection sensor should be rapid, disease specific and sensitive to the initial stages of disease infection (Lopez et al., 2003). The spectroscopic and imaging techniques are non destructive, fast and inexpensive.
The spectroscopic and imaging sensors could be integrated with an aero plane, micro copter or agricultural vehicle (manual or autonomous) that can monitor plant health and detect anomaly in early stage to control the spread of plant disease.

**Visible Near-Infrared Spectroscopy**

Infrared spectroscopy is a precise, fast developing and non-destructive technology with increasingly wide range of applications. The scope of infrared spectroscopy in detection of plant diseases is very high. Guo et al. (2009) carried out experiments showing the potential of NIR spectroscopy as a tool to classify plant species. They used principal component analysis (PCA) combined with the establishment of a Mahalanobis distance of plants leaves to create the discrimination model of leaves and obtained 100% classification. Ramon et al. (2002) used NIR reflectance using a spectrograph and a neural network classifier to discriminate between weeds and the crop for accurate delivery of the herbicide. Polischuk et al. (1997) made an early detection of Tomato mosaic virus in Nicotiana debneyi plants using spectral reflectance measurements in the visible and near-infrared. Thus, the spectral reflectance between healthy and infected leaves can be used to diagnose plant diseases before visible changes can be observed. Diseases can influence spectral properties of plants at many wavelengths, making different wavebands apt for disease detection (West et al. 2003).

Kobayashi et al. (2000) used multispectral spectroradiometer and airborne multispectral scanner to detect panicle blast in rice. Airborne multispectral scanner consists four bands of 400-460 nm, 490-530 nm or 530-570 nm, 650-700 nm and 950-1100 nm spectral range. Its instantaneous field of view (IFOV) was 2.5 mrad and ground resolution was 0.94 m at an altitude of 300 m. They measured ground reflectance data with multi spectroradiometer (MSR-7000; Opto Research Corp., Tokyo)
with spectral range of 400 to 2000 nm. They concluded reflectance ratios ($R_{470}/R_{570}$, $R_{520}/R_{675}$, and $R_{570}/R_{675}$) decreased significantly as incidence of panicle blast increased at dough stage. They reported ground base sensor data and airborne multispectral scanner are effective in panicle blast detection. Zhang et al. (2008) predicted total phenolics, flavonoid contents and antioxidant capacity of rice grain using NIR spectroscopy. They utilized PLS and modified partial least squares (mPLS) and reported standard errors of prediction were 47.1 and 45.9 nm gallic acid equivalent (GAE) for phenolic content and the coefficient of determination ($R^2$) were 0.849 and 0.864 by PLS and mPLS, respectively.

The feasibility of NIR spectroscopy (1100-2500 nm) to identify waxy wheat was done by Delwiche and Grayboscht (2002) in the lab. They applied linear and quadratic discriminant functions of the scores from principal component and demonstrated near perfect separation of fully waxy wheat from non waxy wheat. Sundaram and Kandala (2009) reviewed the application of NIR spectroscopy to peanut grading and quality analysis. They concluded that NIR spectroscopy could be used for measuring protein, moisture, oil content and fatty acid composition in oil seeds.

Wang et al. (2002) classified damaged soybean seeds using NIR spectroscopy. They measured reflectance spectra ($\log(1/R)$) from 400 to 1700 nm. Partial least squares (PLS) and artificial neural network (ANN) models were developed to classify normal and damaged seeds. They concluded ANN yields higher accuracy than PLS models. Gomez et al. (2006) concluded that by using the VIS-NIR measurement technique in the full spectral range (400-2350 nm), it is possible to assess the quality characteristics of mandarin.
VIS-NIR reflectance spectroscopy (325-1075 nm) was applied to the early detection of *Botrytis cinerea* disease in eggplant leaves before symptoms appear (Wu et al., 2008). PCA was used for dimension reduction. Based on the PCs back propagation neural network (BP-NN) model was developed. Furthermore, PLS was executed to identify seven potential wavelengths. BP-NN model was also developed with these wavelengths. They indicated that it is possible to apply spectral technology to the early detection of *Botrytis cinerea* on eggplant leaves.

Roggo et al. (2003) compared various classification method accuracies using McNemar’s statistical test in sugar beet. Three qualitative characteristics of sugar beet were studied; disease resistance, geographical origins and crop periods. NIR spectroscopy data were compared by eight classification method; Linear Discriminant Analysis (LDA), K-Nearest Neighbors (KNN) method, Soft Independent Modeling of Class Analogy (SIMCA), Discriminant Partial Least Squares (DPLS), Procrustes Discriminant Analysis (PDA), Classification And Regression Tree (CART), Probabilistic Neural Network (PNN) and Learning Vector Quantization (LVQ). They reported SIMCA, DPLS and PDA have the highest classification accuracy. LDA and KNN were not significantly different. Dobrowski et al. (2005) demonstrated that the simple reflectance indices calculated in the red edge spectral region can track temperature and water induced changes in fluorescence.

NIR spectroscopy was applied to predict pre visual decline in eastern hemlock trees (Pontius et al., 2005). An ASD FieldSpec spectroradiometer (350-2500 nm) was used to collect spectral data. PLS and reduced stepwise regression techniques with various vegetation indices (Carter miller index, derivative chlorophyll index, NDVI, RVI)
were used in regression analysis. Their results demonstrated that NIR spectroscopy can detect hemlock decline, before visual symptoms are visible to naked eyes.

**Hyperspectral and Multispectral Imaging**

Lu (2003) studied the detection of bruises in apples using near infrared hyperspectral imaging. He reported that the spectral region between 1000 nm to 1340 nm was most appropriate for bruise detection. The author utilized principal component and minimum noise fraction (MNF) transformation and was able to detect new and old bruises with correct detection rate from 62% to 88% for red delicious and 59 to 94% for golden delicious. Kim et al. (2001) designed and developed a hyperspectral imaging system which is capable to capture reflectance and fluorescence image in the 430 to 930 nm with 1 mm spatial resolution. The adaptability of the hyperspectral imaging system was demonstrated with sample fluorescence and reflectance images of a normal apple and an apple with fungal contamination and bruised spots. Hyperspectral imaging within the wavelength range of 400-1000 nm was used to detect bruises in Jonagold apple (Xing and Baerdemaeker, 2005). The authors utilized PCA and reported the classification accuracy for sound apples between 77.5 to 84.6% for the one day old bruises. Based on the hyperspectral imaging Xing et al. (2005) stated that wavebands centered at 558, 678, 728 and 892 nm have potential to detect bruises in apples. A hyperspectral NIR imaging system (900-1700 nm) was developed to identify bitter pit lesions on apples (Nicolai et al., 2006). Their system was able to identify bitter pit lesions, even when symptoms were not visible to the naked eye, though their system was failed to discriminate bitter pit lesions and corky tissue. Elmasry et al. (2008) also worked in detection of apple bruises on different background colors using hyperspectral imaging and successfully distinguished from the sound apples. They used PLS method.
and stepwise discrimination analysis for dimension reduction and indentify critical wavelengths. They reported three wavelengths in NIR region (750, 820 and 960 nm) are critical for bruise detection in apple.

Moshou et al. (2006) tried to detect plant stress caused by disease infestation and to discriminate it from nutrient deficiency stress in field conditions using hyperspectral imaging. They compared yellow rust infected winter wheat plants from the nutrient stressed and healthy plants. They utilized self organizing maps (SOM) and quadratic discriminant analysis (QDA). The authors demonstrated successfully detection of yellow rust from the nutrient stressed and healthy plants. Mahesh et al. (2008) differentiated different wheat varieties by NIR hyperspectral imaging (960 to 1700 nm). Seventy five relative reflectance intensities were identified from the images and used for differentiation of wheat classes using statistical classifier (LDA & QDA) and ANN classifier. They reported above 90% classification accuracy with statistical classifier and ANN classifier.

Early detection of grapevine phylloxera disease was investigated in Australia (Costa et al. 2007). Their finding showed that at the leaf level, hyperspectral spectroscopy (650-1200 nm) can differentiate phylloxera infested vines. However at the canopy level differentiation is challenging with water deficiency and nitrogen deficiency.

Larsolle and Muhammed (2007) measured crop status using multivariate analysis of hyperspectral field reflectance (360-900 nm). They analyzed their data in two step: a preprocessing step where data was normalized and a classification steps for estimating the crop variable. They demonstrated that hyperspectral analysis method can be used to extract spectral signatures of disease severity and plant density.
Late blight caused by the fungal pathogen (*Phytophthora infestans*) in tomato was successfully identified in field conditions (Zhang et al., 2005). They used ADAR (airborne data acquisition and registration, using system 5500 airborne sensor from positive system, Inc. Idaho, USA) broadband system to acquire multispectral image of four broad bands (blue, green, red and NIR). Various vegetation indices (combination of red and NIR bands) were used to discriminate late blight. Sour skin disease in vidalia sweet onions was detected by NIR Imaging (Wang et al., 2009). They observed the significant change in mean reflectance spectra in the region 1150 to 1280 nm when the onion was stored 3 days after inoculation.

Blasco et al. (2007) evaluated NIR, ultraviolet (UV) and fluorescence techniques to identify the most common defects in citrus. With the NIR system anthracnose and sooty mould were detected. Using UV system only stem-end injury was detected, while fluorescence images were able to detect damages caused by green mould, scarring or thrips. The infection due to two plant pathogens (*Phytophthora citrophthora* and *Penicillium italicum*) in orange using laser induced fluorescence (LIF) and hyperspectral imaging (400-800nm) was studied by Sighicelli et al. (2009). They observed band sensitivity temporally as infection increases. They reported that both techniques are promising.

**Application of Spectroscopy and Imaging in Citrus**

Earlier Gaffney (1972) worked on the spectral characteristics of citrus. He reported that a wavelength band of 580 nm to 610 nm is suitable for sorting out defects in pineapple oranges. For valencia oranges, a wavelength band of 570 to 600 nm could possibly be used to detect the defects. A significant (p≤0.05) negative relationship was
observed between canopy reflectance and the severity due to Rhizoctonia blight or gray leaf spot (Green et al., 1998).

Citrus greening (Huanglongbing or HLB) disease was tried to detect by Lee et al. (2008) using aerial hyperspectral imaging. An aerial hyperspectral images were collected from HLB infected groves having spectral range 400 to 1000 nm in 128 different spectral bands with 5 nm spectral resolution and 0.7 m spatial resolution. Spectral angle mapping (SAM) and spectral feature fitting (SFF) classification techniques were used in ENVI software. Due to much variability of healthy and HLB infected tree canopies with geo referencing error, these classification techniques did not yield good results.
CHAPTER 2
REVIEWS ON HLB AND OTHER SIMILAR DISEASE AND DEFICIENCIES

Citrus is one of the most important agricultural products in Florida as it is the largest citrus producing state in United States and second largest in the entire world. Citrus production being a multi-million dollar industry accounts significantly for Florida’s agricultural economy. But recently, it has been threatened by Huanglongbing (HLB), a devastating and rapidly spreading disease of citrus. This chapter reviews various citrus diseases and nutrient deficiencies that may be confused with HLB. This chapter also discusses various micro activities during chlorosis in senescence, toxicity, deficiencies and diseases.

**Chlorosis**

In chlorosis, leaves fail to synthesize sufficient chlorophyll resulting in pale, yellow or yellow-white appearance of leaves. Chlorosis may occur due to nutrient deficiency, disease infection, poor drainage, damaged or compacted roots, high alkalinity, or excess use of fertilizers. Low level of nutrients in the soil or their unavailability for reasons like injured roots or high pH can cause nutrient deficiencies in plants. Chlorosis hinders carbohydrates synthesis through photosynthesis in plant that may lead to plant death unless it is treated for the cause of its chlorophyll insufficiency. The following leaf abnormalities have been associated with certain chemicals.

**Perchlorate Chlorosis**

This chlorosis is induced by impurities in chilean nitrate of soda potash. The yellow tipping of citrus leaves has been observed in Florida for many years and was once thought to be associated with excess boron in certain fertilizers. The symptoms first
develop at the leaf tip and may be confused with boron toxicity, but careful examination reveals that the yellow tipping that is, the chlorotic areas, do not blend with adjoining green tissue. The transition from green to chlorotic areas is sharp and abrupt, producing a patchy appearance; whereas the boron toxicity pattern shows a gradual change from green to chlorotic areas, producing a blend of colors. The yellow tipped pattern has been experimentally proven to be due to perchlorate impurities in chilean nitrate of soda potash (Stewart et al., 1952).

**Biuret Toxicity**

The chlorosis has been experimentally shown by Oberbacker (1954) to be due to biuret impurities in commercial grades of urea. The symptoms first develop at the leaf tips and margins, and the early stages may be confused with the early stages of perchlorate chlorosis. The color of biuret chlorosis is yellow compared to an orange color with the perchlorate chlorosis (Figure 2-1). The advanced cases of biuret chlorosis may show a burning effect which is more severe on immature than mature leaves. The yellow color of the biuret chlorosis is similar to that of boron toxicity, but the biuret colors are somewhat patchy and free from guming on the under surface.
The chlorotic leaf patterns frequently observed after arsenic sprays is commonly known as arsenic toxicity. The degree of chlorosis is usually in the order of the amount of arsenic applied. The symptoms show a loss of chlorophyll without any distinct pattern except that of chlorosis. Arsenic deficiency may be confused with manganese deficiency pattern but close examination indicates that the chlorosis due to arsenic extends across the veins whereas the chlorosis from the manganese deficiency is interveined.

**Fluorine Toxicity**

The early stages of the toxicity are somewhat similar to boron toxicity but the under surface of the leaves show no resinous excretion with the fluorine toxicity whereas it is generally true for boron (Figure 2-12). The fluorine toxicity shows considerable blends of different shades of green which may be confused with
manganese deficiency. No fluoride problems have been reported where liberal amounts of finely ground rock phosphate carrying high levels of fluorine have been applied to the soil. This indicates that insoluble fluorides added to the soil are not injurious.

**Mechanism of Chlorosis**

Achor and Albrigo (2005) reported that the severe biuret induced chlorosis affected leaves have very few chloroplasts and their average size were about one fifth of that in the normal healthy plants. They found amount of cytoplasm was reduced even more, with the central vacuole filling 60% of the viewed surface area of the palisade cells. The plastids looked more like chloroplasts with no grana or other internal membranes and large numbers of plastoglobuli (lipid bodies). Following are the general changes that occur during leaf senescence.

**Ultra structural Changes in Chloroplasts during Senescence**

The earliest and most striking anatomical changes associated with leaf senescence occur in chloroplasts (Woolhouse, 1984). These organelles undergo ordered sequential changes of their photosynthetic capability from maturity through the process of senescence.

**Autonomous Degradation of Chloroplasts**

Two models have been proposed to explain photosynthetic activity during senescence (Gepstein, 1988). First hypothesis assumes that the chloroplasts number per mesophyll cell decline during senescence. The other hypothesis is that the autonomous and sequential degradation of the individual chloroplasts constituents leads to the decline in photosynthetic activity. Achor and Albrigo (2005) found both situations in natural senescence and biuret chlorosis. They found fewer and smaller chloroplasts
in the mesophyll cell and gradual breakdown and release into the vacuole or cytoplasm of the internal constituents of the chloroplasts.

**Chlorophyll Degradation**

Disappearance of chlorophyll is one of the most important processes of senescence, and eventually the rate of chlorophyll degradation is usually considered to be reliable criteria of leaf senescence and a measure of the age related deterioration of the photosynthetic capacity (Thomas and Stoddart, 1980).

**Changes in Lipids during Chloroplasts Senescence**

The striking ultrastructural changes of thylakoids and the concomitant rise in the size and number of plastoglobuli during senescence suggest that fundamental changes occur in the chloroplasts membranes during senescence. The lipids in other membranes show both quantitative and qualitative changes with the advance senescence (Thompson, 1987).

**Changes in Stromal Enzymes during Leaf Senescence**

RuBPCase, the enzyme of the photosynthetic carbon reduction cycle constitutes 50% or more of total soluble leaf protein. Due to the loss of photosynthetic activity during senescence, activity of RuBPCase also decrease (Woolhouse 1984).

**Changes in the Components of the Chloroplast Thylakoid Membranes During Foliar Senescence**

The light harvesting and energy transducing functions of the chloroplasts are now believed to be associated with five main protein complexes in the inner membranes of the chloroplasts. (Anderson and Anderson, 1982).
**Chloroplasts Protein Degradation**

Net loss of both thylakoid and stomal proteins during senescence is the result of balance between two opposite processes i.e. synthesis and degradation.

**Leaf Conductance and CO$_2$ Assimilation in Senescence Leaves**

Stomata are main entryways for CO$_2$ from the atmosphere to the mesophyll cells, where CO$_2$ assimilation takes place. It was found that leaf diffusion conductance decreases with the progress of senescence. Insufficient CO$_2$ supply as a result of reduction in leaf conductance may account for the decreased rates of assimilation, especially when leaves are exposed to high irradiation or stress.

In brief, during chloroplasts senescence, changes in the molecular organization of the thylakoids, differential and sequential changes in the main protein complexes of thylakoid, changes in the activities of key enzymes in the Calvin cycle and changes in the rates of protein synthesis and/or degradation of certain chloroplasts proteins takes place (Gepstein, 1988).

Senescence process observed in citrus was slightly different. There is a loss of plastoglobuli by their liberation in association with membrane vesicles or directly through the double membrane in place of the build up and loss of plastoglobuli at the last step when the membrane dissipates. Matile (1992) reported that the plastoglobuli are the final depository of thylakoidal lipids while Wittenbach (1982) stated that the vacuole and cytoplasm may be the final depository within the cell. These bodies were observed in both the cytoplasm, associated with membranes and in the vacuoles.

Acher and Albrigo (2005) also found that the chloroplasts lost their store of plastoglobuli and internal membranes in biuret chlorosis and in senescence whereas in Zn deficient leaves, plastoglobuli and internal membranes both were retained. Therefore,
they concluded that biuret chlorosis is more similar to chlorosis due to senescence than chlorosis due to nutrient deficiency in citrus.

**Chlorosis Due to Nutrient Deficiency**

**Iron**

The most obvious effect of iron deficiency is that it produces a marked decrease in the amount of green pigments (Abadia, 1986). Total carotenoids are also decreased by iron deficiency, but to a lesser extent than chloroplasts (Terry, 1980). The characteristic yellow color of chlorotic leaves is a consequence of this relative enrichment in carotenoids (Figure 2-2).

![Figure 2-2. Iron deficiency in Orange leaves (Courtesy: Steve Futch, CREC)](image)

Mildly affected plants become unsightly and grow poorly. Severely affected plants fail to flower or fruit and may even die from lack of iron. Iron chlorosis may occur as a result of one or a combination of causes. The condition is often due to high pH, which makes it possible for other elements to interfere with the absorption of iron, rather than
lack of iron in the soil. This occurs in neutral to alkaline soils when the pH is above 6.5. If overwatering or poor drainage are possible causes, they should be corrected. Iron chelates are water-soluble forms of iron that remain in the solution once added to the tree. Some formulations of iron chelates can be applied to the foliage; however, this approach is usually not as permanent as soil applications. Follow the manufacturer’s recommendations for amount of use. Some fertilizers contain iron chelates, and use of these with plants susceptible to iron deficiency is recommended.

**Nitrogen**

A deficiency of nitrogen in citrus is first characterized by a uniform loss of chlorophyll over the entire leaf, with occasional vein chlorosis in early stages (Figure 2-3). The symptoms range from a pale yellowish-green color in early stages, to old ivory color in the advanced stages. The deficiency extends over the entire plant, with the greatest severity on fruiting branches, the leaves of which may show a slight mottling effect in acute cases. Severely affected trees show stunting, sparse foliage
Calcium deficiency in citrus fruit is shown in Figure 2-4. Calcium deficiency symptoms are characterized by a marked stunted and hard condition of the tree, with small leaves. In severe cases the leaves become chlorotic at the margins and tips, which progress towards the leaf center and base. The calcium deficiency pattern may be confused with an advanced case of biuret toxicity. The differences consist of smaller leaves with calcium deficiency, and the chlorosis following the leaf margins, whereas the biurate toxicity is somewhat patchy in early stages, beginning in the tip of the leaf and spreading inward. The tips of calcium deficient leaves are often blunt and sometimes under developed.
Manganese

The symptoms of manganese deficiency in citrus are usually less distinct than those of magnesium and zinc. The symptoms occur on both young and mature leaves, without affecting leaf size, whereas zinc deficiency has a marked reduction on size of leaves and magnesium deficiency pattern is characterized by green veins with light green background and may be confused with iron deficiencies (Figure 2-5). As the leaves become mature, the leaf develops pattern with bands of green along the main and lateral veins with light green tissue.
Magnesium deficiency in citrus is characterized by a type of leaf chlorosis as bronzing (Figure 2-6). This discoloration or loss of chlorophyll occurs only on mature leaves, and is more prevalent on heavily fruiting trees and branches, and is more noticeable in late summer and fall. In atypical case, yellow chlorotic areas develop in the initial stage on each side of the mid-rib. Later these areas enlarge often at an angle to the midrib and usually coalesce to form a yellow zone surrounding a wedge shaped green area at the leaf base. As the deficiency advances, the entire leaf becomes yellow or bronze like.
Figure 2-6. Deficiency symptoms of magnesium in grapefruit (Courtesy: Steve Futch, CREC)

**Molybdenum**

The symptom of molybdenum (Mo) deficiency appears first as water soaked areas in the spring flush, later developing into interveinal circular chlorotic areas (Figure 2-7). It is more noticeable during the summer and early fall months. Molybdenum deficiency is found on acid sands far more than on heavier and better types. Acid fertilizer aggravates the deficiency, whereas neutral fertilizers and lime usually relieve it. The amount of molybdenum necessary for plant growth, including citrus is very small. The actual amount to correct deficiency ranges from 1 to 2 ounces of sodium molybdate per 100 gallons of spray of equivalent amounts from other soluble sources.
Potassium, Phosphorus and Sulfur

Potassium and phosphorus deficiencies are shown in Figure 2-8 and 2-9. The chloroplasts from potassium deficient plants have a regular ellipsoidal shape and contain osmiophillic globules. They observed starch grains in almost every chloroplasts.

In phosphorus deficient maize plants, Hall et al. (1972) observed a regular outline and osmiophillic globules in chloroplasts. An extensive system of grana and stroma lamellae were present. The grana lamellae were organized into irregular grana stacks. The grana discs within a single stack vary considerably in length and many were longer than the disc seen in the chloroplasts of healthy plants. The most important phenomenon they observed was the absence of starch globules in the phosphorus deficient plants.
Figure 2-8. Potassium deficiency in Orange leaves (Courtesy: Mongi Zekri, LaBelle)

Figure 2-9. Phosphorus deficiency (Courtesy: Steve Futch, CREC)
The shape of chloroplasts in sulfur deficient plants was irregular and possessed long projection during advanced stage deficiency. Table 2-1 summaries the effect of mineral deficiencies on chloroplasts structure (Vesk et al. 1965).

Figure 2-10. Sulfur deficiency in Orange leaves (Courtesy: Mongi Zekri, LaBelle)
Table 2-1. Summary of the effects of mineral deficiencies on chloroplasts structure

<table>
<thead>
<tr>
<th>Deficiency</th>
<th>Grana</th>
<th>Intergranum connections</th>
<th>Stroma</th>
<th>Star bodies</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>Reduced no</td>
<td>Long frets, parallel or branching</td>
<td>Relative increase</td>
<td>Increased no</td>
<td>Absent</td>
</tr>
<tr>
<td>Ca</td>
<td>Reduced no, Swelling</td>
<td>Extensive parallel frets Swelling</td>
<td>-</td>
<td>-</td>
<td>Increased amount</td>
</tr>
<tr>
<td>S</td>
<td>Swelling</td>
<td>Swelling</td>
<td>-</td>
<td>-</td>
<td>Present</td>
</tr>
<tr>
<td>N</td>
<td>Reduced no</td>
<td>Frets and long lamellae</td>
<td>Relative increase</td>
<td>-</td>
<td>Present</td>
</tr>
<tr>
<td>P</td>
<td>Swelling</td>
<td>Increased no of long parallel frets, Swelling</td>
<td>-</td>
<td>-</td>
<td>Present occasionally</td>
</tr>
<tr>
<td>B</td>
<td>No grana</td>
<td>Reduced no</td>
<td>Relative increase</td>
<td>Increased no</td>
<td>Absent</td>
</tr>
<tr>
<td>Zn</td>
<td>Reduced no</td>
<td>Reduced no</td>
<td>Relative increase</td>
<td>-</td>
<td>Starch &quot;vacuoles&quot;</td>
</tr>
<tr>
<td>Cu</td>
<td>Swelling</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Absent</td>
</tr>
<tr>
<td>Mn</td>
<td>Reduced no and size, Swelling</td>
<td>Reduced no, replaced by vesicles, Swelling</td>
<td>Relative increase</td>
<td>-</td>
<td>Present occasionally</td>
</tr>
<tr>
<td>Fe</td>
<td>Greatly reduced no and size</td>
<td>Reduced no replaced by vesicles, Swelling</td>
<td>Relative increase</td>
<td>-</td>
<td>Increased amount</td>
</tr>
<tr>
<td>Mg</td>
<td>Reduced no and size</td>
<td>Reduced no of frets</td>
<td>-</td>
<td>-</td>
<td>Present</td>
</tr>
<tr>
<td>N</td>
<td>Reduced no swelling</td>
<td>Plastids smaller in size Reduced no of frets</td>
<td>Reduced amount</td>
<td>Present</td>
<td>Starch &quot;vacuoles&quot;</td>
</tr>
<tr>
<td>P</td>
<td>Reduced no and size, Swelling</td>
<td>Reduced no of frets, Swelling</td>
<td>-</td>
<td>Increased no</td>
<td>Starch &quot;vacuoles&quot;</td>
</tr>
<tr>
<td>B, Zn, Cu, Mo</td>
<td>No results</td>
<td>Coalescence of compartments, swelling</td>
<td>Very dense granular</td>
<td>Present granular</td>
<td>Present occasionally</td>
</tr>
<tr>
<td>Mn</td>
<td>Reduced no, extremely Swollen</td>
<td>Reduced no replaced by vesicles, Swelling</td>
<td>Relative increase, formation of tails</td>
<td>-</td>
<td>Absent</td>
</tr>
<tr>
<td>Fe</td>
<td>Absent or greatly reduced in no and size</td>
<td>Show tubules and vesicles swelling</td>
<td>Relative increase</td>
<td>-</td>
<td>Absent</td>
</tr>
<tr>
<td>K</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S</td>
<td>-</td>
<td>-</td>
<td>Increased no</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mg</td>
<td>Reduced no and size, Swelling</td>
<td>Reduced, branching frets, Swelling</td>
<td>Tails</td>
<td>Absent</td>
<td>-</td>
</tr>
<tr>
<td>Fe</td>
<td>Greatly reduced in no and size, may be absent</td>
<td>Plastids smaller in size, Extensive parallel lamellae</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Zinc

The initial stages of the deficiency appear as irregular chlorotic areas in the leaf tissue, between the main and lateral veins (Figure 2-11). The tissue immediately adjoining the veins remains green, while the chlorophyll disappears from the rest of leaf. This results in an irregular, mottled or variegated mixture of vivid green and white to yellow colors. In the early stages of the deficiency, the characteristic leaf pattern may occur on apparently normal sized leaves, but as the deficiency becomes more acute, the new leaves are small, narrow and pointed, with a greater loss of chlorophyll.

Figure 2-11. Zinc deficiency in orange leaves (Courtesy: Mongi Zekri, LaBelle)

Boron

Fruit symptoms most indicative of boron deficiency include dark spots in the white albedo of fruit and sometimes in the central core (Figure 2-12). Boron deficient fruit turns hard and dry due to lumps in the rind so this deficiency is also known as "hard
fruit”. Boron deficiency results in premature shedding of young fruits that have brownish discolorations and unusually thick albedo. Older fruit remain undersized and misshapen with an unusually thick albedo. Seeds do not develop and the terminal growing point of the main stem dies. Other symptoms include slight thickening and downward curling of leaves. Damp spots are found on young leaves that turn translucent as the leaves mature. Defoliation begins at the top of the tree and continues until tree dies.

Figure 2-12. Boron deficiency (Courtesy: Mongi Zekri, LaBelle)

Borax is commonly used to treat boron deficient citrus. It can be applied to soil or to the foliage. Boric acid is preferred for foliar application as it is more soluble than Borax. Foliar spray application is effective in Florida. The spray may be applied either during the dormant period or post-bloom. Unlike other micronutrient deficiencies, boron can impact fruit quality and should therefore not be allowed to occur. Slight excess can
cause toxicity, so maintenance or correctional applications should involve ground or foliage applications, but not both.

**Copper**

Copper deficiency affects the formation of grains, seeds and fruit much more than it affects vegetable growth (Figure 2-13). The main reason for the poor development of seeds and fruits is that a high percentage of the pollen from copper-deficient plants is not viable. When extractable copper exceeds 100 pounds per acre, trees may begin to decline. Unusually large dark green foliage with a "bowing up" of the midrib are among the primary symptoms for copper deficiency. Fruit symptoms are most evident on oranges. Fruits bear brown spotted areas of hardened gum on rind and fruit splitting is commonly found on the trees with Cu deficiency. The brown-stained areas on the fruit may become almost black over the time and fruit sheds by summer eventually. Leaf and twig symptoms may not be observed when Cu deficiency is present along with Zn or Mg deficiency but the typical fruit symptoms will be evident. Therefore, fruit symptoms are considered reliable and consistent indicator of Cu deficiency. Foliar sprays or soil applications of Cu fertilizer can prevent or cure Cu deficiency. Spraying a solution containing 2 to 3 lbs per acre of elemental Cu applied during flowering usually results in recovery followed by a normal fruit set.
Diseases in Citrus

Alternaria Brown Spot

Alternaria brown spot causes serious losses of susceptible tangerine and tangerine hybrids. A similar leaf and fruit spot affects rough lemon and Rangpur lime. This disease affects young leaves, twigs and fruit, and produces brown to black lesions which vary in size from small dots to large expanding lesions (Figure 2-14). Diseased fruit may abscise, and lesions on remaining fruit may vary from small spots to larger lesions (Whiteside, 1976).
Figure 2-14. Alternaria brown spot in orange fruit (Courtesy: Moongi Zakri LaBelle)

Moderate to high temperatures and rainfall favors the disease but since heavy dews are sufficient for infection, fruit blemishes occur even in semi arid areas where no rainfall occurs after flowering (Timmer *et al.*, 2000). Minimizing the period of leaf wetness of the tree canopy can reduce disease incidence. Nursery trees, free of the disease should be used for new plantings, and overhead irrigation should be avoided. Excessive nitrogen fertilization and irrigation that promote abundant growth flushes should be avoided. Foliar fungicide applications are needed in most affected orchards, with frequency based on disease severity.

**Black Spot**

This disease causes fruit loss and a serious external blemish of citrus fruit. Black spot is widespread in the humid to semi-arid citrus growing areas in the southern
hemisphere that have summer rainfall. Black spot produces lesions on fruit varying from small brown to black spots to large sunken lesions (Figure 2-15). Symptoms may appear in the orchard on fruit, and cause premature fruit drop, or infections may remain quiescent until harvest.

Figure 2-15. Black spot (Courtesy: Michael Rogers, CREC)

Infections usually occur from early to mid-summer and remain latent for some time. Moisture is essential for infection. Fungicide applications are the primary means for managing black spot. Often a single, late summer spray of benomyl will provide sufficient disease control except where resistant strains occur.

**Canker**

Citrus canker is a serious bacterial disease in humid tropical and subtropical areas. The disease causes external blemishes on the fruit, making them unsuitable for the fresh market, and may cause fruit drop (Figure 2.16). This disease is widespread in
Asia and is spreading in southern South America and in South Florida. Canker affects young leaves, stems and fruit of most citrus species, producing water soaked lesions of variable size.

Figure 2-16. Citrus canker (Courtesy: Jamie Yates, CREC)

This disease is dependent on storms and windblown rain not only for dispersal, but also to force the bacteria into wounds and stomata. Canker is most serious in areas with severe thunderstorms, hurricanes and typhoons. The presence of leaf miners exacerbates canker because tunnels provide entry points for the bacterium and expose additional tissue in which it multiplies. Citrus canker is controlled by quarantine and eradication in countries in which it is absent or has limited distribution. Movement of citrus fruit and budwood from infested areas is restricted (Schubert et al., 2001). Diseased trees are burned in place, and the area is kept free from citrus root sprouts for
6-12 months. Wind breaks are quite effective in reducing spread of the disease and in limiting the amount of infection. Copper fungicides are effective in preventing fruit infection if applied frequently (Stall et al., 1981).

**Mal Secco**

This disease is primarily a problem of lemons, but can also affect tangerines and their hybrids. Oranges and grapefruit are seldom affected. Mal secco can result in losses of tree limbs or, in severe cases, of the entire tree. Infected leaves develop a veinal chlorosis (Figure 2-18). As the infection proceeds downward in the vascular system, leaves wilt and the shoots die back. Eventually, limbs or the tree may die. When the bark of affected branch is removed, the wood shows a characteristic orange or orange red discoloration. Disease trees and branches should be removed and burned to reduce inoculum. Foliar sprays of benomyl or copper fungicides in the spring and autumn reduce new infection.

![Figure 2-17. Mal secco](image)
Melanose

Melanose is most severe on lemon and grapefruit. It is an important disease of fruit produced for the fresh market in humid subtropical areas, but is not major concern in Mediterranean climates or in high rainfall tropical areas. Melanose appears as raised, brick red to brown pustules on the leaves, twigs and fruit (Figure 2-19). Spores carried down the side of the fruit by water may cause lesions to form in a tearstain or droplet pattern.

Relatively long period of wetting (12-18h) are required for infection even at high temperatures. Copper fungicides are the most widely used means to control melanose (Timmer and Zitko, 1996) because they are highly effective and have a high residual. However, they must be applied frequently when fruit growth is rapid.

Figure 2-18. Melanose (Courtesy: Jamie Yates, CREC)
**Powdery Mildew**

Powdery mildew occurs throughout the humid areas of Asia and USA. It reduces yield by debilitating trees and causing fruit drop. Whitish powdery patches of mildew occur on the upper surface of leaves, especially at the edges and on young fruits (Figure 2-20). Immature leaves and entire shoots may shrivel and drop, and infected young fruit falls permanently.

![Powdery Mildew](image)

**Figure 2-19. Powdery mildew (Courtesy: Megh Singh, CREC)**

**Scab**

Scab disease affect only the external quality of the fruit of susceptible citrus and are important primarily on fruit that are grown for fresh market (Figure 2-21). Citrus scab affects many mandarins and their hybrids, lemons and grapefruit, and occurs in all areas where conditions are favorable. The first symptoms are clear to slightly pink, water-soaked areas on leaves or fruit. These grow rapidly to raise pustules that become warty and grey with age. Lesions on fruit tend to flatten with age, especially on
grapefruit, and lesions of sweet orange scab tend to be flatter than those of citrus scab. Fruits are susceptible to scab until they reach approximately 3 cm diameter. Fungicide application during this period is effective in controlling the disease. The most effective products include the sterol biosynthesis-inhibiting fungicides, benomyl, ferbam and copper materials (Timmer and Zitko, 1997).

Figure 2-20. Scab (Courtesy: Mongi Zekri, LaBelle)

**Huanglongbing (Greening)**

Huanglongbing (HLB) was reported in mainland China in 1919, and in South Africa in 1937 as citrus greening disease (da Graca, 1991). Its name translates as ‘yellow shoot disease’, and it has been reported under different names in many countries. ‘Greening’ is the most common name in english speaking countries. HLB has destroyed an estimated 60 million trees in Africa and Asia.
The most characteristic symptom of HLB is green patches on the pale green background that often begins in one part of the canopy (Figure 2-26). Leaf yellowing and leaf drop result in twig die back. Fruit on affected trees are small, lopsided and poorly colored, hence the name greening. Juice is bitter, low in soluble solids and high in acid. Nursery trees are stunted, terminal leaves are yellowed, new leaves are small, leathery and upright and old leaves are mottled. As these symptoms take 4-6 months to appear; symptomless trees may be distributed from affected nurseries.

Schneider (1968) proposed that when leaves are invaded by the greening virus, necrosis of localized pockets of phloem in the leaf vascular system is the first degenerative change induced. This occurs in mature leaves or in the leaves nearing maturity. Several reactions to the necrosis may occur in the leaf. During this phase
starch granules become unusually large and stretch the chloroplast’s outer membrane to a thin film that encloses them. Granules occur in the leaf that gives a leathery feel. It is assumed that grana and chlorophyll of the chloroplasts may be destroyed by being stretched, due to enlargement of the granule within and by crowding chloroplasts due to enlargement of granules. It was assumed that weak chlorotic growth is not a direct result of virus activity, but that results from the disturbed state of the old shoot from which new shoots grow. Mineral, nutrients and various organic compounds in old leaves moves to newly forming shoots where they support growth.

Transmission of HLB occurs by grafting and by African citrus psyllid, *Trioza erytreae*, and the Asian psyllid, *Diaphorina citri*. Each psyllid is able to transmit *L. africanus* and *L. asiaticus*. Polymerase Chain Reaction (PCR) and DNA-RNA hybridization techniques can now be used to detect the two species.

**Leprosis**

Leprosis causes chlorotic to necrotic areas on the fruit, leaves and twigs of susceptible cultivars. Initial symptoms are chlorotic lesions that often become necrotic and gum impregnated and show concentric patterns (Figure 2-23). A chlorotic zone around the lesion may still remain. Leaf and fruit drop occurs when infections are abundant.
Infections are localized and apparently associated with feeding activity of mites that carry the causal virus. The virus does not infect citrus systemically and trees do not develop symptoms on new growth after infective mites are removed.

**Citrus Variegated Chlorosis**

The trees affected with the citrus variegated chlorosis (CVC) have mottled leaves on one or more branches, and in chronic stage may be stunted and show twig dieback (Figure 2-24). Fruits are small and hard and change color prematurely. They are frequently sun burned and may also have sunken brown areas on the surface of the rind.
The disease is caused by a strain of the bacterium Xylella fastidiosa that inhabits xylem and impairs its normal function. It may spread by infected bud wood or by leaf hopper vectors. Control measures include avoiding propagation of CVC infected bud wood for new plantings, removing infected limbs from recently affected trees and removal of affected trees in young plantings. Mandarins, grapefruit and lemons appear to be less sensitive to CVC than sweet orange and are more susceptible for areas that are severely affected by CVC.
CHAPTER 3
SPECTRAL CHARACTERISTICS OF CITRUS GREENING (HUANGLONGBING)

Introduction

Citrus is one of the most important agricultural products in Florida as it is the largest citrus producing state in United States and second largest in the entire world. Citrus production being a multi-million dollar industry accounts significantly for Florida’s agricultural economy. But recently, it has been threatened by Huanglongbing (HLB), a devastating and rapidly spreading disease of citrus.

Spectral reflectance characteristics of leaves have been shown to be highly correlated with their chemical composition. Carter and Knapp (2001) showed the importance of chlorophyll concentration on the spectral signature of leaves. The optical response to stress near 700 nm, as well as corresponding changes in reflectance that occur in the green-yellow spectrum (400-500 nm), was explained by the general tendency of stress to reduce leaf chlorophyll concentration. The reflection of incident radiation from within the leaf interior of stressed trees increases such that stressed trees appear brighter in the visible region of the spectrum than healthy trees (Cibula and Carter, 1992). Riedell and Blackmer (1999) found that leaf reflectance in the 625-635 nm and the 680-695 nm wavebands, together with the Normalized total Pigment Chlorophyll Index (NPCI) were significantly correlated with the total chlorophyll concentrations in both green bug and Russian wheat aphid-damaged trees. Boochs et al. (1990) suggested that high-resolution reflectance spectra, especially in the red edge area (reflectance between 680-760 nm), would be useful for the identification of small differences in the chemical and morphological status of the trees in the field. Optimal
reflectance at wavebands of 825 nm and 980 nm were determined using stepwise linear discriminate analysis to detect bruises in strawberries (Tallada et al., 2006). Borel and Gerstl (1994) pointed out that canopy architecture strongly influences illuminated areas for different sun angles, and thus reflectance. This could affect the spectral signature of trees in the field.

The use of first, second and higher orders derivatives have become an established technique for reduction of low frequency background noise and for resolution of overlapping spectra (Butler and Hopkins, 1970). In remote sensing, mostly the first derivative has been used to facilitate the location of critical wavelengths such as the ‘red edge’. Horler (1983) used the first derivative of leaf reflectance spectra to locate the red edge. Hence, derivative analysis may have the potential to discriminate HLB-infected trees.

**Objective**

This research was aimed at developing a spectral method for the detection of HLB. The specific objective was to identify optimal wavebands (400 nm to 2500 nm) for accurate detection of HLB-in citrus.

**Materials and Methods**

The study was conducted at a commercial grove in Lake Placid, FL (approx. 27.34386° N and 81.38387° W). Twenty infected leaf samples and 20 non-infected leaf samples were collected.

Canopy reflectance spectral data was collected with an ASD FieldSpec® spectroradiometer (FieldSpec® UV/VNIR, Analytical Spectral Devices, Boulder, CO). This spectroradiometer is a compact and field portable, with a spectral range of 350-2500 nm and a rapid data collection time of 0.1 second per spectrum. All data files were
collected in an ASD file format that can then be viewed and post-processed in ViewSpec™ Pro. The sampling interval was 1.4 nm for the spectral region 350-1000 nm and 2.0 nm for the spectral region of 1000-2500 nm. Bare fiber optic cable was used in data collection. The integration time was optimized using optimize options within the software. Dark and white calibrations were conducted prior to data collection in the field. The data were collected between 11:00 a.m. to 12:00 noon to limit the variability due to change in sun angle. Every 10 minutes, a reference reading was collected to reduce the error due to atmosphere. Bare fiber optic was kept about 50 to 80 cm from the tree canopy. During data collection, the area scanned by the ASD spectroradiometer was approximately 385 to 988 cm².

During raw spectral data processing in ViewSpecPro (Analytical Spectral Devices, Boulder, CO), outliers were identified by two criteria. First, spectra from one variety of trees should only contain random errors. Secondly, if a spectrum had a different shape or curve, it was removed from the data set. Since the sensor was viewing the tree canopy nearby parallel to ground, any background effect due to soil was negligible.

Data Analysis

Discriminability

The discriminability of two probability density functions (pdf) with the same standard distribution is defined by (Duda et al., 2000) as:

\[
d = \frac{\mu_2 - \mu_1}{\sigma}
\]

(1)

Where, \(d\) = discriminability,

\(\sigma\) = standard deviation, and
μ₂ and μ₁ = mean spectra of HLB affected trees and healthy trees, respectively.

In this case, the standard deviations could be different between the two pdfs at the same wavelength, thus the standard formula could not be used. Equation 2 displays an example of two pdfs with different standard deviations from the sample data used. For this reason following equations was used.

\[
d' = \frac{(μ₂ - μ₁)}{(σ₁ + σ₂)/2}
\]

Discriminability may be one method by which can determine optimal wavelengths to discriminate HLB trees with healthy trees. Averaging the standard deviations of both pdfs allow the discriminability to scale with magnitude changes in the standard deviation. For better discriminability of HLB-infected trees from healthy tree, d (or d') should be large. Since reflectance properties at one wavelength shares common properties with neighboring wavelengths, a second wavelength was required to be outside a threshold of 100 nm, allowing two distinct features (Kane and Lee 2006).

Data analysis was performed with Statistical Analytical Software (SAS). Procedure “PROC STEPDISC” was used to identify the critical bands. This procedure performs a stepwise discriminant analysis to select a subset of the quantitative variables for use in discriminating categories.

**Spectral Derivative Analysis**

Among the techniques that have been developed in spectroscopy, derivative analysis is particularly promising for use with remote sensing data. Demetriades-Shah et al. (1990) showed that derivative analysis was better than ratio or difference vegetation indices. Spectral derivative analysis was used to examine the spectral
differences more closely in reflectance at specific wavelengths. A derivative analysis separated the differences more clearly (Rundquist et al., 1996).

The simplest numerical method for generating derivatives is to divide the difference between successive spectral values by the wavelength interval separating them (Demetriades-Shah et al., 1990). This provides an approximation of the first derivative at the central point between the values whose difference is used to calculate the slope. The first order derivative provides information on the rate of change in reflectance, which is the slope, with respect to wavelength, while a second order derivative gives the change in slope with respect to wavelength.

**Spectral Ratio Analysis**

Spectral ratio analysis was used to identify the wavelengths that are sensitive to tree stress caused by HLB-infection. Spectral derivative analysis magnifies the differences in spectral reflectance. By calculating a spectral ratio of healthy trees with HLB-infected trees, the effect of noise can be reduced (Zhang et al. 2002). If the ratio of these spectra is close to 1, it means there is no significant difference between the healthy and HLB trees at particular wavelengths. The more deviation of the ratio from one, the more likely separation is possible at particular wavelengths.

Taking the mean spectrum of HLB-infected trees as the numerator and mean spectrum health tree as the denominator, the spectral ratio ($S_{\text{ratio}}$) was calculated.

$$S_{\text{ratio}} = \frac{\text{Mean spectrum of HLB plant}}{\text{Mean spectrum of healthy plant}} \quad (3)$$
Results and Discussion

Discriminability

The discriminability, $d$, of all samples is presented in Table 1. At the wavelengths of 695 to 705 nm, a discriminability of 0.86 was obtained. It seems that the visible region (400-700 nm) has good (0.89 to 0.85) discrimination. The wide range suggests a great amount of inconsistency with samples. This could indicate a need for more samples or a slight change in reflectance during data collection.

Table 3-1. Discriminability of wavelengths for HLB and healthy trees

<table>
<thead>
<tr>
<th>Wavelengths (nm)</th>
<th>Discriminability ($d$)</th>
<th>Wavelength (nm)</th>
<th>Discriminability ($d'$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>695-705</td>
<td>0.86</td>
<td>695-705</td>
<td>0.89</td>
</tr>
<tr>
<td>585-595</td>
<td>0.83</td>
<td>585-595</td>
<td>0.85</td>
</tr>
<tr>
<td>405-415</td>
<td>0.78</td>
<td>405-415</td>
<td>0.78</td>
</tr>
<tr>
<td>2345-2350</td>
<td>0.71</td>
<td>2345-2350</td>
<td>0.69</td>
</tr>
<tr>
<td>1980-1990</td>
<td>0.68</td>
<td>1980-1990</td>
<td>0.68</td>
</tr>
</tbody>
</table>
Figure 3-1. A sample spectra of a healthy and HLB-infected tree canopy

**Spectral Derivative Analysis**

To examine the differences in spectral reflectance, derivative analysis was used. The rate of change in reflectance in the first derivative, within a 2.0 nm range, was distinctly different for healthy and HLB-infected trees. Likewise, the second derivatives are also different for healthy and HLB-infected trees. Derivative analysis was performed by using the finite difference method and the Savitzky-Golay method. The results of both the first and second derivative analysis reveal spectral ranges where the response of HLB-infected trees has the opposite sign compared to healthy trees.
The derivatives computed by the finite difference method and the Savitzky-Golay were noisy. The first derivative spectra seem to be less a function of noise than second derivative spectra. The finite difference method seems have more potential to differentiate HLB-infected trees over the Savitzky-Golay method. SAS output gave a large range of wavelengths where first derivative spectra and second derivative spectra reveals good separation of HLB-infected trees with healthy trees (Table 3-2). The finite difference method computed 747 nm, 1041 nm, 1283 nm, 1601 nm and 2283 nm in first derivative. Results from the finite difference second derivative method revealed that wavelengths of 480 nm, 590 nm, 754 nm, 1041 nm, and 2071 nm have the potential to differentiate HLB. The Savitzky-Golay method gave similar results as the finite difference method (Table 3-2).

### Spectral Ratio Analysis

An example of spectral ratio was illustrated in Figure 3-2. Large magnitude differences among spectral ratios can be observed for the wavelength range of 400 nm to 2350 nm. The results of ratio analysis showed the wavelengths that are most sensitive to HLB and can be better utilized for discriminating healthy and HLB-infected trees (Figure 3-2).

| Table 3-2. Identified wavelengths for separating HLB trees from healthy trees |
|---------------------------------|-------------------------------|---------------------------------|-------------------------------|
| **Finite difference method**    | **Savitzky-Golay method**     |
| First derivative                | Second derivative             | First derivative                | Second derivative             |
| 747 nm                          | 590 nm                        | 747 nm                          | 653 nm                        |
| 1041 nm                         | 754 nm                        | 1671 nm                         | 754 nm                        |
| 2283 nm                         | 1041 nm                       | 2014 nm                         | 1039 nm                       |
| 1283 nm                         | 480 nm                        | 1010 nm                         | 487 nm                        |
| 1601 nm                         | 2071 nm                       | 487 nm                          | 2073 nm                       |
The spectral ratios of 1.3 to 1.5 correspond to 530 to 564 nm. These wavelengths correspond to the green peak. The HLB-infected trees are less green than healthy trees. Reflectance of HLB-infected trees at 530-564 nm was higher than that of healthy trees. Therefore, the ratio is higher in this range.

A second sensitive point was observed at 710 to 715 nm (red edge). In this range, the ratio was 1.3 to 1.6. This range is sensitive due to the chlorophyll absorption. Healthy trees have more chlorophyll; hence, they will absorb more light in this range than HLB-infected trees resulting in higher reflectance of HLB-infected trees than healthy trees.

The wavelengths of 1450 nm and 1990 nm correspond to the water absorption band.

![Figure 3-2. Spectral ratio of HLB-infected and healthy trees](image)

This work provides a better understanding of the spectral properties of HLB-infection in citrus canopy. The identified wavelengths in the green region and near infrared sensitive to the change of chlorophyll content and water content in the ratio analysis are consistent with previous work reported by Gitelson and Merzylyak (1997).
The HLB-infected trees contain lower chlorophyll which leads to a low photosynthesis rate and lower water content. The changes of these pigments and water content are often indicators of tree stress, which can be used to monitor the conditions of crop growth and site characteristics.

**Conclusion**

The practical implication of this result is that hyperspectral spectroscopy has the potential to identify HLB-infected trees from healthy trees. Further study is necessary to confirm the potential to detect HLB infected trees from healthy trees. Discriminability to separate HLB-infected trees from healthy trees was 0.83 to 0.86 in visible region (695nm to 705 nm and 585nm to 595 nm). Results of second derivative analysis with the finite difference method and the Savizky-Golay method were the same. Results of first derivative analysis with these two methods were slightly different. Higher ratios (1.3 to 1.6) were obtained at green bands (530nm to 564 nm) and red edge region (710nm to 715 nm). Spectral ratio analysis supports the results obtained from the discriminability analysis.
CHAPTER 4
SPECTRAL DISCRIMINATION OF HEALTHY VS. HLB-INFECTED CITRUS TREES IN THE VIS-NIR RANGE

Introduction

Huanglongbing (HLB) or greening is one of the most serious diseases of citrus threatening Florida’s multi-billion dollar citrus industry. It was first reported in Florida in August 2005 in South Miami Dade County. It affects all citrus cultivars and causes rapid deterioration of trees. Ron Muraro (2007) reported that the total production costs of fresh fruit increased from $ 1115.19 to $ 1711.20 per acre in southwest Florida.

At present, Polymerase Chain Reaction (PCR) is the only determinant method to detect HLB. For the selection of leaves for PCR testing, an iodine based starch test can be used. HLB infected trees show an increased level of starch accumulation (Schneider, 1968). Leaves with strong blotchy mottle symptoms of HLB infection stain very dark grey to black along cut surfaces when immersed in iodine solution for two minutes, while healthy citrus leaves show no or very little staining after their immersion in iodine for two minutes (Etxeberria et al., 2007).

Currently, there is no cure available for HLB. Groves are scouted regularly and affected trees are removed as soon as possible. Generally scouting is recommended at least four times a year but more frequent identification and removal of HLB infected trees would be desirable. Annual field inspection costs for identifying HLB infected trees was reported as $90.92 per acre in 2006-2007 (Muraro, 2007).

Near-infrared (NIR) spectroscopy has been utilized for fruit quality assessment and disease detection for many years. Visible and NIR spectroscopy have been largely used to detect plant status such as moisture content, nutrient stress and disease detection. Thomas and Oerther (1972) found a non-linear relationship between
reflectance at 550 nm and leaf nitrogen content of sweet pepper leaves with a correlation coefficient of -0.93.

Hyperspectral spectroscopy is a technique that utilizes hundreds of narrow contiguous spectral bands for the assessment of plant health. The data obtained from hyperspectral spectroscopy may detect many plant attributes that were not detectable with multispectral spectroscopy. Spectral properties of potato, bean and barley disease were reported by Lorenzen and Jensen (1989) and Malthus and Madeira (1993). There is very little known about the spectral characteristics of HLB in the visible and NIR regions.

Gaffney (1972) worked on the reflectance properties of citrus. He concluded that a wavelength band of 580 nm to 610 nm is suitable for sorting out defects in Pineapple oranges. Hyperspectral imaging was used to detect chill-induced damage in whole cucumbers under a variety of conditions (Liu et al., 2005). Band ratio algorithms and principal component analysis (PCA) were attempted to discriminate the area damaged by chilling injury. They found that a dual band ratio algorithm (R-811nm/R-756 nm) and a PCA model from a narrow spectral region of 733-848 nm can detect chill-injured skins with a success rate of over 90%. Zacro-Tejada et al. (2005) showed that the best indicators for chlorophyll content estimation in V. vinifera L. leaves were narrow-band hyperspectral indices calculated in the 700-750 nm spectral region, with $R^2$ ranging from 0.8 to 0.9, with poor performance of traditional indices such as the Normalized Difference Vegetation Index (NDVI).

Partial least squares (PLS) is a multivariate analysis technique that is commonly used in analyzing the spectral data. It is sometimes referred as “soft modeling”. This is
in contrast with an ordinary least squares regression that makes “hard” assumptions including lack of multicolinearity among the predictor variables, with well-understood relationships to the response variable. PLS balances the two objectives of explaining response variation and predictor variation. Since the focus of PLS is prediction and not explanation, lack of well-understood relationships of the response to the predictor variable is not a problem. The number of extracted factors depends on the data. Latent vectors, i.e. successive linear combinations of the predictors, explain response variation and predictor variation. Sometimes too many extracted factors can cause over-fitting.

Discriminate analysis is a technique for classifying a set of observations into predefined classes. It is a one way classification based on the known values. The technique is based on how close a set of measurement variables are to the multivariate means of the levels predicted. This technique can also be used to discriminate HLB. This technique includes stepwise selection of variables, choice of linear, quadratic or regularized parameter analyses and a discriminant score to show each point close to a particular group.

Therefore, the objectives of this study were as follows:

- Collect reflectance data from HLB and healthy leaves.
- Investigate the potential of the PLS technique and discriminate analysis in identifying the spectra of HLB infected trees from the spectra of healthy trees.
- Evaluate the possibility of using the narrow NIR spectral range (400 nm to 900 nm) instead of the full range (400 nm to 2500) in discriminating the spectra of HLB infected citrus leaves from healthy leaves.
Material and Methods

This study was conducted near Lake Alfred, FL. There were two sets of data collected on June 13 and 14, 2007. Two trees were reported positive with HLB. The canopy reflectances of two neighboring healthy trees were also collected for the comparison. Another two sets of data were collected from a greenhouse on Aug 2 and 3, 2007. The spectra were collected in the presence of artificial lights on Aug 2, 2007 and in the presence of natural light on Aug 3, 2007. Healthy and HLB infected plants of eureka lemon, mandarin, madam vinous (MV), and sunchusha were used on Aug 2, 2007 for spectral data collection. Spectra of healthy and HLB infected plants of calamondin, Duncan grapefruit, trifoliate orange, Madam Vinous, Cleopatra mandarin, Mexican Lime, sweet lime, Valencia, Sunchusha and sour orange were collected on Aug 3, 2007.

Canopy reflectance was collected with a FieldSpec® 3 spectroradiometer manufactured from Analytical Spectral Device (Boulder, CO). This ASD spectroradiometer collects reflectance data from 350 to 2500 nm with rapid data collection rate of 10 scans per second. The ASD spectroradiometer transmits the spectral data wirelessly to a laptop computer. Each individual scan was the result of an average of 10 scans automatically made by the equipment. Bare fiber optic cable with 25 degree field of view was used in data collection. The integration time was optimized using optimization options within the software. Dark and white calibrations were conducted prior to and during data collection in the field. The data were collected between 11:00 a.m. to 2:00 pm to limit the variability due to change in sun angle. Spectral reflectance was collected from two sides of the tree to compensate for the effect of shade. On June 14, 2007, data was also collected from the top of the canopy.
Reference readings were collected every 10 minutes to reduce the error due to the atmospheric changes. The bare fiber optic was kept about 50 to 80 cm from the tree canopy. During data collection, the area scanned by the ASD spectroradiometer was approximately 385 to 988 cm$^2$.

Raw spectral data processing was performed in ViewSpecPro (Analytical Spectral Devices, Boulder, CO). The percent reflectance value was obtained in 350-2500 nm range. JMP 7 (Cary, NC) was used for partial least squares modeling.

The initial canopy reflectance was obtained from 350 nm to 2500 nm with intervals of 1 nm. Reflectance data was reduced by averaging 50 reflectance values. For example, the reflectance value at 400 nm was calculated by averaging the reflectance values at 375 nm to 424 nm. This reduced the total number of data point from 2152 to 40. PLS modeling was applied to the full range from 400 nm to 2450 nm and narrow range from 400 nm to 900 nm.

There were 113 spectra (49 HLB, 62 healthy) evaluated on June 13, 2007. Out of 49 HLB spectra, 27 spectra were used for calibration and 22 were used for validation. For healthy trees, 32 spectra were used for calibration and 30 spectra were used for validation. A total of 128 spectra (70 HLB, 58 healthy) were collected on June 14, 2007. For HLB trees, 35 spectra were used for calibration and 35 spectra were used for validation. In the case of healthy trees, 30 spectra were used for calibration and 28 spectra were used for validation.

In JMP 7, specified measurement variables were specified (reflectance at various wavelength) as Y effects and classification variables (healthy or HLB) as a single X
effect. The multivariate fitting gives estimation of the means and the covariance matrix for the data, assuming that the covariance is the same for each group.

**Results and Discussion**

Figure 4-1 shows an example of spectral differences between healthy and HLB infected tree. The healthy and HLB infected tree canopies were measured from northeast (NE) and southeast (SE) orientations. Each measurement is an average of ten measurements. In the near-infrared region, low reflectance was observed. Noise was observed in bands 1350 to 1500, 1750 to 1950 nm and bands after 2350 due to the presence of atmospheric moisture.

![Figure 4-1. Canopy reflectance of healthy and HLB infected tree with FieldSpec® 3 spectroradiometer. Healthy and HLB infected canopies were measured from northeast and southeast direction](image)
The results of PLS calibration and validation for HLB are summarized in Table 4-1 for June 13, 2007. Correct (full range) and correct (narrow range) are the number of spectra classified correctly for healthy and HLB in full NIR range (400 nm to 2450 nm) and narrow NIR range (400 nm to 900 nm), respectively.

Table 4-1. PLS modeling for HLB and healthy trees showing total samples, correct classifications (June 13, 2007).

<table>
<thead>
<tr>
<th></th>
<th>Healthy-1</th>
<th></th>
<th>Healthy-2</th>
<th></th>
<th>HLB-1</th>
<th></th>
<th>HLB-2</th>
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<td>NE</td>
<td>SE</td>
<td>NE</td>
<td>SE</td>
<td>NE</td>
<td>SE</td>
<td>NE</td>
<td>SE</td>
</tr>
<tr>
<td>Calibration</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>8</td>
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<td>Validation</td>
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<td>8</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
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<td>15</td>
<td>16</td>
<td>16</td>
<td>10</td>
<td>9</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Correct (full range)</td>
<td>6</td>
<td>6</td>
<td>8</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Correct (narrow)</td>
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<td>7</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

The percentages of correctly classified spectra are shown in Fig 4-2. The PLS model classified 87.1% healthy and 95.5 % of HLB spectra correctly in full range. The PLS model classified 93.5 % healthy and 90.9% HLB spectra correctly in the narrow NIR range. These results support that the narrow NIR range (400 to 900 nm) has almost equal potential to discriminate HLB as the full range (400 to 2450 nm).
Figure 4-2. Percentage of correct classification of HLB and healthy trees in full NIR range (400-2450 nm) and narrow NIR range (400-900 nm) on June 13, 2007

PLS modeling results from June 14, 2007 are shown in Table 4-2. Canopy reflectances were collected from the northeast, southeast, and top of the canopy. Figure 4-3 shows the percentage of spectra classified correctly with PLS modeling on June 14, 2007. In the full range, PLS classified 66.7% of healthy and 74.3% of HLB trees, correctly. PLS has classified, 78.6% healthy and 54.3 % HLB spectra correctly in the narrow NIR range.
Table 4-2. PLS modeling for HLB and healthy trees showing total samples, correct classifications (June 14, 2007)

<table>
<thead>
<tr>
<th></th>
<th>Healthy-1</th>
<th>Healthy-2</th>
<th>HLB-1</th>
<th>HLB-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NE  SE  T</td>
<td>NE  SE  T</td>
<td>NE  SE  T</td>
<td>NE  SE  T</td>
</tr>
<tr>
<td>Calibration</td>
<td>5   5   5</td>
<td>6   5   9</td>
<td>5   5   5</td>
<td></td>
</tr>
<tr>
<td>Validation</td>
<td>5   5   5</td>
<td>5   5   10</td>
<td>5   5   5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10  10  10</td>
<td>11  10  19</td>
<td>10  10  10</td>
<td></td>
</tr>
<tr>
<td>Correct (full range)</td>
<td>2   3   4</td>
<td>4   3   8</td>
<td>3   3   5</td>
<td></td>
</tr>
<tr>
<td>Correct (narrow range)</td>
<td>3   4   4</td>
<td>3   3   5</td>
<td>1   3   6</td>
<td>4   2   3</td>
</tr>
</tbody>
</table>

Figure 4-3. Percentage of correct classification of HLB and healthy trees in full range (400-2450 nm) and narrow NIR range (400-900 nm) on June 14, 2007.

PLS modeling for Eureka lemon, mandarin, Madam Vinous and Sunchusha citrus types are given in Table 4-3. Figure 4-4 shows the percentage of spectra classified correctly with PLS modeling on Aug 2, 2007. PLS classified 78.3%, 100% in full range and 78.6%, 54.3% in narrow range respectively, for healthy and HLB trees. PLS modeling for HLB and healthy trees from the greenhouse in natural light (Aug 3, 2007) with
various citrus is shown in Table 4-4. Full range and narrow NIR ranges were almost the same in identifying healthy and HLB infected trees except healthy trees of calamondin and sour orange. In the full range, PLS classified 79.5% healthy and 86.1% HLB trees correctly (Figure 4-5), while in the narrow range, it has classified 74.5% of healthy and 79.8% of HLB trees correctly.

Table 4-3. PLS modeling for HLB and healthy trees showing total samples, correct classifications in a greenhouse with artificial light (Aug 2, 2007).

<table>
<thead>
<tr>
<th>Variety</th>
<th>HLB</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calibration</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Validation</td>
<td>5</td>
</tr>
<tr>
<td>Eureka lemon</td>
<td>Total no of spectra</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Correct (Full)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Correct (Narrow)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Calibration</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Validation</td>
<td>5</td>
</tr>
<tr>
<td>Mandarin</td>
<td>Total no of spectra</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Correct (Full)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Correct (Narrow)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Calibration</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Validation</td>
<td>2</td>
</tr>
<tr>
<td>Madam Vinous</td>
<td>Total no of spectra</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Correct (Full)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Correct (Narrow)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Calibration</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Validation</td>
<td>2</td>
</tr>
<tr>
<td>Sunchusha</td>
<td>Total no of spectra</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Correct (Full)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Correct (Narrow)</td>
<td>2</td>
</tr>
</tbody>
</table>
Figure 4-4. Percentage of correct classification of HLB and healthy trees in full range (400-2450 nm) and narrow NIR range (400-900 nm) on Aug 2, 2007.
Table 4-4. PLS modeling for HLB and healthy trees showing total samples, correct classifications in a greenhouse with natural light (Aug 3, 2007)

<table>
<thead>
<tr>
<th>Variety</th>
<th>HLB</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calamondin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibration</td>
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<td>5</td>
</tr>
<tr>
<td>Validation</td>
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<tr>
<td>Total no of spectra</td>
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<td>10</td>
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<tr>
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<td>4</td>
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<tr>
<td>Correct (Narrow)</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Duncan grapefruit</td>
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<td></td>
</tr>
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<td>Calibration</td>
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</tr>
<tr>
<td>Validation</td>
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<td>5</td>
</tr>
<tr>
<td>Total no of spectra</td>
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<td>10</td>
</tr>
<tr>
<td>Correct (Full)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Correct (Narrow)</td>
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<td>5</td>
</tr>
<tr>
<td>Madam Vinous</td>
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<td></td>
</tr>
<tr>
<td>Calibration</td>
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<td>5</td>
</tr>
<tr>
<td>Validation</td>
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<td>Total no of spectra</td>
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<td>4</td>
</tr>
<tr>
<td>Mexican lime</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibration</td>
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</tr>
<tr>
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<tr>
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<td>5</td>
</tr>
<tr>
<td>Correct (Narrow)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Trifoliate Orange</td>
<td></td>
<td></td>
</tr>
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<td>Validation</td>
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<tr>
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<td>5</td>
</tr>
<tr>
<td>Sweet lime</td>
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<tr>
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<td>Correct (Narrow)</td>
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Table 4-4. Continued

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</tr>
<tr>
<td></td>
<td>Validation</td>
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</tr>
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</tr>
<tr>
<td></td>
<td>Correct (Narrow)</td>
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<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>Validation</td>
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<tr>
<td>Sunchusha</td>
<td>Total no of spectra</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Correct (Full)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Correct (Narrow)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Calibration</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Validation</td>
<td>5</td>
</tr>
<tr>
<td>Sour orange</td>
<td>Total no of spectra</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Correct (Full)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Correct (Narrow)</td>
<td>5</td>
</tr>
</tbody>
</table>

Figure 4-5. Percentage of correct classification of HLB and healthy trees in full range (400-2450 nm) and narrow NIR range (400-900 nm) on Aug 3, 2007.
The results of discriminant analysis are shown in Table 4-5. Overall, it shows that discriminant analysis can correctly classify the HLB spectra. It is more accurate at the full range than the narrow range.

Table 4-5. Number of misclassified spectra in discriminant analysis. Values given in the parenthesis are percentage of misclassified spectra in full range and narrow range.

<table>
<thead>
<tr>
<th>Date (2007)</th>
<th>Total no of spectra</th>
<th>HLB No of misclassified spectra</th>
<th>Healthy No of misclassified spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Full range</td>
<td>Narrow range</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 (0)</td>
<td>10 (20.4)</td>
</tr>
<tr>
<td>June 13</td>
<td>49</td>
<td>0 (0)</td>
<td>5 (7.8)</td>
</tr>
<tr>
<td>June 14</td>
<td>70</td>
<td>3 (4.3)</td>
<td>18 (25.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 (0)</td>
<td>18 (31.0)</td>
</tr>
<tr>
<td>Aug 2</td>
<td>40</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 (0)</td>
<td>2 (5.0)</td>
</tr>
<tr>
<td>Aug 3</td>
<td>158</td>
<td>16 (10.1)</td>
<td>54 (34.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 (8.9)</td>
<td>53 (33.5)</td>
</tr>
</tbody>
</table>

Figure 4-6 shows the canonical plot of the points and the multivariate means of healthy and HLB infected trees in the full spectral range for Aug 2, 2007. Each multivariate mean is a labeled circle. The size of circle corresponds to a 95% confidence limit for the mean. Groups that are significantly different tend to have non intersecting circles. Discriminant analysis showed that it has good potential to classify HLB.
The reason behind misclassifying healthy and HLB infected trees might be the degree of infection in HLB infected trees. Some HLB infected trees show severe infection all over the tree while some trees show symptoms in only a small portion of the tree. Thus we need to collect data very precisely with all the information about its degree of infection. If the miss classification at narrow range can be tolerated, it is quite possible to develop a low-cost rugged sensor to collect reflectance spectra at the narrow range. Such a sensor can be used in assisting the scouting process. However, a sensor that can cover the full range would have a significantly higher cost.

**Conclusions**

Partial least squares (PLS) modeling and discriminant analysis techniques identified HLB under field conditions and in a greenhouse with artificial lights. Results supports that these techniques have the potential to discriminate HLB for different types of citrus. Overall, the full range of data gave more accurate results compared to narrow range with both techniques. However, the narrow range (400 nm to 900 nm) data gave better results with PLS modeling. In contrast, discriminant analysis was better overall.
using the full spectral range. It seems that the narrow range can produce very good results if the HLB symptoms are visible, but a major goal is to detect HLB before visible symptoms appear.
CHAPTER 5
IDENTIFICATION OF CITRUS GREENING (HLB) INFECTED CITRUS TREES USING SPECTROSCOPY AND STATISTICAL CLASSIFICATION

Introduction

One of the most important diseases found in citrus in Florida is citrus greening, also known as Huanglongbing or HLB. It is a systemic bacterial disease transmitted by Asian citrus psyllids (*Diaphorina citri*). The disease causes substantial economic losses to the citrus industry by shortening the life span of infected trees. In the infected citrus orchards, trees are decimated and the productive duration of fruit-bearing is reduced. As there is no cure reported for citrus greening so far, the growers have to remove the infected trees. The elimination and removal of infected trees due to citrus canker and greening diseases contributed to the gross loss of 19,918 acres on Florida (NASS, 2009). Muraro (2007) reported that the total grower costs of fresh fruit increased from $1,657 to $2,283 per acre due to HLB.

Yellow angular blotching has been considered a symptom specific to the HLB disease and consists of blotches of yellow on dark greenish-grey leaves. By the time these symptoms are apparent, a plant can already be severely affected. Takushi et al. (2007) reported that starch content of HLB affected leaves can be 20 times higher than leaves from healthy leaves. Etxeberria et al. (2009) studied anatomical distribution of abnormally high levels of starch in HLB infected valencia orange trees. They reported phloem collapse in HLB infected leaves in addition to starch accumulation. They found multiple starch grains per chloroplast in HLB infected leaf palisade cells whereas healthy leaf chloroplasts include a small number of lipid inclusions and occasional smaller starch grains. They further reported that HLB infected leaves have corky texture due to the thicker photosynthetic cell walls.
Accurate diagnosis of HLB is essential before applying control strategies like tree removal to prevent a major outbreak. HLB diagnosis is difficult based on field observations as the symptoms bear resemblance to nutrient deficiencies such as zinc deficiency (Etzeberria et al. 2007). Electron microscopy and bioassay can be used to diagnose HLB but are time consuming and cannot be done under field conditions (Chung and Brlansky, 2005). Molecular methods like real-time polymerase chain reaction (PCR) based assays are used to detect the presence of HLB. However, identification of plants as suspect by foliar and fruit symptoms is required by a trained scouting crew prior to real-time PCR assays. The current methods being expensive, time consuming and tedious necessitate developing a rapid and reliable method to identify HLB infected trees from healthy trees.

Visible (VIS) and near infrared (NIR) spectrum of a leaf contains information on plant pigment concentration, leaf cellular structure, and leaf moisture content (Borengasser et al. 2001). Previous studies have shown that VIS-NIR spectroscopy has the potential to identify plant anomaly due to disease or malnutrition (Bravo et al. 2004; Zhang et al. 2002; Zhang et al. 2005). Smith et al. (2005) studied the plant stress caused by elevated levels of natural gas in the soil, dilute herbicide solution, and extreme shade. They found that the red edge position was strongly correlated with chlorophyll content across all the treatments. The ratio of reflectance centered on the wavelengths 670 and 560 nm was used to detect increases in red pigmentation in gas- and herbicide stressed leaves. Stress due to extreme shade could be distinguished from the stress caused by natural gas and herbicide by analyzing the change in spectral features. Liu et al. (2007) characterized and estimated rice brown spot disease severity
using stepwise regression, principal component regression, and partial least squares regression. With PCA they predicted disease severity with root mean square errors (RMSE) of 16.3% and 13.9 % for the training and testing dataset while with PLS with seven extracted factors they predicted disease severity with RMSE’s of 4.1% and 2.0% for training and testing dataset, respectively. They concluded that it was feasible to estimate the disease severity using hyperspectral reflectance from the leaves. Lee et al. (2008) used aerial hyperspectral imaging to detect HLB. They used spectral angle mapping (SAM) and spectral feature fitting (SFF) methods. They reported that it was difficult to obtain good results with SAM and SFF because of the positioning errors in GPS ground truthing and aerial imaging, and the spectral similarity between non-symptomatic HLB infected trees and healthy trees. Delalieux et al. (2007) used hyperspectral imaging and parametric approaches such as logistic regression, partial least squares, discriminant analysis and tree based modeling to detect biotic stress (Venturia inequlalis) in apple trees.

A fast method for detecting HLB in the field will assist growers to better manage and control the disease, resulting in significant production and economical benefits. The long-term goal of this study is to develop a ground-based method to detect HLB at early stages of development in the orchard. The specific objective of this study was to investigate the possibility of identifying HLB infected trees using VIS-NIR spectroscopy and to determine the best classification techniques.

**Materials and Methods**

**Field experiments**

A total of 1,239 spectra were collected from 135 (80 HLB, 55 healthy) Valencia orange trees. Table 5-1 shows detailed information on the data used in this study. The
age of all the healthy and HLB infected trees, used in this study, were 15-20 years old. Spectral data were collected using two portable spectroradiometers. The first series of data were collected with a FieldSpec® 3 spectroradiometer (Analytical Spectral Devices (ASD), Boulder, CO). The reflectance data from 350 nm to 2,500 nm were collected using the spectroradiometer and transmitted wirelessly to a laptop computer. The equipment was set up to collect 10 scans, average the scans and represent it as a single observation. Bare fiber optic cable with 25º field of view (FOV) was used for data collection. The ASD spectroradiometer has a circular field of view. The integration time was optimized using the optimization options within the software. Optimization values depend on the response to light in a particular spectral region. The bare fiber optic cable was placed at a distance of approximately 50 to 80 cm from the tree canopy, where sensor can see canopy clearly. During the data collection, the area scanned by the spectroradiometer was approximately 385 to 988 cm². The second series of data were collected using a SVC HR-1024 portable spectroradiometer (Spectra Vista Corporation, Poughkeepsie, New York). Spectral range of SVC is 350 nm - 2,500 nm with a spectral

Table 5-1. Spectral data from healthy and HLB-infected trees used in this study

<table>
<thead>
<tr>
<th>Locations in FL</th>
<th>Data collection date</th>
<th>No. of trees</th>
<th>No. of Spectra</th>
<th>Type of Spectroradiometer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Alfred</td>
<td>June 13, 2007</td>
<td>2</td>
<td>119</td>
<td>ASD</td>
</tr>
<tr>
<td>Lake Alfred</td>
<td>June 25, 2007</td>
<td>4</td>
<td>90</td>
<td>ASD</td>
</tr>
<tr>
<td>Lake Placid</td>
<td>Feb 22, 2007</td>
<td>4</td>
<td>24</td>
<td>ASD</td>
</tr>
<tr>
<td>Immokalee</td>
<td>Jan 16, 2009</td>
<td>10</td>
<td>100</td>
<td>SpectraVista</td>
</tr>
<tr>
<td>Immokalee</td>
<td>Feb 5, 2009</td>
<td>10</td>
<td>100</td>
<td>SpectraVista</td>
</tr>
<tr>
<td>Immokalee</td>
<td>Feb 27, 2009</td>
<td>10</td>
<td>100</td>
<td>SpectraVista</td>
</tr>
<tr>
<td>Southern Garden</td>
<td>Mar 24, 2009</td>
<td>40</td>
<td>200</td>
<td>SpectraVista</td>
</tr>
</tbody>
</table>
resolution of 3.5 nm (350-1000 nm), 9.5 nm (1000-1,850 nm), and 6.5 nm (1,850-2,500 nm) which was similar to the ASD spectroradiometer. The SVC HR-1024 spectroradiometer communicated with a handheld PDA through a Bluetooth. All the data were collected with 4°FOV. The FOV of the SpectraVista spectroradiometer was rectangular, which covered an area of 26.6 x 9.8 cm from a distance of 665 cm. A minimum integration time of one millisecond was used. In the field condition, scan time was set at 4 s. Laboratory tests were conducted with various objects to compare the spectral signatures of ASD and SVC HR-1024 spectroradiometers. Similar results were obtained from both spectroradiometer.

Dark current measurements were automatically taken immediately prior to the reference or target scans using the ASD and SVC spectroradiometers. The spectral data were collected from sunlit canopy between 11:00 am and 2:00 pm with no additional source of light. Spectral reflectance was collected from tree canopy from two sides of the tree to compensate for the effect of shade. Both spectroradiometers were handheld and trees were scanned by their sides. Reference readings of white panel were collected every 10 min to reduce the error in the reflectance due to the atmospheric changes. Both spectroradiometers provides relative reflectance values of the tree canopy based on the reference value.

Data Analysis

Spectral pretreatment and feature selection

The Multiplicative Scatter Correction (MSC) was performed on the NIR portion of the spectra. Then, the local average of the spectrum, and its first and second derivatives were calculated at selected wavelengths along the spectra. To ensure complete use of the information in the spectra, it was decided to compute these values
at every 25 nm along the entire spectrum, except in the noisy regions. Therefore, the local mean, and the first and the second derivatives were calculated at 25 nm intervals from 375 nm to 1,325 nm, from 1,500 nm to 1,750 nm, and from 2,050 to 2,300 nm. At each of these 61 points, the local mean was computed by averaging the spectral reflectance values for that wavelength and its four neighbors. For example, the local mean at 375 nm was computed by averaging from 373 nm to 377 nm. In order to avoid noise amplification, which results from differentiation, the Savitzky-Golay method (Orfanidis 1996) was used to compute the first and second derivatives. A quadratic polynomial and a window size of 21 were used. As three values were computed for each of the 61 points mentioned above (i.e. the mean, first and second derivatives), each spectrum was represented by a feature vector of 183 elements. Because the number of features was significantly high and many of these features could be correlated, Principal Component Analysis (PCA) was performed to reduce the number of features (Figure 5-1). The 183 spectral elements were reduced to 25 principal components (PCs) that accounted for more than to above 90% variance within the data. From the Fig. 5-1, it can be seen that the first 25 PCs contribute to about 90% variance. The data analysis described in this paper was performed in Matlab (Mathworks Inc., Natick, MA).
Classification

The principal components (25) were used as the input features for the classification algorithm. The output classes obtained from the classification models were the ‘diseased’ and ‘healthy’ trees. For the classification models, 75% of the randomized data were used for training, while 25% of the data were used for testing the classification algorithms.

Weighted K-Nearest Neighbors (KNN)

Weighted k-Nearest Neighbors (KNN) is an instance-based classification algorithm. These types of algorithms do not develop an explicit function or model for predicting the target classes, instead all the training samples are stored and computation is delayed until a new unknown sample must be classified. For a new sample, the Euclidian distance between its feature vector and the feature vectors of the training samples is computed. The computed distances are then used to find the k training samples that are closest to the unknown sample and a prediction is made.
based on these nearest neighbors. For a classification problem, this can be done simply by a majority vote. A more sophisticated approach used in this study, was to give a different weight to the contribution of each of the K-nearest neighbors in the prediction. Commonly, a weight that is inversely proportional to the square of the Euclidian distance is used (Mitchell 1997):

$$\hat{f}(x_{unknown}) = \arg \max_{v \in \mathcal{V}} \sum_{i=1}^{k} w_i \delta(v, f(x^i))$$

(1)

Here, $$w_i = \frac{1}{\sum_{j=1}^{n}(x^j_{unknown} - x^i_j)^2}$$

(2)

In these equations, $$\hat{f}$$ indicates the predicted class for the new unknown feature vector $$x_{unknown}$$, $$x^i_j$$ denotes the $$j^{th}$$ element of the $$i^{th}$$ feature vector, $$w_i$$ are the weights, and the function $$\delta$$ is defined as follows:

$$\delta(a, b) = \begin{cases} -1, & \text{if } a = b \\ 0, & \text{otherwise} \end{cases}$$

(3)

A k of ‘12’ was used in this study based on some preliminary analysis of the data.

**Logistic Regression (LR)**

Logistic regression provides a model for the probability of occurrence of an event by fitting the data to the logistic curve. As shown in Fig. 5-2, this curve maps the entire real axis onto the interval [0, 1], making it ideal for modeling the probability of an event. When used in classification, the variable $$z$$ is usually defined as a linear combination of the features (Witten and Frank 2005):

$$g_\theta(x) = f(\theta^T x) = \frac{1}{1 + e^{-\theta^T x}}$$

(4)
In this equation, $\theta$ is the parameter vector, $x$ is the feature vector, and $f(t) = 1$ if $t \geq 0$ and $f(t) = 0$ if $t < 0$. A batch gradient descent approach was used for training the model, as shown in the following pseudocode:

$$
\theta_{j+1} = \theta_j + \varepsilon \sum_{i=1}^{N} x_i^j (y_i - g_\theta(x_i^j))
$$

This iteration continued for all $j$ till the gradient converges to local minima. Here, $N$ is the number of features, $\varepsilon$ is the learning rate, and $y$ is the true label (either 1 or 0). A sufficiently small $\varepsilon$ will ensure that the global optimum will be achieved, although the computation time will increase by decreasing the value of $\varepsilon$. In this study a value of $\varepsilon = 0.001$ was used.

**Support Vector Machines (SVM)**

Support Vector Machines (SVM) is one of the most successful machine learning algorithms that is widely used in various fields. The basic SVM solves a classification problem with only two target classes. However, it can be generalized to solve problems
that involve more than two classes. For a linearly separable data, such as the data that is shown in Fig. 5-3, one can draw many different hyperplanes that can separate the data. The idea in SVM, however, is to find a hyperplane that separates the data with the largest possible margin as shown in Fig. 5-3b. SVM uses numeric labels 1 and -1 to identify the two classes. In this paper, the following notation will be used to show the SVM classifier (Webb, 2002):

\[ y = g_w(x) = f(w^T x + w_0) \]  

(6)

Figure 5-3. A) An example of a linearly separable set of data, and B) the maximum margin classifier for this data set.

The parameters \( w \) and \( w_0 \) define the decision boundary (i.e., the separating hyperplane). The intercept \( w_0 \) is a scalar, while \( w \) and the feature vector \( x \) are n-dimensional vectors. In this equation, \( f(t) = 1 \) if \( t \geq 0 \), and \( f(t) = -1 \) if \( t < 0 \). The equation defining the separating hyperplane is as follows:

\[ w^T x + w_0 = 0 \]  

(7)
The geometric margin of the $i^{th}$ example from the separating hyperplane can be calculated using the following equation:

$$D^i = y^i \left[ \frac{w^T x^i + w_0}{\|w\|} \right]$$

(8)

Here, a positive $D^i$ would mean that $y^i$ and $w^T x + b$ are of the same sign, or equivalently, the $i^{th}$ example is correctly classified. The geometric margin of the classifier with respect to the set of all training samples, $\{(x^1, y^1), \ldots, (x^N, y^N)\}$, is defined as the minimum of the geometric margins for each sample:

$$D = \min_{i=1:N} D^i$$

(9)

Therefore, the goal of SVM is to find a separating hyperplane that maximizes $D$. In mathematical terms, SVM will pose the following optimization problem:

$$\max_{w, \ w_0} D$$

$$\text{Such that} \left\{ \left( y^i (w^T x^i + w_0) \right) \geq D, i = 1, \ldots, N \right\} \quad \|w\| = 1$$

(10)

The second constraint $\|w\| = 1$, is a non-convex constraint and this optimization problem cannot be directly solved. It can be shown (Gunn 1998) that this optimization problem can be replaced by the following equivalent problem:

$$\min_{w, \ w_0} D$$

$$\left( y^i (w^T x^i + w_0) \right) \geq D, i = 1, \ldots, N$$

(11)
In the above equation, \( y^i (w^T x^i + w_0) \) is called the functional margin and is shown by \( d^i \). The difference between functional margin and the geometric margin is that, multiplying \( w \) and \( w_0 \) by a constant will not change the geometric margin but will change the functional margin. Functional and geometric margins are related as follows:

\[
D^i = \frac{d^i}{\|w\|}
\]  

Equation 11 means that the optimal margin classifier sought by SVM can be found by minimizing the norm of \( w \), under the constraint that the functional margin for all examples is at least equal to 1. It is easy to see that the minimum margin always belongs to the points that are on the edge of the widest strip that separates the data as shown in Fig. 5-3b. Only for these points \( y^i (w^T x^i + w_0) = 1 \); for the remaining points, \( y^i (w^T x^i + w_0) > 1 \). These points are called the “support vectors” and usually comprise a very small fraction of the total number of points, resulting in huge reduction in the computational cost.

Although the optimization problem in Eq. 11 can be solved by commercially available quadratic programming code, an easier equivalent problem can be found by using the method of Lagrange multipliers (Strang, 1991). Using this method, the following dual optimization problem will be obtained:

\[
\max_{\alpha} \sum_{i=1}^{N} \alpha_i - \frac{1}{2} \sum_{i,j=1}^{N} y^i y^j \alpha_i \alpha_j (x^i, x^j)
\]

such that \( \{\alpha_i \geq 0, i = 1, \ldots, N\} \)

\[
\sum_{i=1}^{N} \alpha_i y^i = 0
\]
In this equation, $\alpha_i$s are the Lagrange multipliers and $(x^i, x^j)$ indicates the inner product between the $i^{th}$ and $j^{th}$ feature vectors. The interesting point is that all of the Lagrange multipliers are zero except for the ones that correspond to the support vectors. Once the above optimization problem is solved, $w$ and $w_0$ can be found using the following formulas:

$$
\begin{align*}
W &= \sum_{i=1}^{N} \alpha_i y^i x^i \\
W_0 &= \max_{i} y^i \min_{y^j} \frac{w^T x^i + w^T x^j}{2}
\end{align*}
$$

It is important to note that the new optimization problem in Eq. 13 is only in terms of the inner products of the feature vectors. Moreover, with the new definition of $w$ in terms of Lagrange multipliers (Eq. 14), for a new example $x$, the prediction of the SVM classifier can be written as follows:

$$
y = g_w(x) = f \left[ \sum_{i=1}^{N} \alpha_i y^i (x, x^i) + w_0 \right]
$$

In other words, the entire algorithm can be written in terms of the inner product of feature vectors. In many practical applications, the data are not linearly separable or, even if it is, the optimal margin classifier described so far may not be the best choice because it can be very sensitive to outliers. Therefore, a modified version of SVM problem is defined as follows (Webb, 2002):

$$
\min_{w, w_0} \frac{1}{2} \|w\|^2 + C \sum_{i=1}^{N} \xi_i
$$
such that \[ \begin{cases} y^i (w^T x^i + w_0) \geq 1 - \xi_i, \\ \xi_i \geq 0, \quad i = 1, \ldots, N \end{cases} \]

With this definition, some of the examples are allowed to fall on the wrong side of the separating hyperplane. However, for each such example, a cost, \( \xi_i \), will be considered. The parameter \( C \) determines the importance that we attach to these errors. Once again, the method of Lagrange multipliers can convert this problem into an easier one. The resulting problem will be as follows:

\[
\max_{\alpha} \sum_{i=1}^{N} \alpha_i - \frac{1}{2} \sum_{i,j=1}^{N} y^i y^j \alpha_i \alpha_j (x^i, x^j)
\]

\[
\text{such that } \begin{cases} C \geq \alpha_i \geq 0, \quad i = 1, \ldots, N \\ \sum_{i=1}^{N} \alpha_i y^i = 0 \end{cases}
\]

This is the same as the previous optimization problem in Eq. 12, except that the first constraint has changed from \( \alpha_i \geq 0 \) to \( C \geq \alpha_i \geq 0 \). Although the SVM algorithm described so far is already a very powerful method, significant improvements would result by introducing kernels. The idea is to map the feature vector \( x^i \) into a higher dimensional space using a mapping function \( \Phi(x^i) \). As mentioned before, the entire SVM algorithm can be written in terms of the inner products of the feature vectors. After mapping feature vectors using \( \Phi \), all instances of the inner products of the feature vectors \( (x^i, x^j) \) will be replaced by the inner product of the corresponding mapped feature vectors, \( (\Phi(x^i), \Phi(x^j)) \). This inner product is called a kernel:

\[
K(x^i, x^j) = \Phi(x^i), \Phi(x^j)
\]
The idea of kernels significantly improves the SVM technique in terms of both its accuracy and the scope of problems that it can solve. Various different kernels have been introduced and used in practice (Cherkassky and Mulier 2007). Following the suggestion of (Hsu et al. 2008), a Gaussian kernel was used in this study:

\[ K(x^i, x^j) = \exp\left(\gamma \|x^i - x^j\|^2\right) \]  

(19)

Therefore, the SVM problem that was solved in this study was of the following form:

\[
\max_{\alpha} \sum_{i=1}^{N} \alpha_i - \frac{1}{2} \sum_{i,j=1}^{N} y^i y^j \alpha_i \alpha_j K(x^i, x^j)
\]

such that \[
\begin{cases}
C \geq \alpha_i \geq 0, \quad i = 1, \ldots, N \\
\sum_{i=1}^{N} \alpha_i y^i = 0
\end{cases}
\]

(20)

where: \[K(x^i, x^j) = \exp\left(\gamma \|x^i - x^j\|^2\right)\]

To solve this problem, the sequential minimal optimization (SMO) algorithm was used. This algorithm solves the above optimization problem by a coordinate ascent approach. Because of the second constraint in the problem, i.e. \[\sum_{i=1}^{N} \alpha_i y^i = 0\], it is not possible to change only one of the Lagrange multipliers. The SMO algorithm optimizes the function with respect to two Lagrange multipliers simultaneously. The advantage of this approach is that the innermost loop is very fast compared to other algorithms (Platt, 1998).
The parameters $C$ and $\gamma$ in the problem are unknown. Therefore, the first step in solving the problem is to find the optimum values for these parameters. The grid search procedure suggested by Hsu et al. (2008) was used for this purpose. First, the approximate values of the optimum $C$ and $\gamma$ were found on a coarse grid with $C = 2^{-8}, 2^{-4}, \ldots, 2^{30}$ and $\gamma = 2^{-14}, 2^{-10}, \ldots, 2^{24}$. After finding the approximate values for the best $C$ and $\gamma$, a finer grid was defined to search for more accurate values of the optimum $C$ and $\gamma$.

**Reducing the Classification Error by Using Multiple Measurements**

The goal of the present study was to develop classification algorithms that can be used for automatic detection of HLB infected citrus trees in the field. The preliminary results indicated that the classification accuracy from the statistical methods to classify HLB infected trees were low when data with single measurements were considered for analysis. This could be due to variability in experimental conditions such as the amount of sunlight and the orientation of the leaves with respect to the sensor. Therefore, it was hypothesized that high detection accuracy can be achieved through multiple measurements on a single tree. In this study, we investigated the accuracy of classification methods (KNN, LR, and SVM methods) using spectral data from one, three, or five measurements from different canopy areas of the same tree.

When more than one measurement was presented to the classifier, a simple method based on majority voting can be used to determine the classification group. The tree is labeled as HLB infected tree when more than half of the measurements from that tree are classified as HLB infected. An improved approach is to consider the confidence of the classifier for its prediction on each of the measurements. For example, if a
classifier predicts “infected” on two measurements out of five measurements with very high confidence and “healthy” on the three remaining measurements with very low confidence, it would be more reasonable to classify the tree as “infected”. Our analysis was based on the majority with confidence of the classifier.

For the KNN algorithm, the following equation was used to estimate the confidence of each prediction:

$$KNN_{confidence} = \frac{\sum w_i'}{\sum w_i}$$

(21)

$w_i$ is already defined in Eq. 2. For the LR method, Here $w_i'$ is for those among the K nearest neighbors that have the same class as the predicted class and $w_i$ is for all the K nearest neighbors. The value of the function $g_\theta(x)$ in Eq. 4 can be used to calculate a measure of confidence in prediction. The closer $g_\theta(x)$ is to either 0 or 1, the more confident is the prediction. Therefore, the value $|g_\theta(x) - 0.5|$ was used to evaluate the confidence of the predictions by LR method:

$$LR_{confidence} = |g_\theta(x) - 0.5|$$

(22)

For the SVM classifier, the value of the function $g_w(x)$ in Eq. 15 can be used as a measure of confidence in prediction. The larger the absolute value of this function, the more confident the prediction. Therefore, $|g_w(x)|$ was used to evaluate the confidence of predictions by the SVM method:

$$SVM_{confidence} = |g_w(x)|$$

(23)
Results and Discussion

Figure 5-4 shows samples of the collected spectra from both healthy and HLB trees. Each observation is an average of ten measurements. Noise was observed in bands 1350 to 1500 nm, 1750 to 1950 nm and bands after 2350 nm due to the presence of atmospheric moisture.

For the SVM method, the first step was to find the optimum values for $C$ and $\gamma$. As mentioned before, this was performed by a grid search. Figure 5-5 shows typical contour graphs that were used to find the optimum parameter values. First, the classification error was evaluated on a coarse grid (Figure 5-5a) to find approximate estimates for $C$ and $\gamma$. Then, a finer grid was defined (Figure 5-5b) and used to determine accurate values of $C$ and $\gamma$ that minimized the classification error. In the
specific case shown in the Table 5-2, the lowest classification error was approximately 18% which was obtained for $C = 28$ and $\gamma = 34$.

![Figure 5-5. Contour plots of classification error for finding the optimum values for the parameters $C$ and $\gamma$.](image)

Analysis showed that MSC correction of the spectra did not improve the classification accuracy. In other words, when MSC was removed as a pretreatment procedure described in the data analysis section of the paper, the classification error did not improve for any of the three classification techniques.

Table 5-2 shows the classification error for each of the three classification techniques without MSC correction. The table shows the percent of misclassified citrus trees when one, three, or five spectral measurements from each tree were used for classification.

<table>
<thead>
<tr>
<th>Classification method</th>
<th>Error with one spectrum</th>
<th>Error with three spectra</th>
<th>Error with five spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weighted KNN</td>
<td>23%</td>
<td>11%</td>
<td>6.5%</td>
</tr>
<tr>
<td>Logistic regression</td>
<td>35%</td>
<td>23%</td>
<td>19%</td>
</tr>
<tr>
<td>SVM</td>
<td>18%</td>
<td>6.2%</td>
<td>3.0%</td>
</tr>
</tbody>
</table>

It can be seen from Table 5-2 that the classification error with a single spectrum was relatively large. However, when three or five spectra from the same tree were used, the classification error significantly decreased. The SVM method demonstrated lower
classification errors than other two methods, especially with five spectra. The classifiers obtained by weighted KNN, logistic regression, and SVM algorithms can be easily programmed on a microcontroller. The three algorithms were also very different in terms of computation time. For SVM based classification, the most time consuming step was to find the optimum values for $C$ and $\gamma$. The grid search method for optimal value selection used in this study took several hours on a PC. Although there are faster methods for finding $C$ and $\gamma$ (Hsu et al. 2008), this step is time consuming. The batch gradient descent algorithm described previously for logistic regression method took approximately 12 s to complete on a PC with a 4.8 GHz processor and 512 MB of RAM. As mentioned before, the KNN method does not develop any model from the data. Therefore, for KNN algorithm there is no computation until a new spectrum is to be classified. Once the model (i.e., the classifier) is obtained, however, the computation time for classification of a new spectrum is very fast for SVM and logistic regression. On the same PC as mentioned above, the time to make a prediction based on three spectra from the same tree was only 0.06 ms for logistic regression classifier, whereas for weighted KNN and SVM methods, this time was 5.5 ms and 5.0 ms, respectively. Therefore, the logistic regression method was computationally much faster. As the KNN and SVM based algorithms yielded better classification accuracy than logistic regression, it would be considered as a more preferable algorithm for HLB detection in citrus groves.

**Conclusion**

The goal of this study was to develop a technique for rapid detection of HLB infected citrus trees. Canopy reflectance spectra were measured from the infected and
healthy trees using a spectroradiometer, and three common classification algorithms were used to classify the infected trees from the healthy ones. The results indicated that a single measurement was insufficient for accurate detection of the infected trees. The classification error was between 18% and 35% using a spectrum. However, using multiple spectral measurements from a single tree, the classification accuracy increased significantly. SVM method showed an accuracy of higher than 97% when it was provided with five spectra from the same tree. Under real field conditions, varying sunlight and other environmental factors can produce noise that might reduce the classification accuracy. Under these conditions, multiple measurements will be necessary to ensure acceptable classification accuracy.
CHAPTER 6
AN ACTIVE OPTIC SENSOR FOR DETECTION OF HUANLONGBING (HLB) DISEASE

Introduction

Florida accounts for 70 percent of total U.S. citrus production and produced 7,236 tons of citrus from 224,358 hectare (554,400 acre) of bearing orchards in 2006-2007 (FASS, 2008). Huanglongbing (HLB), also known as greening, a systemic bacterial disease transmitted by the Asian citrus psyllid (Diaphorina citri), is considered one of the most devastating citrus diseases in the world. Since HLB is a relatively new disease in the USA, very little published information is available on its dynamics, epidemiology, and molecular characteristics.

Figures 6-1 and 6-2 show leaves of HLB infected and healthy tree, respectively.

Figure 6-1. HLB symptomatic
Figure 6-2. Healthy leaves

Reflectance spectra of vegetation, measured in the visible and infrared regions, contain information on plant pigment concentration, leaf cellular structure, and leaf moisture content (Borengasser et al., 2001). A multi-band sensor measures reflected radiation at specific wavebands. Malthus and Madeira (1993) studied the spectral reflectance of field bean leaves attacked by *Botrytis fabae*. They found the most significant changes in spectral reflectance due to *Botrytis fabae* were flattening of the response in the visible region and a decrease in the near infrared reflectance, around 800 nm. Correlation between percentage infection and reflectance in the visible region (peaks at 525 nm and 589 nm) were higher in the first order derivative spectra than for the original reflectance spectra (zero order). The study indicated the potential for using spectral information for disease detection. Such techniques can be used to identify HLB in citrus trees. However, very little is known about the spectral characteristics of HLB.
infected leaves in the visible and near infrared (NIR) regions. This band-specific information could discriminate HLB infection in the citrus grove.

Currently, the detection of HLB relies on scouting groves for visible symptoms and following up with off-site diagnosis of the disease using the polymerase chain reaction (PCR) technique. HLB infection increases the amount of starch accumulation in leaves (Schneider, 1968). Therefore, on immersing the HLB infected leaves in iodine solution for one or two minutes, the leaves stain very dark grey to black along cut surfaces while healthy citrus leaves show no or very little staining (Etxeberria et al., 2007). However, this procedure is too slow for testing of every tree. Infected trees are removed and insecticides are sprayed to control the population of Asian citrus psyllids. Scouting is laborious, time consuming, often subjective, and prone to errors. Knowledgeable growers in Brazil estimate that at least 50% of infected trees with visible symptoms go undetected by trained scouts. Similar results, even if using an observation platform, have been reported in Florida (Futch et al., 2009).

Rapid, early, and accurate diagnosis, especially at the orchard level, is essential to eliminate the disease at early stages of infection. Non-uniform distribution of disease organisms complicates the tests that are based on detecting the causal agent or visual symptoms. In the case of HLB, it has been shown that high incidence of Candidatus Liberibacter asiaticus (CaLas) in psyllids can be found in an area well before symptomatic plants are found (Manjunath et al., 2008). Presently, there is a need for rapid detection techniques to prevent the spread of disease. The present work evaluates a spectroscopic method for early detection of HLB infected trees. The specific
objective of this study was to develop and evaluate a multi-band active optic sensor for detecting HLB-infected citrus trees under field conditions.

**Material and Methods**

**Data Collection with Multi Band Sensor**

The sensor was composed of four narrow-band (active optic) light sources with four different wavelengths; two in the visible region (at 570 nm and 670 nm) and two in the near infrared region (at 870 nm and 970 nm), with accuracy of 1% reflectance. All the four bands have epoxy lens type illuminator manufactures by Marubeni America Corporation (Santa Clara, CA 95054). These illuminators are wide viewing and extremely high output power illuminators assembled with a total of 60 high efficiency aluminum gallium arsenide (AlGaAs) diode chips, mounted on metal stem TO-66 with aluminum nitride (AlN) ceramics and covered with double-coated clear silicone and epoxy resin. Sampling frequency was set at 10 Hz and that was also the averaging time. It was not influenced by ambient conditions. All the four detectors were arranged to view same area of target. Vibrating leaves or moving while sampling, the ambient conditions may vary and have very low frequency content. Sunlight of course even with clouds will have only a fraction of 1 Hz. The ambient conditions then are essentially negligible in their effect. Only the original transmitted light signal is passed for measurement. Design of this sensor eliminates the need for dark operation by using modulated illumination. The sensor was calibrated by measuring irradiance over a BaSO4 coated “white” plate with the sensor at 1 meter from the plate. This material has a reflectance of approximately 0.98 in the four selected bands. The sensor also incorporates compensation for temperature and variations in the supply voltage. These effects are measured by detecting the level of light output from the LED illuminators and
the reflected irradiance is adjusted accordingly. The sensor automatically generated the
values of reflectance for each of the four wavelengths. The sensor was interfaced with a
hand-held personal digital assistant (PDA) through a serial port. The PDA saved the
reflectance data and the internal temperature of the unit at the time of reading. A similar
sensor was used in plant physiology studies at International Maize and Wheat
Improvement Center (CIMMYT) in Mexico. Stone et al. (2010) detected nitrogen status
in winter wheat using similar sensor. The general design of this sensor is identical to the
Greenseeker® manufactured by the NTech division of Trimble Navigation Inc. (Stone et
al., 2003). Figures 6-3 and 6-4 depicts the four-band sensor used in this study.

The spectral data from the multi band sensor were collected in a grove at Fort
Basinger (longitude 08220.5248 W, 2721.9617 N), Immokalee (longitude 08126.5901
W, 2628.0339 N), and a third location (longitude 8126.5915 W, 2628.0241 N) near
Clewiston in Florida. Valencia orange trees were measured in Immokalee and
Clewiston, and Mid-sweet orange trees were evaluated in Fort Basinger. A total of 10
trees (5 HLB, 5 healthy) in Fort Basinger, 10 trees in Immokalee (10 HLB), and 58 trees
(37 HLB, 21 healthy) near Clewiston in Florida were evaluated in the data analysis.
Since there were no healthy trees of Valencia available in the grove at Immokalee, 10
healthy trees of Valencia near Lake Alfred were evaluated. Ten readings from both HLB
symptomatic and healthy tree leaves were collected from different locations in the same
tree. For the HLB infected trees the data collected from the same branch that was
confirmed HLB positive by the PCR test. These data were collected in Fort Basinger
during fall 2008 and in Immokalee and Clewiston during spring 2009.
The four-band sensor was build from Applied Technology (Stillwater, OK). The sensor was composed of four narrow-band (active optic) light sources with four different wavelengths: two in the visible region (at 570 nm and 670 nm) and two in the near infrared region (at 870 nm and 970 nm). The band width for each spectral band was about 50 nm. After lighting the target, the reflected light was captured by a receiver located in the center of the device. The sensor automatically generated the values of reflectance for each of the four wavelengths. The sensor was interfaced with a hand-held personal digital assistant (PDA) through a serial port. The PDA saved the reflectance data and the internal temperature of the unit at the time of data collection. Figures 6-3 and 6-4 depict the multi-band sensor used in this study. Measurements of reflectance were made at a distance of about one meter between the sensor and the target with the sunlight intensity below 900 lux. Based on the preliminarily tests, it was determined that these were the best conditions for acquiring spectral readings with the multi-band sensor.
Figure 6-3. Multi-band active optic sensor

Figure 6-4. Field measurements using the four-band sensor
Data Analysis

Each measurement was composed of four reflectance values at 570, 670, 870, and 970 nm. Using these values, 11 different vegetation indices were computed. Table 6-1 shows the details of the vegetation indices computed in this study.

Table 6-1. List of vegetation indices used in analysis.

<table>
<thead>
<tr>
<th>Vegetation Index (VI)</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normalized Difference Vegetation Index (NDVI₁), Rouse et al. (1974)</td>
<td>( NVDI_{870} = \frac{R_{870} - R_{670}}{R_{870} + R_{670}} )</td>
</tr>
<tr>
<td>NDVI₂</td>
<td>( NVDI_{970} = \frac{R_{970} - R_{670}}{R_{970} + R_{670}} )</td>
</tr>
<tr>
<td>Simple Ratio Index (SR₁), Rouse et al. (1974)</td>
<td>( SR_{870} = \frac{R_{870}}{R_{670}} )</td>
</tr>
<tr>
<td>Simple Ratio Index (SR₂)</td>
<td>( SR_{970} = \frac{R_{970}}{R_{670}} )</td>
</tr>
<tr>
<td>Modified Triangular Vegetation Index (MTVI₁), Haboudane et al. (2004)</td>
<td>( MTVI_1 = 1.2[1.2(R_{870} - R_{570}) - 2.5(R_{670} - R_{570})] )</td>
</tr>
<tr>
<td>Modified Triangular Vegetation Index (MTVI₂), Haboudane et al. (2004)</td>
<td>( MTVI_2 = \frac{1.5[1.2 \times (R_{870} - R_{570}) - 2.5 \times (R_{670} - R_{570})]}{\sqrt{(2 \times R_{870} + 1)^2 - (6 \times R_{870} - 5 \times \sqrt{R_{670}} - 0.5)}} )</td>
</tr>
<tr>
<td>Renormalized Difference Vegetation Index (RDVI), Rougean and Breon (1995)</td>
<td>( RDVI = \frac{(R_{870} - R_{670})}{\sqrt{(R_{870} + R_{670})}} )</td>
</tr>
<tr>
<td>Greenness Index (G)</td>
<td>( G = \frac{R_{550}}{R_{670}} )</td>
</tr>
<tr>
<td>Triangular Veg. Index (TVI), Broge and Leblanc (2000)</td>
<td>( TVI = 0.5 \times [120 \times (R_{870} - R_{570}) - 200 \times (R_{670} - R_{570})] )</td>
</tr>
<tr>
<td>Modified Chlorophyll Absorption in Reflectance Index (MCARI₁), Haboudane et al. (2004)</td>
<td>( MCARI_1 = 1.2 \times [(2.5 \times R_{870} - R_{670}) - 1.3 \times (R_{870} - R_{570})] )</td>
</tr>
<tr>
<td>Structure Intensive Pigment Index (SIPI), Zacro-Tejada (2000)</td>
<td>( SIPI = \frac{(R_{870} - R_{570})}{(R_{870} + R_{670})} )</td>
</tr>
</tbody>
</table>

Outliers in the data were detected and removed prior to the classification of diseased and healthy trees. This was done by performing a principal component analysis (PCA) on the data and plotting the first four principal components. Among a
total of 1552 measurements, 20 measurements were recognized as outliers (larger than 3 standard deviation) from PCA analysis and removed. After the removal of outlier, the four reflectance values (raw data) and the vegetation indices were used for the classification. Five different classification techniques were applied to the data. In the following paragraphs, these techniques are briefly described.

**Decision Trees**

The iterative dichotomiser 3 (ID3) algorithm (Coppin, 2004) was used to build decision trees. The number of decision tree layers was seven. This number was selected based on some preliminary analysis to find the optimum number of layers for best results, i.e. to avoid over fitting. No pruning was performed on the trees. Because decision trees are inherently unstable classifiers, it was decided to develop an ensemble of decision trees using a stacking scheme (Polikar, 2006). More specifically, first 20 decision trees were built, and then another decision tree was used to learn the output pattern of these decision trees.

**k-Nearest Neighbors (KNN)**

A weighed k-nearest neighbor scheme was used, with weights inversely proportional to the square of Euclidian distance (Fukunaga, 1990). In our analysis, k was chosen as 25 based on the preliminary analysis.

**Logistic Regression**

In this method, the goal is to adjust the parameters ($\theta$) of the logistic curve in order to best fit the curve to the training data (Larose, 2006).

$$f_{\theta}(x) = \frac{1}{1 + e^{-\theta^T x}}$$  \hspace{1cm} (1)
A batch gradient descent method was used to find the optimum values for the parameter \( \theta \).

**Neural Networks**

A feed forward neural network with a single hidden layer containing 20 neurons and 2 output neurons (equal to the number of classes, i.e. healthy and HLB infected) were used. Sigmoid transfer functions were used in the hidden and output layers. The scaled conjugate gradient method was used to train the network (Haykin, 1998).

**Support Vector Machines (SVM)**

This technique aims to find the hyperplane that separates the data with the largest possible margin. In this study a modified SVM method (Webb 2002) with a Gaussian kernel was used. This will lead to an optimization problem of the following form:

\[
\max_{\alpha} \sum_{i=1}^{N} \alpha_i - \frac{1}{2} \sum_{i,j=1}^{N} y^i y^j \alpha_i \alpha_j (x^i, x^j) \tag{2}
\]

Such that \( \alpha_i \geq 0, 1= 1,\ldots, N \)

\[
\sum_{i=1}^{N} \alpha_i y^i = 0 \tag{3}
\]

Where, \( K(x^i, x^j) = \exp \left[ y \| x^i - x^j \|^2 \right] \) \tag{4}

On solving this optimization problem, the optimal margin classifier will have the following form:

\[
y = f \left( \sum_{i=1}^{N} \alpha_i y^i (x, x^i) + w_0 \right) \tag{5}
\]

where: \( w = \sum_{i=1}^{N} \alpha_i y^i x^i \) \tag{6}

\[
w_0 = \max_{y^i = 1} \min_{y^j = -1} \frac{w^T x^i + \min_{y^j = 1} w^T x^j}{2} \tag{7}
\]
In equation 5, \( f(t) = 1 \) if \( t \geq 0 \), and \( f(t) = -1 \) if \( t < 0 \), where labels “1” and “-1” represent the two classes that the classifier separates. In this study, we used the sequential minimal optimization (SMO) algorithm (Platt 1998) to solve this problem.

**Results and Discussion**

Table 6-2 shows the results for the five classification methods used in this study. For all the models, 75% of the data was used for training, while 25% of the data was used for testing. (Haykin, 1998). The Table 6-2 shows the classification error for each of the classification methods when only one measurement was presented to the classifier in the testing phase. The misclassification errors were between 17% and 40%. Because the classification errors were high, it was decided to evaluate the performance of the classifiers with more than one measurement as input in the testing phase. Table 6-2 shows the classification error when three and five measurements from the same tree were used as an input to the classifier. These multiple measurements were taken from the different locations of the same tree. In this case, the final class label (i.e. “HLB-infected” or “healthy”) was selected on a majority basis, i.e. if the classifier labeled more than half of the measurements as “HLB-infected”, then the final prediction was also “HLB-infected”; otherwise the final prediction was “healthy”. The classification error decreased significantly with multiple measurements. This is due to large variability in the field measurements caused by environmental factors (such as the orientation of the leaves with respect to the sensor or the wind) and by the human operator (such as the non-constant distance between the sensor and the leaves). Using multiple measurements eliminates these sources of noise and allows higher classification accuracies. Decision trees, SVM, and KNN achieved an accuracy of higher than 95% with five measurements from each tree.
Table 6-2. Average classification error for different classification techniques.

<table>
<thead>
<tr>
<th></th>
<th>Neural networks</th>
<th>Logistic regression</th>
<th>k-nearest neighbors</th>
<th>Support vector machines</th>
<th>Decision trees</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error with one set of measurements (%)</td>
<td>25</td>
<td>40</td>
<td>18</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Error with three sets of measurements (%)</td>
<td>16</td>
<td>35</td>
<td>8.5</td>
<td>7.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Error with five sets of measurements (%)</td>
<td>10</td>
<td>32</td>
<td>4.5</td>
<td>3.5</td>
<td>4.5</td>
</tr>
</tbody>
</table>

A closer examination of the decision tree classifiers reveals important information regarding the power of each of the vegetation indices in separating the healthy and infected leaves. Investigation of ten different decision trees showed that the top-level (i.e. root) node always tested on the RDVI index. This means that if we seek to separate the data into healthy and HLB-infected classes by testing on only one variable, RDVI would be the best choice. This result is in accordance to Roujean and Breon (1995) findings. They observed NDVI was less affected by spectral and view geometry and DVI was less affected by soil background. Therefore, they combined these two indices and introduced RDVI which minimizes the effect of soil background as well as the view geometry. Other vegetation indices that were frequently used by the decision trees, especially in the upper layers, include NVDI$_{970}$, SR$_{970}$, MTVI$_2$, and MCARI$_1$. On the other hand, SR$_{870}$ and SIPI were not used much in the decision trees, which indicate that these indices contain little or no information regarding HLB infection. Among the original reflectance values, the two values in the NIR region (i.e. 870 and 970 nm) were used much more frequently than the ones in the visible range (i.e. 570 and 670 nm). This signifies that the canopy reflectance in the NIR region contains more information with respect to HLB infection compared to the visible range.
Conclusions

The results indicated that the multi-band optic sensor used in this study has a very good potential for detecting HLB infected citrus trees under field conditions. However, to obtain high classification accuracy, it is necessary to acquire multiple measurements from a single tree. The sensor can be integrated with the scouting practice, to improve the effectiveness in HLB disease detection. Obtaining multiple readings using this sensor is easy and fast, and can be performed by a human or an automated vehicle system. The measurements in this study were performed on Valencia and mid sweet orange cultivars. It would be interesting and useful to know the performance of the sensor and the classification algorithms on other orange cultivars or other citrus types.
CHAPTER 7
APPLICATION OF HYPERSPECTRAL IMAGING FOR THE DETECTION OF HLB IN THE FIELD

Introduction

Citrus is one of the most important agricultural products in Florida as it is the largest citrus producing state in United States. Citrus production being a multi-million dollar industry accounts significantly for Florida’s agricultural economy (NASS 2010). But recently, it has been threatened by Huanglongbing (HLB), a devastating and rapidly spreading disease of citrus.

It was first confirmed in Florida in August 2005 (Halbert, 2005; Bouffard, 2006). Asian Citrus Psyllid, *Diaphorina citri*, is the vector of citrus greening or HLB. The bacteria are restricted to the sieve tubes of infected plants, and are acquired and transmitted by nymphs and adults of Asian citrus psyllid during feeding (Garnier and Bové, 1983). Psyllids prefer feeding and breeding on younger leaves (Halbert and Manjunath, 2004) so younger trees are at higher risk of infection as they produce newer leaves and flushes throughout the year. Symptoms of HLB infected citrus include a blotchy mottle or asymmetrical chlorosis, and yellowing of leaf veins due to inefficient production of chlorophyll (Brlansky et al., 2007). The fruits from HLB infected trees fall prematurely.

Hyperspectral imagery offers a better solution for the early detection plant diseases. In the hyperspectral imaging, the spectral reflectance of each pixel is acquired for a range of wavelengths in the electromagnetic spectra. Application of hyperspectral images in NIR- VIS region (450-930 nm) was investigated to detect citrus canker and other damages in ruby red grapefruit. The authors reported 96% classification accuracy.
in determining disease and damaged fruits. Early detection of yellow rust disease in winter wheat was investigated by Bravo et al. (2003) by using hyperspectral imaging. The authors reported 92-98% classification accuracy in discriminating diseased plants. Application of hyperspectral imaging was used by Safri and Hamdan (2009) to detect ganoderma basal stem rot disease in oil palm trees. They used various vegetation indices and red edge techniques to identify the infected trees. Conventional spectrometers or spectrophotometers measure optical spectra from a specific field-of-view that is often restricted spatially. Therefore, spectral data collection from tree crops such as citrus would require measurement of the spectrum at several spatial locations. This problem can be overcome using a hyper-spectral camera. The hyper-spectral camera scans a scene one line at a time and disperses light to its spectral components in each pixel in the line. Hyperspectral camera captures the line image of the tree and disperses it to a spectrum. Thus, each image frame includes the line pixels in one direction (spatial axis) and spectral distribution (light intensities in spectral elements) in another dimension (spectral axis), as illustrated in Figure 7-1.
Material and Methods

Data collection

Hyperspectral images were collected from the grove at Fort Basinger (27° 20' 29.826" N, 81° 10' 57.528" W), Southwest Florida Research and Education Center (SWFREC) at Immokalee (26° 27' 59.004" N, 81° 26' 34.830" W), and a grove near Clewiston (26° 28' 1.446" N, 81° 26' 35.490" W), Florida. Figure 7-2 shows the location of the study areas. Valencia was evaluated in this study. These data were collected in Fort Basinger during Fall 2008 and in Immokalee and Clewiston during Spring 2009.
Images were collected with a Specim hyperspectral camera (Autovision Inc., Los Angeles, CA, USA) (Figures 7-3 and 7-4) having a spectral range from 306.5 nm to 1067.1 nm with 2.7 nm spectral resolution. SpectralCube spectral imaging software (version 2.7) provided by Specim, Ltd. and AutoVision Inc. was used for capturing hyperspectral images. The windows based SpectralCube application conducts data collections by creating and storing sequences of spectral images into files that can be used for data analysis. Spectral cube has several controls like video control to start or pause spectral video, data cube setup to open/close the data recording control window, color view to open/close the scrolling color image window, spectral view to open/close the spectral plot window, band selection to activate band selection option, GPS information etc. to facilitate image capturing. The camera consists of 135 bands.
Figure 7-3. Hyperspectral camera (Dimension (16x3x6 inches (length x width x depth))

Figure 7-4. Hyperspectral image acquisition in field
A total of 40 images 13 images (8 HLB, 5 Healthy) in Fort Basinger, ten images (HLB) in Immokalee the and 20 images (10 HLB, 7 Healthy) at the third location were evaluated in this study. There was no healthy tree of Valencia available in Immokalee grove.

Generally, this study is comprised of two important parts. The first part includes pre-processing and second includes processing. The hyperspectral images were

![Flow chart of methodology](image)

Figure 7-5. Flow chart of methodology
imported in remote sensing software ENVI 4.5 (ITT Visual Information Solutions, Boulder, Colorado). Figure 7-5 shows the overall flow of the process that has been involved in this study.

**Preprocessing**

Preprocessing involved several steps. Raw image acquired by hyperspectral camera is shown in Figure 7-6. Since noisy data was acquired between 306 nm to 420 nm, and bands after 870 nm, these bands were eliminated from the data and a total of 80 bands were used in the analysis. Now dark subtraction was applied by subtracting minimum band value. In the next step, image was calibrated with flat field method. Flat field method normalize image with the area of known reflectance. In each image capturing, spectralon white reference was included. By selecting the pixels of spectralon white panel in the image, hyperspectral images were calibrated.

In the next step, background objects such as soil, sky, grasses etc. were removed before data extraction. For isolating citrus leafy area, two masks were created. First mask was created with the help of unsupervised classification. Unsupervised classification classified images into various categories. The mask based on the unwanted objects were created and applied on the calibrated image. Now still there are few objects remain in the image needs to remove before final processing.
The region of interests (ROI) was selected for remaining unwanted objects and second mask was created (Figure 7-7). This mask was applied on the image obtained after applying first mask. Finally, image contains only leafy area of citrus is ready to analyze. (Figure 7-8)
Figure 7-7. Final mask for removing background as sky, soil, grass etc.
Figure 7-8. Processed image used for data analysis

**Processing**

Processing part involved calculation of vegetation indices. List of all the vegetation indices, given in table 6-1, were evaluated in this study. Statistics for all individual bands computed. In this study band 970nm were replaced by 800 nm because of the noise.

**Results and Discussion**

Statistical analysis was performed to see the difference between vegetation indices of HLB-infected tree and healthy trees. ANOVA were performed to find out whether the calculated vegetation indices were significantly different from one another among two class (healthy and HLB). Among the estimated vegetation indices (Table 6-
1), few vegetation indices were found to be statistically significant to differentiate between HLB-infected trees and healthy trees. Normalized difference vegetation index (NDVI₁ & NDVI₂), simple ratio index (SR₁ & SR₂), modified triangular vegetation index (MTVI₂), renormalized difference vegetation index (RDVI), modified chlorophyll absorption in reflectance index (MCARI₁) and structure intensive pigment index (SIPI) were found significant from each other and could be utilized to discriminate HLB trees from healthy trees.

Table 7-1. Means of vegetation indices of HLB-infected and healthy trees showing statistically significant difference at α =0.05 (Same letter in row shows no significant difference between healthy and HLB samples)

<table>
<thead>
<tr>
<th>Vegetation indices</th>
<th>HLB</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normalized Difference Vegetation Index (NDVI₁)</td>
<td>0.47a</td>
<td>0.71b</td>
</tr>
<tr>
<td>Normalized Difference Vegetation Index (NDVI₂)</td>
<td>0.49a</td>
<td>0.72b</td>
</tr>
<tr>
<td>Simple Ratio Index (SR₂)</td>
<td>3.75a</td>
<td>6.44b</td>
</tr>
<tr>
<td>Simple Ratio Index (SR₁)</td>
<td>3.97a</td>
<td>6.80a</td>
</tr>
<tr>
<td>Modified Triangular Vegetation Index (MTVI₁)</td>
<td>0.83a</td>
<td>0.94a</td>
</tr>
<tr>
<td>Modified Triangular Vegetation Index (MTVI₂)</td>
<td>0.50a</td>
<td>0.72b</td>
</tr>
<tr>
<td>Renormalized Difference Vegetation Index (RDVI)</td>
<td>0.45a</td>
<td>0.63b</td>
</tr>
<tr>
<td>Greenness Index (G)</td>
<td>1.73a</td>
<td>1.99a</td>
</tr>
<tr>
<td>Triangular Vegetation Index (TVI)</td>
<td>32.16a</td>
<td>37.00a</td>
</tr>
<tr>
<td>Modified Chlorophyll Absorption in Reflectance Index (MCARI₁)</td>
<td>0.83a</td>
<td>0.94a</td>
</tr>
<tr>
<td>Structure Intensive Pigment Index (SIPI)</td>
<td>0.34a</td>
<td>0.58b</td>
</tr>
</tbody>
</table>
Both tests concluded that modified triangular vegetation index (MTVI$_1$), greenness index (G), triangular vegetation index (TVI) and modified chlorophyll absorption in reflectance index (MCARI$_1$) are not significant to discriminate HLB among healthy trees. All these indices suggested that reflectance at 870, 800, 670 and 570 nm are very critical. It seems that G index is insignificant to discriminate HLB trees from healthy trees because of the absence of NIR band. Though 870, 670 and 570 nm are critical wavebands their combination in vegetation indices are also very critical. It seems MTVI$_1$, TVI and MCARI$_1$ fail to discriminate HLB trees from healthy trees because of the difference of reflective values narrows the mean difference of HLB and healthy trees.

**Conclusions**

40 Hyperspectral images (28 HLB, 12 Healthy) were evaluated for detection of HLB trees in field condition. They demonstrated good potential to discriminate HLB-infected and healthy trees. Since the sizes of hyperspectral images are large,
preprocessing consumes longer time. Software of hyperspectral camera is also not very user friendly and have communication issues with computer. Normalized difference vegetation index (NDVI₁ & NDVI₂), simple ratio index (SR₁ & SR₂), modified triangular vegetation index (MTVI₂), renormalized difference vegetation index (RDVI), modified chlorophyll absorption in reflectance index (MCARI₁) and structure intensive pigment index (SIPI) showed good potential to discriminate HLB infected trees. Future studies involve the evaluation of the imaging and optical sensor for discriminating nutrient deficient tree and trees infected with other diseases. The measurements in this study were performed on one cultivar of orange. It will be necessary to assess the hyperspectral images and the classification algorithms for their performance with respect to other orange cultivars and citrus trees.
CHAPTER 8
SUMMARY AND RECOMMENDATIONS

The goal of this research was to develop a technique for rapid detection of HLB infected trees in the field condition. In this study canopy reflectance spectra were measured on infected and healthy trees using FieldSpec® 3 spectroradiometer (Analytical Spectral Devices, Boulder, CO), SVC HR-1024 portable spectroradiometer (Spectra Vista Corporation, Poughkeepsie, New York), multiband sensor (Applied Technology Stillwater, OK) and Specim hyperspectral camera (Autovision Inc., Los Angeles, CA, USA). Various classification algorithms like, KNN, logistic regression, support vector machine, neural network and decision tree were used to classify the infected trees from the healthy ones.

Chapter two describes the brief overview of all suspected diseases similar to HLB and nutrient deficiencies that may be confused with HLB. A very careful and rigorous inspection with trained people is required in the grove to identify HLB. Citrus trees also need to supply proper fertilizer and water for proper growth and to avoid nutritional deficiencies. This chapter explains chlorosis, types of chlorosis, causes of chlorosis, various mechanism of chlorosis due to diseases and nutritional deficiencies. It also discusses the diseases in citrus caused by vectors, virus, pathogens and post harvest decays.

Chapter three identifies the critical wavelength that may be helpful in developing low cost sensor. Discriminability, derivative analysis and spectral ratio analysis were performed before applying machine learning techniques. Results were promising and supports our hypothesis that spectroscopy can detect HLB.
Chapter four reports the partial least squares modeling and discriminant analysis to identify HLB in the field with ambient light and in greenhouse with artificial light. Results showed that these techniques are promising in HLB detection for various varieties of citrus. Overall, the full range of data gave more accurate results compared to narrow range with both techniques. However, the narrow range (400 nm to 900 nm) data gave better results with PLS modeling. In contrast, discriminant analysis was better in overall use of the full spectral range. It seems that the narrow range can produce very good results if the HLB symptoms are visible, but a major goal is to detect HLB before visible symptoms appear.

Chapter five discusses application of visible NIR spectroscopy in HLB detection. The goal of this study was to develop a technique for rapid detection of HLB infected citrus trees. Canopy reflectance spectra were measured on infected and healthy trees using a SpectraVista spectroradiometer. Three machine learning techniques (KNN, logistic regression and support vector machine) were used to classify the infected trees from the healthy ones. The results concluded that a single measurement was insufficient for accurate detection of the infected trees. The classification error was between 18% and 35% using a single spectrum. However, using multiple spectral measurements from a single tree, the classification accuracy increased significantly. SVM method showed an accuracy of higher than 95% when it was provided with five spectra from the same tree. Under real field conditions, varying sunlight and other environmental factors can produce noise that might reduce the classification accuracies. Under these conditions, multiple measurements will be necessary to ensure acceptable classification accuracy.
Chapter six illustrates the potential of multiband sensor to detect HLB in the field. The results indicated that the multi-band optic sensor has a very good potential for detecting HLB infected citrus trees under field conditions. However, to achieve high classification accuracy, it requires several measurements from a single tree. The sensor can be incorporated with the scouting practice, to increase the efficacy in HLB disease detection. Collecting multiple readings using multi band sensor is easy and fast, and can be used by a human or an automated vehicle system. The results indicated that the multi-band optic sensor used in this study has a very good potential for detecting HLB infected citrus trees under field conditions. However, to obtain high classification accuracy, it is necessary to acquire multiple measurements from a single tree. The sensor can be integrated with the scouting practice, to improve the effectiveness in HLB disease detection. The measurements in this study were performed on valencia and mid sweet orange cultivars. It would be interesting and useful to know the performance of the sensor and the classification algorithms on other orange cultivars or other citrus types with similar diseases and nutrient deficiencies symptoms.

Chapter seven concluded the application of hyperspectral imaging to detect HLB in the field conditions. 40 hyperspectral images (28 HLB, 12 Healthy) were used in this study. They demonstrated good potential to discriminate HLB-infected and healthy trees. Hyperspectral images require good storage space in computer and good processor to play with hyperspectral images. Normalized difference vegetation index (NDVI<sub>1</sub> & NDVI<sub>2</sub>), simple ratio index (SR<sub>1</sub> & SR<sub>2</sub>), modified triangular vegetation index (MTVI<sub>2</sub>), renormalized difference vegetation index (RDVI), modified chlorophyll absorption in reflectance index (MCARI<sub>1</sub>) and structure intensive pigment index (SIPI)
showed good potential to discriminate HLB infected trees. Future studies involve the evaluation of the imaging and optical sensor for discriminating nutrient deficient trees and trees infected with other diseases. Several other classification algorithms can be used in future to see their effectiveness. The measurements in this study were performed on one cultivar of citrus. It will be necessary to assess the hyperspectral images and the classification algorithms for their performance with respect to other citrus varieties.


Kane, K. E. and W. S. Lee. 2006. Spectral sensing of different citrus varieties for precision agriculture. *In Proceeding of ASABE annual meeting*, July 9-12, Portland, OR ASABE paper no: 061065


BIOGRAPHICAL SKETCH

Ashish Ratn Mishra was born in Allahabad city in the state of Uttar Pradesh, India, in 1980. He graduated from Allahabad Agricultural Institute Deemed University, India, in 2001 with a bachelor’s degree in agricultural engineering with silver medal. He moved to University of Arkansas, Fayetteville, Arkansas, United States to pursue his graduate studies, in 2003. He graduated with a Master of Science degree in agricultural and biological engineering in 2005. To continue his higher education he joined the Ph.D. program in the University of Florida’s department of agricultural and biological engineering, where he specialized in hyperspectral imaging, spectroscopy and geographical information systems (GIS).