EARLY DETECTION OF OAK WILT DISEASE IN QUERCUS SPP.:  
A HYPERSPECTRAL APPROACH

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ABSTRACT

Initial work has begun on a long-term study of the application of spectroradiometric techniques to spectral discrimination of oak wilt disease in Quercus woodlands of central Texas. In situ hyperspectral data at the leaf, branch, and canopy level of the most commonly affected species will be collected, processed, and analyzed for discriminatory signatures of the disease in various stages of disease pathology. A 2 x 2 factorial design inoculation experiment is in progress that will provide the leaf-level spectral response to drought stress and oak wilt infection stress in forty 2 cm. caliper potted specimens of Q. virginiana var. fusiformis. Measurements were made of xylem water potential, leaf water content, and reflection spectra before, during, and after an initial 6 day water withdrawal to test the effects of drought stress. Spectral vegetation indices (SVI) and red-edge inflection point (REP) parameters were extracted from the leaf reflection spectra. ANOVA results indicated that all SVI and REP parameters successfully (P < 0.05) detected water stress at the leaf level. Results for the inoculation treatments are pending – at the time of this writing, no evidence is apparent of an oak wilt infection. Data obtained from the inoculation experiment and from the field will lead to the development of classification methods that can be applied to hyperspectral imagery that will be obtained over the study area in a scheduled acquisition from the Hyperion satellite. The ultimate goal of this research is to determine the feasibility of early disease detection through the application of hyperspectral technology.

INTRODUCTION

Remote sensing plays a pivotal role in the characterization of important ecological and environmental variables from local to global scale. The ability to monitor and track changes in nutrient, carbon, and water cycles, land use and land cover, forest structure and function, habitat, and invasive species is critical to the understanding of ecosystem and climate models (Kerr and Ostrovsky, 2003). Remote sensing platforms such as Landsat have made great contributions to environmental research over the past three decades. However, as environmental scientists probe ever deeper into ecosystem processes and models, the importance and need of higher spatial, spectral, and temporal resolutions for our orbital and airborne sensors has become apparent (Wulder, et al. 2004). The development of high spectral resolution (Hyperspectral) image sensor technology (Hyperion, AVIRIS, HyMap) in the mid-1990’s has revolutionized both environmental and geologic analysis of the earth’s surface for its ability to discriminate subtle spectral signatures of mineralogy, soil moisture, vegetation type, and vegetation stress (van der Meer, 1998; Kruse, Boardman, Huntington, 2003; Mumby, et al., 2004; Schmidt and Skidmore, 2003; Apan, et al. 2004). Hyperspectral remote sensing and field-based spectrometry, employed in concert, offer significant opportunities to delineate and discriminate particular environmental variables – variables previously considered difficult, if not impossible, to isolate using existing multispectral, broadband imagery. The goal of this research project is to develop and conduct an environmental investigation of oak wilt disease in the woodlands of central Texas using field-spectrometry, a controlled inoculation experiment, and classification of hyperspectral imagery. In the course of searching for the discriminating signature of oak wilt-induced physiological stress on afflicted oak species, I will be collecting and cataloging significant data on the general spectral signatures of these species as they progress through their phenological cycles in a variety of environmental settings. The spectral libraries that will be
built of central Texas woodland species will begin to fill a much-needed data gap in vegetation spectral information. Building a spectral library, however, is only the groundwork for the spectral analysis needed to adequately assess and classify hyperspectral data. The spectra of vegetation materials must be statistically separable; if not, then little possibility exists that the endmembers they represent will be spectrally distinct in the hyperspectral imagery (Herold, M., et al., 2004).

**Background of Oak Wilt**

Disease pathogens such as Dutch Elm Disease and Chestnut Blight have wreaked havoc on North American forests during the 20th century. A less virulent pathogen, but potentially as devastating in its impact, is the pathogen responsible for the destructive vascular disease in *Quercus ssp.*, the fungus *Ceratocystis fagacearum* (Wilson, A. D., 2001). Commonly known as oak wilt, this disease claims many thousands of trees annually from the upper midwest to central Texas, resulting in significant property, ecological, and aesthetic losses (Appell, D. N., 1995). Multispectral and high spatial resolution remote sensing have been shown to be effective in identifying oak wilt mortality centers but only in middle to late stages of disease pathology - stages of defoliation and/or leaf discoloration (Everitt, J. H., Escobar, D. E., 1999). Of particular importance in disease management is the ability to detect disease centers in their earliest stages of development. My approach, using in-situ hyperspectral technology, is to construct a spectral library of the pathology of oak wilt in the most commonly affected oak species native to central Texas. Filtered by a spectral baseline of healthy trees as they progress through their phenological stages, my goal is to look for a hyperspectral signature of the disease, data that will ultimately be convolved to airborne and satellite hyperspectral sensors, such as Hyperion and AVIRIS. Success in this project has ramifications for early detection of numerous forestry pathogens, invasive species, and disease vectors.

**METHOD**

This research project will be based on the collection, processing, and analysis of data from 3 sources – field data from known oak wilt mortality centers, a controlled inoculation experiment, and hyperspectral satellite imagery. All three phases were designed to run concurrently. Field spectroradiometric data were to be collected synchronously with a Hyperion acquisition scheduled for June or July. However, as of August 24, no imagery has been acquired due to Hyperion tasking conflicts. Although some data has been collected from the field sites, that aspect of this project has been put on hold until the imagery is acquired. The oak wilt inoculation experiment, in progress, will be reported on here.

**Experimental design**

Drought stress and oak wilt stress will be examined in a 2x2 factorial design (Table 1). The objective is to find statistically significant spectral discriminants between drought stress and oak wilt infection stress. However, due to the uncertainty of the success of the inoculation and of the timing of the onset of visual oak wilt symptoms it was recognized that the drought stress treatment could not run concurrently with the expected oak wilt onset (20 – 60 days). In other words, drought stress symptoms could completely mask oak wilt symptoms and vice versa. While the 2x2 factorial design allows for interaction effects, such effects were not considered a priority. Therefore, the design would dictate the water stress treatment be conducted in the first week after inoculation, followed by a resumption of normal water allocation. Any interaction between the two stress treatments, of interest to this study, would involve the potential of the initial drought stress to delay or advance the onset of the oak wilt stress. The assessment of such an interaction is problematic given that a diagnosis of an oak wilt infection is entirely visual or requires sample extraction and laboratory incubation. No “test kit” indicators of oak wilt are available.

<table>
<thead>
<tr>
<th>Drought Stress</th>
<th>Oak Wilt Stress</th>
<th>No Inoculation</th>
<th>Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal water</td>
<td>Treatment 1</td>
<td>N = 10</td>
<td>Treatment 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N = 10</td>
</tr>
<tr>
<td>Water withdrawal</td>
<td>Treatment 3</td>
<td>N = 10</td>
<td>Treatment 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N = 10</td>
</tr>
</tbody>
</table>
Materials and preparation

Forty sapling specimens of 5-year-old *Q. virginiana var. fusiformis* were purchased May 22, 2005, from a local San Antonio, Texas grower. The trees were from locally harvested seed and were selected for uniform height (~1.5 m), trunk girth (2 cm), and leaf morphology. All specimens were repotted in uniform ~ 15 liter pots with a sandy loam soil mix. The specimens were placed in an outside, fenced 4 x 6 m enclosure, approximately 0.6 meters apart, under full sun and allowed to acclimate to local conditions. No transplant shock was noticed and all trees responded within three weeks with a new flush of growth. Trees were initially hand-watered on a watering schedule of 1 liter per day, increased to 2 liters per day as temperatures climbed and drier conditions prevailed in June. Tests of daily water consumption were run on sample trees, taking into account 6 cm of mulch added to each pot. An automatic drip irrigation system was setup to deliver 1.5 liters/day total to each tree incrementally at 10 am, 2 pm, and 5pm. An impervious, solar reflective bubble wrap (Reflectix ®) shield was placed tightly around the tree's trunk, overlapping the sides of the pot, to exclude rainwater and to moderate pot temperatures. Inoculum was obtained from the plant pathology laboratories of Texas A&M University as a 0.9% saline suspension of *Ceratocystis fagacearum* spores prepared on June 22, 2005 from a culture of a recently acquired wood tissue specimen. The specimen originated from an active oak wilt infection center near Dallas, Texas.

Trees were randomly assigned a position in the enclosure and to each of 4 treatment groups, 10 trees per group. The 2 x 2 factorial design analyzed water stress (normal water and a 6 day withdrawal) and oak wilt stress (inoculated and not inoculated). The inoculation technique involved excising a 3 x 0.5 cm. strip into the trunk through the cambium and into the xylem tissue at approximately 15 cm. above the root collar. Under aseptic conditions as much as possible, correspondingly sized strips of cellulose sponge (previously autoclaved) were dipped in the suspension, placed into the trunk wound, and taped to prevent moisture loss. The inoculation was carried out on the evening of June 26. The following day, a 6 day water withdrawal was begun for two treatment groups (treatments 3 and 4).

Measurements

Reflectance spectra were collected of individual leaves using an Analytical Spectral Devices (ASD) FieldSpec Pro spectroradiometer. This instrument records in 2150 interpolated wavebands over the range of 350 – 2500 nm. Prior to each sampling, the instrument is optimized for dark current levels, and then calibrated against a Labsphere Spectralon white reference. Each reflectance file consists of 10 consecutive reflectance spectra collected over a period of 3 seconds, auto-averaged to reduce ambient noise. All on-tree leaf samples are placed individually against a flat-black background to minimize additive reflection, and illuminated from an ASD xenon light source leaf probe, in direct contact with the leaf. Spectra were collected from 5 to 10 mature and, if available, emergent leaves from each tree in each sampling run at locations from basal branches to tree apex.

To further assess drought conditions, pre-dawn xylem water potentials (XWP) were collected, using a pressure chamber, on all trees the day after inoculation (the first day of water withdrawal), and a week later on the 6th day of water withdrawal. Normal watering of all trees was resumed on the 7th day after inoculation due to the severe stress imposed – 5 trees were totally dessicated, others were suffering from extensive leaf drop. Xylem water potential collection was resumed on the 14th, 17th, and 23rd day after inoculation to assess the recovery from the water withdrawal.

A second assessment of drought conditions was accomplished by deriving leaf water content (LWC) on or about the same days as the water potential collections. LWC is expressed as

\[
LWC = \frac{FW - DW}{FW}
\]

where FW is the fresh weight in g and DW is the dry weight in g. Leaves were oven dried at 60 °C for a minimum of 24 hours.

Data handling and processing

Data files collected were input into the Spectral Analysis and Management System (SAMS v. 2.0) developed by the Center for Spatial Technologies and Remote Sensing, at the University of California, Davis. Data files were initially corrected for calibration offsets between sensors (the FieldSpec Pro has 3 separate sensors), using the jump correction algorithm in SAMS. Each set of leaf spectra from a single tree was averaged and a mean spectra with standard deviation was output. This mean reflectance file for each tree then became the basis for the calculation of the red edge inflection point (REP) and the peak value at the red edge inflection point (REP_max) from the first derivative spectra. Using the extraction algorithm of SAMS, reflectance values at specific wavelengths were
generated and used to calculate a set of spectral vegetation indices – indices expected to be particularly sensitive to water stress or to other biophysical stresses strongly influenced by water stress (Table 2).

<table>
<thead>
<tr>
<th>Name</th>
<th>Formula</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normalized DifferenceVegetation Index 1</td>
<td>((R750–660)/(R750+R660))</td>
<td></td>
</tr>
<tr>
<td>Disease-Water Stress Index 1</td>
<td>(R800/R1660)</td>
<td>Apan, et al., 2003</td>
</tr>
<tr>
<td>NDWI-Hyperion</td>
<td>((R1070–R1200)/(R1070+R1200))</td>
<td>Ustin, et al., 2002</td>
</tr>
<tr>
<td>Moisture Stress Index MSI</td>
<td>(R1600 / R820)</td>
<td>Hunt &amp; Rock, 1989</td>
</tr>
</tbody>
</table>

\(R\) = reflectance

Statistical Analysis

I assessed the performance of both derivative REP parameters and spectral vegetation indices as statistically significant indicators of water stress using a two-way analysis of variance (ANOVA) with the statistical analysis software JMP v 5.1. ANOVA’s were run on two sets of baseline spectral data, the first collected on May 26, just after repotting the trees, and again on June 20, about 1 week prior to the inoculation. Follow-up ANOVA’s were run on post-inoculation data collected on August 2, the date of maximum water stress, and on August 12, and18 - the latter two to assess the degree of recovery of the water stress treatment group. Statistical significance was set at \(P < 0.05\).

RESULTS

Baseline conditions

Baseline leaf water content (LWC) of fully turgid leaves (1 leaf per tree soaked 24 hours in 4 °C water), sampled July 27, the day after the inoculation, was 53.9 ± 2.4 % (mean ± 1 standard deviation). LWC of the same leaves in their fresh state was 52.0 ± 2.2 %.

Baseline pre-dawn xylem water potential (XWP) for all trees, sampled July 28, was -0.28 ± 0.13 MPa (mean ± 1 standard deviation).

Baseline spectra from each tree (the mean of 10 leaf spectra per tree) in all treatment groups, collected July 19 – 21, were statistically analyzed for differences between treatment group means. No significant difference was observed, and none was expected, using several key spectral indices as parameters: MSI (\(P = 0.8465\)), NDWI (\(P = 0.9388\)), DSWI(1) (\(P = 0.8207\)), and REP\(_{\lambda}\) (\(P = 0.997\)), indicating considerable uniformity of tree spectral properties across the treatment groups.

Drought stress conditions

Treatment groups 3 and 4 were subjected to a 6 day water withdrawal beginning on the day after treatment groups 2 and 4 were inoculated. Reflectance spectra and samples for the LWC calculation were collected on the morning of day 6, and XWP measurements were taken pre-dawn on the morning of day 7. For LWC, 3 leaf samples from each tree were taken. ANOVA results indicated highly significant differences among treatment means for LWC (\(P < 0.0001\)). For XWP, one sample per tree was taken at a common midpoint position from each tree apex. ANOVA results indicated highly significant differences among treatment means for XWP (\(P < 0.0001\)). Drought stress was of sufficient intensity and duration that 5 trees died rather abruptly on day 7, 4 from treatment group 3 and 1 from group 4. All 5 trees had shown pre-dawn water potentials in excess of –7.0 MPa the previous morning. For the surviving trees (n = 15), the mean water potential was -5.83 MPa. In the normal watered groups 1 and 2, the mean water potential was –0.68 MPa.

Statistical analysis using the derivative spectra parameters, peak value at the red-edge inflection point (REP\(_{\text{max}}\)) and the wavelength at the red-edge inflection point (REP\(_{\lambda}\)) indicated significantly different means (Table 3).
Table 3. Mean ± one SD first derivative value at REP<sub>max</sub> (P = 0.001) and at REP<sub>λ</sub> (P = 0.0133)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± one SD REP&lt;sub&gt;max&lt;/sub&gt;</th>
<th>Mean ± one SD REP&lt;sub&gt;λ&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Δ reflection/nanometer)</td>
<td>(nanometers)</td>
</tr>
<tr>
<td>1</td>
<td>0.0108 ± 0.0003&lt;sup&gt;A&lt;/sup&gt;</td>
<td>715.91 ± 2.86&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>0.0108 ± 0.0002&lt;sup&gt;A&lt;/sup&gt;</td>
<td>717.62 ± 1.26&lt;sup&gt;AM&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>0.0112 ± 0.0004&lt;sup&gt;H&lt;/sup&gt;</td>
<td>714.60 ± 2.75&lt;sup&gt;H&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>0.0113 ± 0.0003&lt;sup&gt;H&lt;/sup&gt;</td>
<td>713.44 ± 3.77&lt;sup&gt;H&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Levels with same letter are not significantly different (Student’s T pair test)

Additional analysis of spectral vegetation indices (SVI) specifically selected for their utility as indicators of water or drought stress, all showed statistically significant differences in treatment means (Table 4).

Table 4. Mean ± one SD spectra ratio indices for 4 treatments

<table>
<thead>
<tr>
<th>Spectral Index</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
<th>Treatment 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDVI(1) P = 0.0043</td>
<td>0.8692 ± 0.0104&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.8770 ± 0.0102&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.8593 ± 0.0125&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.8635 ± 0.0090&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>MSI P &lt; 0.0001</td>
<td>0.5810 ± 0.0251&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.5710 ± 0.0239&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.6266 ± 0.0313&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.6233 ± 0.0298&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>DSWI(1) P &lt; 0.0001</td>
<td>1.7182 ± 0.0766&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.7468 ± 0.0725&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.5909 ± 0.0772&lt;sup&gt;B&lt;/sup&gt;</td>
<td>1.5986 ± 0.0787&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>NDWI(hyp) P &lt; 0.0001</td>
<td>0.0526 ± 0.0042&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.0549 ± 0.0041&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.0446 ± 0.0056&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.0454 ± 0.0056&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Levels with same letter are not significantly different (Student’s T pair test)

**DISCUSSION**

It is a bit premature to draw any supportable conclusions regarding the anticipated outcome of this inoculation experiment – that of being able to find a discriminating spectral response to stress from oak wilt infection. The results of the analysis of variance of the treatment means do indicate, however, that the selected spectral parameters respond directly or indirectly to plant water stress through water withdrawal. Leaf pigments primarily control leaf level reflectance in the visible spectrum (VIS), while leaf mesophyll structure controls reflectance in the near infrared (NIR). Reductions in leaf water content does not directly impact reflectance in the VIS and NIR, although it is likely to have indirect effects through impacts to chlorophyll chemistry in the VIS and structural changes in the NIR. The NDVI spectral index utilizes spectral bands from only the VIS and NIR, yet that parameter statistically discriminated (P = .0043) the water stressed treatment from the non-stressed treatment groups. The spectral region between the VIS and NIR, known as the red edge region, yields the derivative REP parameters that are also highly significant in predicting water stress. In the middle or short wave infrared (SWIR), the effect of water stress is a dominant factor in leaf reflectance. This was clearly supported by the statistical significance of the other spectral indices.

Leaf water stress should be a key indicator of an oak wilt infection as the primary pathology of the disease involves a fungal invasion of water-conducting tissue in the xylem which trigger the formation of tyloses and gums that plug the conducting vessels (Appel, 1995). Whether the mechanics of water stress from drought versus the mechanics of water stress from a fungal invasion of the xylem, and the plant’s physiological response to both, are distinguishable at the leaf level through spectral analysis remains to be seen. At the time of this writing, 4 weeks after inoculation, the spectral parameters analyzed have largely returned to baseline values. Data collection will continue in the hopes of a return of a water stress signal in the spectra. At such time work will commence on a more detailed and exhaustive search for specific spectral information capable of discriminating the oak wilt infection stress from the drought stress.
REFERENCES CITED


ACKNOWLEDGEMENTS

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